The Myocardial Energetic Active State

I. Oxygen Consumption during Tetanus of Cat Papillary Muscle

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SUMMARY The potential role of active state maintenance as a determinant of myocardial oxygen consumption (MVO₂) has not been defined. Right ventricular papillary muscles from 15 cats were studied in a polarographic myograph at 23°C in a Krebs-Ringer solution containing 7.5 mM Ca²⁺ and 10 mM caffeine. MVO₂ was determined for isometric tetani at Lmax of 1-5 seconds duration. Increases in tetanus duration related linearly to increments in both active tension·time (active tension) and MVO₂. In order to examine the oxygen cost of active state maintenance not attributable to associated tension generation, both the same isometric and 2.5- to 10.0-second lightly preloaded isotonic tetani were produced in nine muscles. For each tetanus duration the contribution throughout the contraction of developed force (preload) to MVO₂ could be subtracted from overall isotonic MVO₂. In the absence of the MVO₂ associated with force development, the active state duration was related linearly to MVO₂, with a mean active state MVO₂ of 2.42 ± 0.29 nI O₂/mg dry muscle/sec of isotonic tetanus; this MVO₂ is 68% of the value of 3.58 ± 0.42 nI O₂/mg dry muscle/sec that was obtained for isometric tetanus at Lmax. This study identifies active state maintenance as the major determinant of MVO₂ during myocardial tetanus and, furthermore, suggests the possibility that alterations in active state intensity and duration may be the biochemical mechanism by which other determinants of MVO₂ act in a more physiological setting.

THE HEART is almost exclusively an aerobic organ; therefore, steady state oxygen consumption provides a reasonably precise estimate of overall myocardial metabolism. In a recent review¹ tension development, contractile state, and heart rate were stated to be the major determinants of myocardial oxygen consumption, with current information suggesting that other factors are less important. However, tetanus of cardiac muscle, with variation of active state duration independent of changes in other determinants of myocardial oxygen consumption, has not previously been possible. Therefore, the potential contribution of active state maintenance to myocardial oxygen consumption has not been determined. The purpose of this investigation was to combine two recently described methods for myocardial tetanization²³ with the polarographic papillary muscle bath¹⁴ to define what role active state maintenance may have in determining myocardial oxygen consumption.

Methods

MEASUREMENT OF MECHANICAL ACTIVITY AND OXYGEN CONSUMPTION OF CAT PAPILLARY MUSCLES

The procedures and myograph used were essentially those described by Coleman,² with any modifications described below. Rapid cardiectomy was performed on adult cats (weighing 1.36-2.72 kg) after anesthesia was induced with sodium pentobarbital (30 mg/kg, ip). The infundibulum of the right ventricle then was opened and flushed with Krebs-Ringer solution saturated at 25°C with 95% O₂-5% CO₂. A papillary muscle was excised from the right ventricle and mounted in the polarographic myograph as previously described.³ Mean papillary muscle dimensions at Lmax (that length at which maximum isometric tension is generated) were: length, 6.11 mm (range 4.27-9.56 mm), and cross-sectional area, 0.77 mm² (range 0.46-1.12 mm²). Muscles of these dimensions are not diffusion-limited so that adequate metabolic support is assured.⁴ The ratio of resting tension to total tension (active tension + resting tension) at Lmax has been used as an index of the mechanical suitability of any particular cat papillary muscle preparation.⁵ This ratio for these 15 muscles, contracting at Lmax at a rate of 0.2 Hz at 23°C in the bathing solution described below, was 15.34 ±

1.14% (mean ± se), with a resting tension at \( L_{\text{max}} \) of 12.28 ± 0.80 milliNewtons per square millimeter (mN/mm²). This value for the ratio is comparable to Brutsaert's value for 11 muscles of 15.64 ± 1.29%.

