Influence of Length Changes on Myocardial Metabolism in the Cat Papillary Muscle

GEORGE COOPER, IV

SUMMARY Myocardial oxygen consumption (MVO₂) increases in proportion to the cumulative product of active tension and time throughout the normal isometric contraction. However, rapid shortening at any time during a contraction prevents further tension generation and oxygen consumption during the remainder of that contraction. My hypothesis is that, under physiological conditions, shortening produces this effect by reducing the extent and duration of the energy-dependent events subserving contraction. I tested this hypothesis by minimizing the potential reduction of metabolism via muscle shortening during contraction; this was done by lowering temperature (23°C), reducing shortening rate (0.8 muscle length/sec) and increasing myoplasmic calcium (7.5 mM Ca²⁺ + 10 mM caffeine). Twelve right ventricular cat papillary muscles were released from that length at which maximum active force is developed to a slack length at increasing intervals after stimulation. Under these conditions, absolute MVO₂ increased significantly, but the relative increase with progressively longer stimulus-release intervals was greatly attenuated. At a 0-msec stimulus-release interval under the present conditions, MVO₂ was 8.26 nl/mg dry wt per contraction; for a full contraction MVO₂ was only 27% greater. Under the more physiological conditions employed in a prior study, this increase, from 0.41 to 2.97 nl/mg dry wt per contraction, was 624%. The addition of 10⁻⁷ M norepinephrine under otherwise physiological conditions increased MVO₂ at all times during contraction, but the relationship between MVO₂ and length was not altered. These data suggest that, both under normal conditions and after inotropic augmentation, shortening of cardiac muscle during contraction decreases the level of the energy-dependent events supporting contraction; this may be the mechanism by which MVO₂ is regulated by length-dependent changes in metabolism throughout contraction. Circ Res 49: 423-433, 1981

IT has been accepted generally (Braunwald, 1971; Lambert and Posner, 1979) on the basis of previous data (Monroe, 1964) that the relaxation phase of myocardial contraction requires very little energy. However, recent studies (Cooper, 1978; Cooper, 1979) on the regulation of myocardial metabolism have led to two new conclusions. First, there is no energy-independent portion of the isometric myocardial contraction. Second, the level of metabolism, measured as the net energy utilization of the processes required to initiate and sustain the myocardial contraction, varies directly with changing muscle length from the time of stimulation through that of complete relaxation.

These two conclusions have led to the hypothesis that length-dependent variation in metabolic activity regulates myocardial oxygen consumption (MVO₂) during the normal cardiac contraction. The present investigation of this hypothesis is based both on my previous finding that MVO₂ is reduced when heart muscle is allowed to shorten during contraction, with a progressive reduction in MVO₂ with earlier shortening, and on the supposition that one possible mechanism for such a reduction would be a decrease in the level of intracellular calcium affecting cross-bridge activation. If oxygen consumption primarily reflects the action of calcium on the contractile apparatus, the effect of such a decrease would be obscured as the calcium concentration approaches the level required to saturate the contractile apparatus. The present experimental conditions include the presence of high calcium and caffeine in an effort to cause such a saturation. The effect of these experimental conditions was assayed both metabolically and mechanically throughout contraction. Under these circumstances, absence of major length-dependent effects on myocardial me-
tabolism would support the hypothesis; persistence of major length-dependent metabolic effects would not. The present results support the hypothesis.

Methods

Experimental Apparatus

This study employed a flow respirometer in which the mechanical and metabolic behavior of a stable, in vitro superfused cat right ventricular papillary muscle preparation could be characterized. The flow respirometer, associated equipment, and attendant methods have been described fully in previous papers (McDonald, 1966; Coleman, 1967; Cooper, 1976; Cooper, 1979). For the particular purposes of this study, the respirometer and the other devices were modified to allow muscle length to be measured and controlled from above (Cooper, 1979), and muscle length, and therefore tension, to be varied during and between contractions in a controlled manner in an attempt to prevent variable length and load effects on mechanical activity (Edman and Nilsson, 1972).

Measurement of Mechanical Activity and Oxygen Consumption

A rapid cardiectomy was performed on adult cats (1.9-3.6 kg) after anesthesia had been induced with sodium pentobarbital (25 mg/kg, ip). Twenty-nine right ventricular papillary muscles 4.12-9.83 mm in length (6.50 ± 0.33, mean ± SE) and 0.38-1.10 mm² right ventricular papillary muscles 4.12-9.83 mm in length and load effects on mechanical activity (Edman and Nilsson, 1972).