The muscles were bathed at 23 ± 0.05°C in a solution of the following millimolar composition: CaCl₂, 7.5; KCl, 4.7; MgSO₄, 1.2; K₂HPO₄, 1.1; NaHCO₃, 24.0; Na acetate, 20.0; NaCl, 98.0; glucose, 10.0; and caffeine, 10.0; with 10 units of insulin added per liter. The solution was equilibrated with 95% O₂-5% CO₂ with a resultant pH of 7.4 and circulated past the muscle from a 1-liter reservoir.

Stimulation (bottom tracing in each panel of Fig. 1) was provided as follows. A digital timer controlled a gated pulse generator which triggered an isolated stimulator to deliver 50-msec rectangular pulses at a frequency of 10 Hz for any desired train duration. These pulses were passed through a field effect transistor analog switch to provide 3.2-mA field stimuli of alternating polarity with zero net current flow between the electrodes. This last condition was necessary to prevent both electrolytic contamination of the bath and baseline drift of the polarographic system.

Tension and displacement were measured with the same transducer systems described previously; the amplified output was displayed on an optical recorder. The total system exclusive of the papillary muscle (bath filled with water, 6-0 silk tie attached to tension and displacement transducers, amplifiers, and recorder) had the following characteristics when measured over the range of load and displacement encountered in this study: A rectangular wave of tension and displacement change was produced by quick release of the preloaded isotonic lever by a solenoid-controlled air jet. The recorded oscillatory transient response predicted a uniform frequency ±5% for the amplitude response of the system of 227 Hz for displacement and 61 Hz for tension. The undamped and damped natural frequency was >200 Hz.

This was directly measured by inducing a known sine wave driving frequency into the silk tie attached both to the tension and displacement transducers and to a stiff metal diaphragm driven by an electromagnet energized by a low frequency function generator. The frequency response for tension measurement was flat ±5% to 70 Hz; this figure for displacement was 90 Hz. Compliance over the range of loads studied was nonlinear but was not greater than 0.7 µm/mN.

The areas under the tension (active tension = N · sec) or displacement (mm · sec) curves were obtained from a digital summing circuit with a frequency response of DC to 500 Hz + 1 dB, -3 dB and a reset time of 5 msec. The rate of change of tension or displacement was obtained from a single-order high pass filter with a low frequency cut off -3 dB of 50 Hz. Tension and displacement were calibrated after each experiment by imposing known loads and motions over the experimental range. The digital summing circuit was then calibrated by square waves from a low frequency function generator, where the y axis was referenced to the above tension and displacement calibrations. Similarly, the rate of change in signal amplitude was calibrated from triangular ramps.

Oxygen consumption was measured polarographically. After each experiment muscle length was measured by a micrometer (±0.1 mm) with a weight of 0.5 g attached to the end of the muscle. This length, together with the passive tension portion of the length-tension curve, allowed calculation of muscle length at \( L_{\text{max}} \). The muscle was then dried at \( 110°C \) for 24 hours and weighed on a balance accurate to ±0.01 mg. Muscle cross-sectional area was calculated from the length at \( L_{\text{max}} \) and the dry weight, assuming a ratio of wet weight to dry weight of 4:1 and a specific gravity of 1. Results were normalized in terms of muscle length at \( L_{\text{max}} \) and cross-sectional area. Thus, tension was expressed as mN/mm², shortening as muscle lengths, and oxygen consumption as nanoliters (nl)/mg dry muscle/contraction.

At the beginning of each study the muscle was preloaded and stimulated at 0.2 Hz until a stable mechanical response was obtained; the studies described below then were performed. Because each papillary muscle was studied over several hours, the stability of the preparation was examined as part of two of the experiments: 24 hours after the experimental protocol was completed, with interim 0.2-Hz lightly preloaded contractions, isometric active tension at \( L_{\text{max}} \) had decreased 11%, and preloaded isotonic shortening had increased 12%.

**TYPES OF EXPERIMENTS**

Three types of experiments were performed.