The muscles were superfused at 23°C with a solution of the following composition (mM): CaCl₂, 7.5; KC1, 4.7; MgSO₄, 1.2; KH₂PO₄, 1.1; NaHCO₃, 24.0; Na acetate, 20.0; NaCl, 98.0; caffeine, 10.0; and glucose, 10.0 with 10 units of zinc insulin added per liter. This solution was equilibrated with 95% O₂-5% CO₂ with a resultant pH of 7.4. The presence of high calcium concentration and caffeine has not been found in the past to limit metabolic support and therefore tension, to be varied during and between contractions in a controlled manner in an attempt to prevent variable length and load effects on mechanical activity (Edman and Nilsson, 1972).

An important feature of this method is that it allows muscle load and length to be altered in a controlled manner. Table 1 summarizes the data for controlled release and lengthening for the nine stimulus-release intervals employed; it is most easily understood in conjunction with the bottom panel of Figure 1. Because of variable active tension during contraction, the unloading rate, that rate at which active tension was removed from the muscle, differed at each stimulus-release interval. This procedure is illustrated in Figure 1 and explained in its legend.

An important feature of this method is that it allows muscle load and length to be altered in a controlled manner. Table 1 summarizes the data for controlled release and lengthening for the nine stimulus-release intervals employed; it is most easily understood in conjunction with the bottom panel of Figure 1. Because of variable active tension during contraction, the unloading rate, that rate at which active tension was removed from the muscle, differed at each stimulus-release interval. That is, the amplitude of the initial negative dT/dt deflection in the bottom panel of Figure 1 was inconstant at different stimulus-release intervals. However, the maximum rate at which the muscles could potentially shorten during contraction, the controlled release rate, did not vary; that is, the amplitude of the positive dL/dt deflection in the bottom panel of Figure 1 was constant at all stimulus-release intervals. The bottom half of this table shows that, after each contraction, when active tension was no longer present, both the rate of lengthening back to L_max, the controlled lengthening rate, and the rate of passive tension reimplosion which produced this length change, the reloading rate, were the same at the varying stimulus-release intervals; that is, the second positive dT/dt deflection and the negative dL/dt deflection in the bottom panel of Figure 1 were constant at all stimulus-release intervals. Thus, these mechanical pertur-
A normal isometric contraction (upper panel) and the following released isometric contraction (lower panel). The calibration bar for each tracing is on the left of the upper tracing, and the identification of each tracing is on the right. $f$ Tension denotes the continuously recorded integral of the tension signal. No deflection of the $f$ Tension tracing is seen for resting tension at $L_{ma}$ (far left on both panels); the cumulative upward deflection (note that the integrator resets to the middle of its range at a fixed upper and lower limit) during active tension generation quantifies the product of active tension and time ($\int AT$). Active tension was developed following a brief latent period after the stimulus. In the lower panel, tension was then unloaded from the muscle by shortening it, as shown by the upper two tracings. Unloading placed the muscle at a slack length, $L < L_{sa}$, as shown by a decrease in tension to zero external tension. Since the muscle was then slack, the tension integrator shows progressive negative deflection. Finally, on the far right, the muscle was returned to $L_{ma}$ prior to the next stimulus.

**Figure 1** A normal isometric contraction (upper panel) and the following released isometric contraction (lower panel). The calibration bar for each tracing is on the left of the upper tracing, and the identification of each tracing is on the right. $f$ Tension denotes the continuously recorded integral of the tension signal. No deflection of the $f$ Tension tracing is seen for resting tension at $L_{ma}$ (far left on both panels); the cumulative upward deflection (note that the integrator resets to the middle of its range at a fixed upper and lower limit) during active tension generation quantifies the product of active tension and time ($\int AT$). Active tension was developed following a brief latent period after the stimulus. In the lower panel, tension was then unloaded from the muscle by shortening it, as shown by the upper two tracings. Unloading placed the muscle at a slack length, $L < L_{sa}$, as shown by a decrease in tension to zero external tension. Since the muscle was then slack, the tension integrator shows progressive negative deflection. Finally, on the far right, the muscle was returned to $L_{ma}$ prior to the next stimulus.

Experimental Protocols, Group I

There were four experimental protocols, the order of which was randomized, for an initial group of 12 muscles. In this group, mechanical and metabolic behavior were characterized simultaneously.
TABLE 1 Controlled Release and Lengthening Data

<table>
<thead>
<tr>
<th>Stimulus release</th>
<th>0</th>
<th>0.4</th>
<th>0.8</th>
<th>1.2</th>
<th>1.6</th>
<th>2.0</th>
<th>2.4</th>
<th>2.8</th>
<th>3.2</th>
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<tbody>
<tr>
<td>Controlled release rate (muscle lengths/sec)</td>
<td></td>
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<tr>
<td>0.80 ± 0.15</td>
<td>0.80 ± 0.10</td>
<td>0.72 ± 0.11</td>
<td>0.73 ± 0.11</td>
<td>0.76 ± 0.13</td>
<td>0.77 ± 0.13</td>
<td>0.78 ± 0.12</td>
<td>0.78 ± 0.11</td>
<td>0.78 ± 0.11</td>
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<td>Unloading rate* (N/mm²·sec⁻¹)</td>
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<tr>
<td>0.11 ± 0.01</td>
<td>0.28 ± 0.03</td>
<td>0.40 ± 0.04</td>
<td>0.41 ± 0.04</td>
<td>0.33 ± 0.04</td>
<td>0.24 ± 0.04</td>
<td>0.16 ± 0.03</td>
<td>0.14 ± 0.03</td>
<td>0.11 ± 0.02</td>
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<tr>
<td>Controlled lengthening rate (muscle lengths/sec)</td>
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<tr>
<td>2.52 ± 0.39</td>
<td>2.55 ± 0.38</td>
<td>2.58 ± 0.39</td>
<td>2.57 ± 0.36</td>
<td>2.51 ± 0.34</td>
<td>2.62 ± 0.46</td>
<td>2.64 ± 0.46</td>
<td>2.67 ± 0.46</td>
<td>2.44 ± 0.41</td>
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<tr>
<td>Reloading rate (N/mm²·sec⁻¹)</td>
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<tr>
<td>0.24 ± 0.03</td>
<td>0.24 ± 0.04</td>
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<td>0.25 ± 0.04</td>
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</table>

For the dependent variable indicated by an asterisk (*) there were significant differences among these values (Winer, 1971). The release and lengthening rates specify the rate of change in muscle length, the unloading and reloading rates specify the rate of change in muscle tension.

MVO₂ and Muscle Mechanics during Isometric Contractions at Lₘₐₓ, Unloaded at Specific Times during Contraction

With the muscles at Lₘₐₓ, groups of 60 contractions (stimulus rate of 0.167 Hz over a 6-minute period for each group of contractions) were produced, and the associated MVO₂ was measured. Tension generation during each contraction in a group was interrupted by controlled release of the muscles at the same particular stimulus-release interval. The stimulus-release interval was determined by solenoid activation starting at 0 msec after stimulus (mechanical release beginning at the end of the latent period) and progressing in 0.4-second increments through 3.2 seconds or, alternatively, at 3.2 seconds after stimulus and progressing through 0 msec. The mechanical and metabolic data were similar with either sequence. An example of the MVO₂ recordings at five of the nine stimulus-release intervals in one muscle is shown in Figure 2.

MVO₂ during Contractions at a Slack Length, L < Lₙ

These experiments were done to define any metabolic effects of changing muscle length or tension during the latent period. The muscles were allowed to remain at the slack length (L < Lₙ) determined by their intrinsic elastic and viscous properties. The MVO₂ produced by 60 contractions at a rate of 0.167 Hz was then measured without release. This MVO₂ value was compared to that observed for the 0-msec stimulus-release interval in the first section of studies, where the muscles were at Lₘₐₓ only during the latent period and slack thereafter.

MVO₂ and Muscle Mechanics during Contractions at Lₘₐₓ without Release

These experiments were done to determine whether the controlled release of contracting muscles affected potential mechanical or metabolic performance. The muscles were held at Lₘₐₓ and stimulated to contract isometrically without being released at any time. Again, 60 contractions during 6 minutes were studied. The mechanical data were compared to those observed for the stimulus-release intervals in the first section of studies where an appropriate comparison could be made. The metabolic data were compared to those observed for the 3.2-second stimulus-release interval in the first section of studies.

MVO₂ during Muscle Release without Stimulation

To assess further the possible effect of the release intervention itself on MVO₂, the muscles were released from L = Lₘₐₓ to L < Lₙ and then returned
to $L = L_{\text{max}}$ 60 times during 6 minutes without being stimulated to contract. $L_0$ is defined in this study as that muscle length on the ascending limb of the active length-tension relationship at which externally measured active tension during contraction is first noted.