1. For 15 muscles, after preloaded equilibration, a length-tension curve was measured. After \( L_{\text{max}} \) was determined, tetani 1 to 5 seconds in duration were studied (Fig. 1) at this

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Isometric tetani at \( L_{\text{max}} \). For each of the five experimental records from one muscle reproduced in this photograph, the bottom tracing is the stimulus train (increasing in 1-second increments from 1 through 5 seconds), the second tracing from the bottom is the signal from the tension transducer, the third tracing up measures active tension during each contraction (no deflection is produced as resting tension, and the cumulative upward deflection during a contraction quantifies the active tension), and the top tracing is the first derivative of the tension transducer signal. Calibration bars from below up for tension, active tension, and rate of tension change are next to the upper right record.
TABLE 1 Contractile State during Isometric Tetani at Lmax

<table>
<thead>
<tr>
<th>Stimulus duration (sec)</th>
<th>Active tension (mN/mm²)</th>
<th>Rate of tension development (mN/mm²/sec)</th>
<th>Time to peak tension (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76.0 ± 4.8</td>
<td>149.4 ± 11.6</td>
<td>1.09 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>78.8 ± 4.9</td>
<td>150.1 ± 11.3</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>78.0 ± 5.6</td>
<td>147.7 ± 11.0</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>77.0 ± 5.8</td>
<td>144.1 ± 11.3</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>75.1 ± 5.7</td>
<td>142.3 ± 10.6</td>
<td>1.04 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE for the 15 studies in this section. For each of the three indexes of contractile state Student's paired t-test revealed no significant difference at any stimulus duration when the greatest difference for each index was compared.

muscle length; a total of 24 tetani over a 4-minute period were summed at each tetanus duration for the measurement of associated M\(\overline{V}O_2\) (Fig. 2).

2. For nine of these muscles, this isometric study was randomly preceded or followed by an isotonic study in which lightly preloaded (4.62 ± 0.35 mN/mm²) tetani were produced for durations of 2.5-10 seconds over an 8-minute period for each tetanus duration (see Fig. 5). The oxygen consumption was summed for 24 tetani at each tetanus duration (see Fig. 6).

3. For nine additional experiments a series of 48 single isometric twitches at Lmax, and their M\(\overline{V}O_2\) were studied over a 4-minute period at 23°C, first in the bathing solution with 2.5 mM CaCl₂ and 0 mM caffeine and then 1 hour after these concentrations had been changed to 7.5 mM CaCl₂ and 10 mM caffeine (see Fig. 9). Resting M\(\overline{V}O_2\), the change in oxygen tension produced by introducing the quiescent muscle into the myograph or by removing it, was also determined in these two bathing solutions.

Results

OXYGEN CONSUMPTION DURING ISOMETRIC TETANI

A photograph of a record of the stimulus trains and resultant mechanical activity of a representative study from this section is shown in Figure 1. Table 1 shows that when these mechanical data for each of the five tetanus durations are summarized for all of the muscles, there is no significant difference between any of the tetanus durations in maximum active tension, rate of tension development, or time to peak tension. Therefore, of the three generally accepted major determinants of myocardial oxygen consumption, i.e., tension development, contractile state (rate of tension development and time to peak tension), and contraction frequency, the first two remained constant, and the third was held constant for each duration of isometric stimulation. Thus, any change in oxygen consumption with greater tetanus duration would necessarily reflect the increasing time integral of developed tension. Figure 2 is a photograph of the oxygen consumption records for each of the five isometric tetanus intervals in one experiment. There is a progressive increase in the area under these curves (M\(\overline{V}O_2\)) with increasing tetanus duration. Figure 3 summarizes the mechanical and metabolic changes during the isometric tetani of the 15 muscles studied. The left panel of this figure, together with Table 1, demonstrates that the only mechani-
cal change associated with increasing tetanus duration is an increase in active tension. The right panel of Figure 3 summarizes the metabolic data for all 15 muscles. There is an increase in M\(\text{VO}_2\) with each increment of isometric stimulus duration. Because both the mechanical and metabolic changes vary directly with tetanus duration, a linear relationship between active tension and M\(\text{VO}_2\) was to be expected. Figure 4 shows this to be the case: when the M\(\text{VO}_2\) of all the experiments is plotted as a function of the time-tension integral a linear relationship is demonstrated. Thus, tension maintenance is shown to be a major determinant of myocardial oxygen consumption in this situation, with a constant relationship between active tension and oxygen cost.