**Experimental Protocol, Group II**

Mechanical and metabolic behavior under two different inotropic conditions were defined in this group of five muscles. The muscles were superfused at 29°C by a solution containing 2.5 mM calcium and no caffeine but otherwise identical to that employed for the group I muscles. The lightly preloaded muscles were stimulated to contract at 0.167 Hz until a stable mechanical response was obtained and brought to $L_{\text{max}}$. $\text{MVO}_2$ and muscle mechanics were then measured during quick release at five stimulus-release intervals beginning at the end of the latent period and proceeding in 200-msec intervals through the time of complete relaxation at 800 msec; the release rate in each instance was 2.0 muscle lengths/sec. At each stimulus-release interval, data were collected for a group of 120 contractions at a stimulus rate of 0.5 Hz. The muscles then were allowed to re-equilibrate at 0.167 Hz in the presence of $10^{-7}$ M norepinephrine, and the same protocol was repeated under otherwise identical experimental conditions. Muscle length and resting tension at $L_{\text{max}}$ did not change with the addition of norepinephrine. Support of metabolism of diffusion under these conditions at this norepinephrine concentration has been found to be adequate before (Coleman et al., 1971).

**Experimental Protocol, Group III**

In this group of 12 muscles, only mechanical behavior was characterized. This was done under the identical conditions of both the previous (Cooper, 1979) and the present studies in an effort to determine through an independent technique whether significant inhibition of length-dependent metabolic effects had been achieved under the conditions employed in the present study. The muscles were brought to $L_{\text{max}}$ and stimulated to contract at 0.5 Hz under the conditions of the previous study or at 0.167 Hz under the conditions of the present study. When a stable mechanical response was obtained, on alternate contractions the muscles were first left at $L_{\text{max}}$ throughout contraction. During the next contraction, they were released to a slack length at the end of the latent period and restored to $L_{\text{max}}$ at the time of peak active tension observed during the preceding contraction. Redeveloped tension on restoration to $L_{\text{max}}$ was used as a qualitative index of the amount of shortening-induced deactivation that occurred during the released contraction.

After this, the muscles were submitted to the alternate set of conditions, allowed to equilibrate until a stable mechanical response was obtained, and the study was repeated. Muscle length and resting tension at $L_{\text{max}}$ did not differ under the two conditions; the order in which the conditions were applied was random.

**Statistical Analysis**

Each value is expressed as mean ± se. For paired comparisons, Student's paired t-test was employed. For comparisons of greater numbers of variables, one-way analysis of variance and multiple intergroup comparisons using polynomial regression were employed (Winer, 1971). A significant difference was said to exist when $P$ was less than 0.05.

**Results**

**MVO$_2$ and Muscle Mechanics during Isometric Contractions at $L_{\text{max}}$ with Variable Stimulus-Release Intervals**

The upper panel of Figure 3 shows the externally measured active tension at the specified instant initiating controlled release. The two points not joined to the others by lines (at 360 ± 10 msec and 1028 ± 48 msec) are at the time of peak active tension and do not represent times at which the muscles were released. The value for peak active tension increased from 69.0 ± 4.7 mN/mm$^2$ under physiological conditions to 83.3 ± 4.2 mN/mm$^2$ under the present conditions. The four major mechanical effects of the present experimental conditions are: (1) a slower rate of rise of active tension, (2) a prolonged time to peak active tension, (3) an increase in active tension, and (4) a prolongation of contraction. The first two effects presumably reflect primarily a temperature-dependent reduction in the rate of calcium entry into the sarcoplasm from the extracellular space; the latter two effects presumably reflect a reduction in the rate of calcium uptake by the sarcoplasmic reticulum (Weber and Herz, 1968). These effects are reflected in the lower panel of Figure 3, which shows the cumulative increase in the area under the active tension curves of the upper panel as these contractions were terminated at progressively later times. The vertical arrows indicate the times of peak active tension. The maximum rate of tension generation did not differ with varying stimulus release intervals; this was also the case in the previous study. Thus, this particular estimate of inotropic state, an important determinant of MVO$_2$ (Braunwald, 1971), did not vary for the eight stimulus-release intervals where it could be measured.

Figure 4 displays the cumulative oxygen consumption associated with contractions in which active tension generation was terminated at the specified stimulus-release intervals. The left panel is reproduced from a previous study (Cooper, 1979). In considering these data, it is important to remember that MVO$_2$ is measured for the entire contraction, as illustrated in Figure 1, regardless of the duration of active tension generation prior to
FIGURE 3  Upper panel: average active tension values at the time of release of isometric contractions at each of the nine stimulus-release intervals studied in 10 muscles in the previous (unfilled circles, dashed lines) study (Cooper, 1979) and in 12 muscles in the present (filled circles, solid lines) study. The points at the times of peak active tension, not connected by continuous lines, are at the time of and show the amount of peak active tension; these are not points at which the muscles were released. Data from the previous study are included to facilitate comparisons. Lower panel: Average fAT values for isometric contractions at each of the nine stimulus release intervals used in the previous (unfilled circles, dashed lines) study (Cooper, 1979) and in the present (filled circles, solid lines) study. The arrows indicate the times of peak active tension.

release during contraction to a slack length. Note that the broken line in the left panel demonstrates a progressive increase in MVO₂ as the duration of active tension increased. At the end of the latent period, i.e., the intercept of the vertical axis for the broken line, MVO₂ is only 14% of that for a complete contraction. At the time of peak active tension, indicated by the vertical arrow, MVO₂ was 64% of that for a complete contraction.

In contrast to the data described by the dashed line, the solid line in the right panel of Figure 4 shows that the relative increase in MVO₂ in the present study as the duration of active tension increased was small. At the end of the latent period, MVO₂ was 73% of that for a complete contraction. At the time of peak active tension, indicated by the vertical arrow, MVO₂ was 93% of that observed for a complete contraction.

MVO₂ during Contraction at a Slack Length, L < L₀

Referring to the pair of points on the vertical axis of the left panel of Figure 4, there is in absolute terms a small, but in relative terms a large and significant, difference between MVO₂ at the two muscle lengths indicated. It should be noted that, in progressively lengthening the muscles to find Lmax, no change in resting MVO₂ was found; it is therefore unlikely that these two different MVO₂ values, which are only slightly greater than that of the resting muscle, represent diffusion limitations based on differing muscle thickness at the two lengths. For contractions in which the muscles were unloaded to zero resting and active tension just before active tension generation began (0 msec, L = Lmax), the MVO₂ was 14% of that observed for the contractions released at the 800-msec stimulus-release interval. For contractions in which the muscles were not released but were at a slack length (0 msec, L < L₀) at which they generated no active tension and demonstrated no resting tension at any time during the 4-minute period of stimulation, the MVO₂ was 8% of the value for the 800-msec stimulus-release interval.

Referring to the pair of points on the vertical axis of the right panel of Figure 4, there is no significant difference between the MVO₂ at the two muscle lengths indicated (L = Lmax vs. L < L₀). Thus, although the absolute difference in MVO₂ at these two lengths was fairly similar in both studies, a significant percentage difference in MVO₂ by Student's paired t-test during the latent period is no longer apparent under the present conditions.

MVO₂ and Muscle Mechanics during Contractions at Lmax without Release

The MVO₂ for 60 full isometric contractions without release at any time was 10.44 ± 0.62 nL O₂/mg per contraction; this was not significantly different from the value of 10.47 ± 0.75 for contractions released at 3.2 seconds after stimulus. Similarly, neither peak active tension, the time-to-peak tension, nor the maximum rate of tension development differed significantly for the unreleased contractions when compared to those released at 1.2 through 3.2 seconds, and the fAT was the same for the unreleased contractions and those released at the 3.2-second stimulus-release interval. The same
relationships were found to obtain when released vs. unreleased contractions of the same type were compared in the previous study (Cooper, 1979).

MVO$_2$ during Release without Stimulation

When the quiescent muscles were released without stimulation 120 times during 4 minutes in the previous study (Cooper, 1979) or 60 times during 6 minutes in the present study, there was no detectable deflection in the MVO$_2$ record.

These last two types of experiments demonstrate that, under the conditions of both studies, the release intervention itself does not have an independent effect on mechanics or energetics; rather, the effects of release are mediated through changes in the length of activated muscle. These effects were large with normal activation; they are small with enhanced activation.

MVO$_2$ under Two Different Inotropic Conditions

Figure 5 shows the oxygen consumption during contraction under control conditions and then, for the same muscles, in the presence of 10$^{-7}$ M norepinephrine. The control conditions are identical to those employed in obtaining the data in the left panel of Figure 4. With the addition of norepinephrine, there were three significant changes in mechanical behavior: active tension increased from 78.6 ± 14.2 to 92.9 ± 10.5 mN/mm$^2$, active tension for a full contraction increased from 4.22 ± 1.06 to 5.13 ± 0.95 N/mm$^2$·sec and the maximum rate of tension generation increased from 0.42 ± 0.11 to 0.56 ± 0.11 N/mm$^2$·sec. Contraction duration did not change.