**OXYGEN CONSUMPTION DURING ISOTONIC TETANI**

The data presented in this section examine the oxygen cost of the tetanic contraction without the potential contribution of associated external tension generation and work. The following approach was used.

During the initial six isometric studies it was found that for each papillary muscle a reasonably accurate determination of M\(\text{VO}_2\)/active tension could be made: for the nine muscles in which both isometric and isotonic tetani were studied the maximum variation in each muscle among the values for M\(\text{VO}_2\)/active tension obtained during the five isometric tetanus intervals was 19.1 ± 3.7 nl \(\text{O}_2\)/mg dry muscle/N/mm\(^2\)*sec. The magnitude of this error may be judged and compared to the overall variation among all the muscles by observing the size and scatter of this value for the points in Figure 4. Since the weight of the light preload (\(\text{N/mm}^2\)) and the duration of the isotonic tetani (sec) were known, for each muscle an accurate value for the oxygen cost of generating the tension required to hold up the preload (nl \(\text{O}_2\)/mg dry muscle/N/mm\(^2\)*sec) throughout the isotonic tetani was available for subtraction from overall isotonic M\(\text{VO}_2\). Thus, if the rate and amount of work performed did not vary during a series of progressively longer isotonic tetani with constant preload, the oxygen cost of myocardial tetanus itself, without the potential contribution of external mechanical events, could be determined.

Figure 5 is a photograph of the experimental record of a
### Table 2  Contractile State during Preloaded Isotonic Tetani

<table>
<thead>
<tr>
<th>Stimulus duration (sec)</th>
<th>Extent of shortening (muscle lengths)</th>
<th>Rate of shortening (muscle lengths/sec)</th>
<th>Time to peak shortening (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.31 ± 0.02</td>
<td>0.57 ± 0.05</td>
<td>1.30 ± 0.12</td>
</tr>
<tr>
<td>5.0</td>
<td>0.32 ± 0.02</td>
<td>0.59 ± 0.06</td>
<td>1.31 ± 0.15</td>
</tr>
<tr>
<td>7.5</td>
<td>0.31 ± 0.02</td>
<td>0.58 ± 0.05</td>
<td>1.27 ± 0.12</td>
</tr>
<tr>
<td>10.0</td>
<td>0.31 ± 0.02</td>
<td>0.57 ± 0.05</td>
<td>1.29 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± SE for the nine studies in this section. For each of the three indexes of contractile state Student's t-test revealed no significant difference at any stimulus duration when the greatest difference for each index was compared.

### Figure 6  M\(\text{VO}_2\) during preloaded isotonic tetani. This is a photograph of the experimental records of oxygen consumption during the four isotonic tetanus intervals shown in Figure 5. The number under each curve is the duration in seconds of the stimulus train used to tetanize the muscle; the area under each of the five curves quantifies the oxygen consumed during 24 tetani over an 8-minute period at each stimulus duration. The calibration box on the original records was 1 cm².

### Figure 7  Average oxygen consumption values (on the ordinate) ± 1 SE for the four isotonic contraction intervals studied in nine muscles. The dashed line describes the linear regression equation for these four points.