The data in Figure 5 show a significant increase in MVO$_2$ after the addition of norepinephrine at each of the stimulus-release intervals examined. The amount of this increase was similar for each of these intervals.

Mechanical Assay of Shortening-Induced Deactivation

To be able to draw firm conclusions about the results presented thus far, it was important to establish through an independent technique that, under the conditions shown in the left panel of Figure 4, shortening produced substantial deactivation of the contracting muscle, especially during the early part of contraction, whereas, under the conditions shown in the right panel of Figure 4, this was largely prevented. For this purpose, activation was studied mechanically under the two sets of conditions.

An example of a study performed on a second group of 12 muscles to substantiate this expectation is shown in Figure 6. The upper panel of this figure shows mechanical behavior under the physiological conditions used for the left panel of Figure 4. After
FIGURE 6. Experimental records obtained under the stated experimental conditions. The vertical arrows on the lower two panels indicate $T_{\text{matched}}$; this refers to identical times after stimulation at which developed and redeveloped active tension were measured.

In contrast, the lower panel of Figure 6 shows the result of the same experiment performed on this same muscle after the conditions had been changed to the conditions of enhanced activation employed in the right panel of Figure 4. It should be noted that no change in resting tension or $\text{MVO}_{2}$ at $L_{\text{max}}$ was detected in the quiescent muscle during the transition to this superfusate, arguing against any significant diastolic activation as measured either metabolically or mechanically. In the lower panel, in which the muscle was slowly released at the end of the latent period and then slowly restored to $L_{\text{max}}$ at the time of expected peak active tension, there is substantial redevelopment of active tension during the second contraction. A significant contribution of stress-relaxation of elastic components to this phenomenon is unlikely: this same release and re-extension without stimulation produced no positive deflection in the tension tracing above resting tension. These data for 12 muscles are summarized in Table 2. Both the absolute amount of redeveloped tension and the relative amount of redeveloped tension with and without Release

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Maximum active tension (mN/mm²)</th>
<th>$%$ Maximum active tension</th>
<th>$%$ Active tension at $T_{\text{matched}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$29^\circ\text{C}, 2.5\text{ mM Ca}^{++}, 0\text{ mM caffeine}$</td>
<td>$52.4 \pm 3.6$</td>
<td>$0.6 \pm 0.3$</td>
<td>$1.2 \pm 0.7$</td>
</tr>
<tr>
<td>$23^\circ\text{C}, 7.5\text{ mM Ca}^{++}, 10\text{ mM caffeine}$</td>
<td>$80.9 \pm 3.5$</td>
<td>$49.9 \pm 3.8$</td>
<td>$62.6 \pm 2.9$</td>
</tr>
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</table>

All four variables under the two experimental conditions were significantly different from each other $T_{\text{matched}}$ refers to the matched time after stimulus at which active tension was measured in both the released and unreleased contractions; the time of $T_{\text{matched}}$ in the time after stimulus of maximum redeveloped tension (the two vertical arrows on the lower panel of Figure 6 indicate $T_{\text{matched}}$, the interval between the stimulus and the arrow is the same for the unreleased and released contractions).
tension when compared to maximum active tension are quite different under the two different experimental conditions. Further, when the maximum redeveloped active tension is compared to active tension during the preceding control contraction at the same time after stimulus, Tm, which as indicated by the vertical arrows in the lower panel of Figure 6, the disparity between the shortening-induced deactivation in the two experimental situations is even more striking. Finally, when even a very brief release-extension step was imposed, beginning at the end of the latent period and lasting only long enough to go from L = Lmax to L < L0 and then back to L = Lmax, a 44% reduction in active tension was seen under the conditions of the previous study (Cooper, 1979); in contrast, no reduction in active tension was seen under the conditions of the present study.

These data show that shortening during contraction produces a marked reduction in the extent and duration of active tension generation under physiological conditions; this effect is largely nullified under the conditions of enhanced activation employed in the present study.

Discussion

This study resulted in four principal new findings. First, increasing the level and duration of myocardial activation and decreasing the velocity of shortening prevent, to a great extent, the influence of shortening during contraction on MVO2. Second, this is true for those energy-dependent events which initiate contraction, as well as for those events which sustain contraction throughout its course. Third, an increase in inotropic state augments MVO2 throughout contraction but does not alter the relationship of shortening to MVO2. Fourth, the first two findings, obtained from measurements of myocardial metabolism, have been independently related to length-dependent activation by measurements of myocardial mechanics.

Time Course of MVO2 during Contraction

The first new finding is best shown by comparing the slopes of the broken and solid lines in the two panels of Figure 4. It is clear from the right panel that, under the conditions of the present study, when the level and duration of activation are greatly increased, there is only a small relative increase in oxygen consumption as the duration of active tension increases during contraction. Not surprisingly, since there was a major increase in the total ΔAT/contraction in the present study, as shown by Figure 3, the absolute increases in total MVO2/contraction under the conditions of the two studies are similar despite the lesser relative increase in MVO2 throughout contraction in the present study. Thus, the slopes of the two lines in Figure 4, presumably reflecting the relative amounts of shortening-induced reduction of metabolism during contraction, differ greatly. The highest MVO2 value in each panel of Figure 4, which did not differ in either instance from that obtained when there was no muscle release, quantifies the metabolism associated with complete, mechanically undisturbed contractions at Lmax under the two sets of experimental conditions. The degree of shortening-induced reduction of metabolism at any particular stimulus-release interval corresponds to the ratio of the MVO2 value at this time to that for a full contraction. The approximately 4-fold increase in the MVO2 for a full contraction under the present conditions probably reflects a greater extent and duration of activation. However, the degree of shortening-induced reduction of metabolism at any time during contraction is clearly less under the experimental conditions shown on the right as opposed to the left panel of Figure 4 when the MVO2 values relative to those for a full contraction are compared at matched fractional intervals throughout contraction.

Figures 4 and 5 and previous data obtained during myocardial tetanus (Cooper, 1976) provide some insight into the variable extent of shortening-dependent regulation of metabolism under different conditions of activation. Under reasonably physiological conditions, such as those used to obtain the data described by the left panel of Figure 4 and the lower, broken line of Figure 5, the calcium-sensitive protein aequorin has been used to show that cardiac muscle stimulation is followed by a rapid rise of myoplasmic calcium concentration to a peak level at about the time of the maximum rate of tension generation, with a gradual decline to the pre-stimulation level at a time approximately coincident with the time of complete relaxation (Allen and Blinks, 1978). In the presence of catecholamines, as in the upper solid line in Figure 5, the aequorin signal shows a more rapid initial rise and a greater amplitude than under control conditions; the signal rapidly returns to the resting level at about the time of complete relaxation. However, during tetanus of skeletal muscle, the aequorin response suggests that the sarcoplasmic calcium concentration may normally exceed that required to saturate the contractile apparatus (Blinks et al., 1978). The conditions (caffeine, increased calcium concentration, and lower temperature) used to obtain the data in the right panel of Figure 4 are the same as those used previously (Cooper, 1975) to tetanize cardiac muscle with repetitive electrical stimulation. Caffeine both releases a labile fraction of membrane-bound calcium in proportion to drug concentration and inhibits calcium uptake by various preparations of sarcoplasmic reticulum (Isaacson and Sandow, 1967; Weber and Herz, 1968). These effects, together with the increased calcium concentration and lower temperature employed in the right panel of Figure 4, should greatly increase the myoplasmic calcium concentration. Under these conditions during twitch contractions (Figure 4, right panel), there
was relatively little effect of shortening on metabolism at any point during contraction; during tetanic contractions, extremes of length and load changes imposed on the myocardium had almost no effect on energetics, and MVO\textsubscript{2} varied directly and linearly with isometric or isotonic contraction duration (Cooper, 1976).

Thus, length-dependent effects on myocardial metabolism may be seen as operating within a framework of progressively changing level and/or duration of activation. Further, the fact that shortening results in a major reduction of metabolism despite widely varying levels of concomitant active tension interruption at different times during contraction suggests that shortening rather than the accompanying tension reduction is the more important factor regulating metabolism. During control conditions and in the presence of catecholamines, length-dependent control of metabolism is both fairly prominent and similar in degree; the shift of the relationship between MVO\textsubscript{2} and stimulus-release interval in Figure 5 is parallel. As the level, and particularly the duration, of activation is increased further, length-dependent metabolic regulation becomes less prominent, such that the lines relating MVO\textsubscript{2} to stimulus-release interval in the two panels of Figure 4 are non-parallel, and previous data obtained during maximal activation with tetanus (Cooper, 1976) show no appreciable effect of muscle length on metabolism.

The contrast between the two sets of data shown in Figure 4 suggests that, under normal conditions, length-dependent effects on myocardial activation during contraction have major effects on underlying events supported by metabolism. This concept is well supported by mechanical data. These data include the demonstration that quick release has a deactivating effect on heart muscle when assayed mechanically (Edman and Nilsson, 1972; Bodem and Sonnenblick, 1974), the finding that the level of activation, judged from developed tension, decreases with decreasing sarcomere length in skinned cardiac cells (Fabio and Fabio, 1975) and the finding that length oscillations during strontium-induced contractions of heart muscle produce deactivation when measured mechanically (Henderson and Cattell, 1976). Further information about this subject is available in a recent review (Jewell, 1977).

There are additional data suggesting that the basis for this effect on energy-requiring events subserving contraction involves a reduction in the interaction of calcium with cross-bridges during shortening (Kaufman et al., 1972), a negative feedback effect of decreasing muscle length on calcium release from the sarcoplasmic reticulum (Ridgway and Gordon, 1975) or length-dependent electromechanical coupling (Gordon and Ridgway, 1975). However, the present data do not provide further insight into which, if any, of these postulated mechanisms relating length to activation is correct.

### MVO\textsubscript{2} during the Latent Period

The second new finding is illustrated next to the intercept of the ordinate in each panel of Figure 4. The length-dependent effect on initial activation energy during the latent period is relatively great under the conditions employed in the left panel, but it is relatively unimportant under the conditions, shown in the right panel, employed in the present study. This metabolic measurement is in accord with a variety of recent mechanical data. These include the demonstration that, in heart as opposed to skeletal muscle, depolarization probably does not normally result in a maximum concentration of myoplasmic calcium, so that such interventions as drug administration or length changes can modulate the level of activation as measured mechanically (Allen et al., 1974). This supposition is supported both by the present data and by other studies (Bianchi, 1968; Blinks et al., 1972) from which it has been concluded that caffeine enhances calcium release from the transverse tubules and terminal cisternae of cardiac sarcoplasmic reticulum and blocks its uptake by the longitudinal reticulum.

### Inotropic Effect on Metabolism

The third new finding, shown in Figure 5, is that the positive inotropic effect produced by norepinephrine is accompanied by an increase in MVO\textsubscript{2} at all stimulus-release intervals, from the end of the latent period through the full contraction. The degree of augmented metabolism for a full contraction at L\textsubscript{max} is comparable to that reported before for this concentration of norepinephrine (Coleman et al., 1971).

Since the MVO\textsubscript{2} increase over the corresponding control value is similar at each stimulus-release interval during contraction, there is a parallel upward shift of the entire solid line in Figure 5 after the addition of norepinephrine. The contrast between this finding and the non-parallel shift in this relationship seen in Figure 4 when the data in the two panels are compared at the same fractional intervals of contraction duration suggests that, in Figure 5, the degree of length-dependent regulation of myocardial metabolism is similar for each experimental condition, whereas in Figure 4, the degree of length-dependent metabolic regulation is greatly reduced under the conditions specified in the right panel. Thus, the augmented level of activation accompanying the inotropic effect of norepinephrine (Allen and Blinks, 1978) does not change the basic relationship between muscle length and MVO\textsubscript{2}; the increased level and duration of metabolism seen in the right panel of Figure 4 greatly attenuates this relationship.

### Mechanical Assay

The fourth new finding is illustrated by Figure 6; the data supporting this finding are summarized in
Table 2. These data demonstrate through a technique independent of metabolic measurements that shortening-induced deactivation was largely prevented under the conditions of the present study, but was prominent under the conditions of the previous study (Cooper, 1979). These mechanical data support the metabolic data shown in Figure 4. Under conditions of normal activation, rapid changes in muscle length during contraction produce prominent effects on myocardial activation, whereas, under conditions of increased activation, slower changes in muscle length during contraction do not have a major effect on myocardial activation.

To conclude, this study, when compared to the prior two studies (Cooper, 1976; Cooper 1979), supports the hypothesis that changing levels of length-dependent activation regulate MVO₂ during the cardiac cycle. Maximizing activation and reducing release velocity did, in fact, demonstrate a marked suppression of any regulatory effect of changing muscle length. The major conclusions of these three studies of the isometric myocardial contraction are: (1) during the maximal activation achieved by tetanus, metabolism is a function primarily of contraction duration and is relatively unaffected by the mechanical conditions during contraction; (2) under physiological conditions with both normal and enhanced inotropic states, there is a strong dependence of metabolism on muscle length during the twitch, and (3) enhanced activation overrides the apparent length-dependent regulation of metabolism during the twitch. The results of these studies directly support the hypothesis that MVO₂ is dynamically regulated by the level of activation under differing mechanical conditions during the myocardial twitch. These results also imply a direct linkage of metabolic demands to muscle length throughout the normal cardiac cycle.

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