### Figure 8  Average values ± 1 SE for the oxygen consumption of the nine papillary muscles studied during both isometric tetani at \(L_{\text{max}}\) (active tension, 78.64 ± 2.94 mN/mm²; length, 6.51 ± 0.43 mm) and isotonic preloaded tetani (preload, 4.62 ± 0.35 mN/mm²; initial length, 5.83 ± 0.45 mm; shortened length, 3.84 ± 0.41 mm). The lines at the bottom of the figure indicate the statistical comparisons being made, and the percent values in the middle and right bars are referenced to the left bar. Corrected isotonic \(\text{MVO}_2\) = isotonic \(\text{MVO}_2\) - preload \(\text{MVO}_2\). See text for explanation of terms.
of this section, a correction is made for the MVO₂ which may be attributed to the active tension required to hold up the preload during these isotonic tetani the cross-hatched bar on the right shows that this corrected MVO₂ is still 68% of that observed at Lₘₐₓ.

**OXYGEN CONSUMPTION DURING ISOMETRIC TWITCHES**

In this section the special conditions required to tetanize myocardium are examined. The left panel of Figure 9 is a photograph of the mechanical response of the same muscle at Lₘₐₓ to a single stimulus, first in a bathing solution having a physiological calcium concentration with no caffeine and, below this, in the solution used for tetanus. The most prominent differences between these two records are in active tension and twitch duration with the resultant increase in active tension shown on the lower record. Table 3 summarizes these mechanical data for nine experiments. The right panel of Figure 9 shows the oxygen consumption curves from a representative muscle during 48 contractions in the physiological and tetanizing solutions; there is an increased MVO₂ during the contractions in the tetanizing solution. Figure 10 summarizes the metabolic data for these nine studies. The left panel shows a slight, but insignificant, increase in resting MVO₂ (the oxygen consumption of the noncontracting muscle) in the tetanizing solution, but the right panel shows that there is no change in the relationship between MVO₂ and active tension in the two solutions.

**Discussion**

A brief consideration of muscle active state and of appropriate ways to measure it is necessary before discussing any relevance of the results of this study to the energetics of the myocardial active state. On the basis of studies of the frog sartorius published in 1924, Gasser and Hill related “active state intensity” to “force development at constant contractile element length.” This mechanical description of muscle active state seems straightforward enough, but the problem of realizing homogeneous constant contractile element length during the contraction of heart muscle has not been clearly solved for a variety of reasons fully reviewed by Brady. Indeed, divergent results for the time course of the myocardial active state obtained by different investigators are very likely due to necessary inferences which must be made about muscle elasticity and muscle models, as Abbott and Gordon recently pointed out. Perhaps most

<table>
<thead>
<tr>
<th>Bathing solution</th>
<th>Active tension (mN/mm²)</th>
<th>Rate of tension development (mN/mm²/sec)</th>
<th>Time to peak tension (sec)</th>
<th>Total twitch duration (sec)</th>
<th>Active tension (N/mm² sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mM Ca²⁺ 0.0 mM caffeine</td>
<td>47.8 ± 3.7</td>
<td>121.4 ± 7.4</td>
<td>0.69 ± 0.04</td>
<td>1.84 ± 0.08</td>
<td>0.89 ± 0.13</td>
</tr>
<tr>
<td>7.5 mM Ca²⁺ 10.0 mM caffeine</td>
<td>74.7 ± 4.6</td>
<td>154.5 ± 11.3</td>
<td>0.97 ± 0.04</td>
<td>4.71 ± 0.31</td>
<td>2.91 ± 0.33</td>
</tr>
</tbody>
</table>

Values are means ± SE for the nine studies in this section. On comparison by Student's paired t-test the difference for each value in the two bathing solutions was significant at a minimum of the P < 0.01 level. There was no difference in resting tension in the two solutions.
The energetic active state would be appropriately measured in skeletal muscle by quantitating ATP hydrolysis during muscular activity. This has been done by a number of investigators, and some of the conclusions of these studies on skeletal muscle were: (1) in isotonic contractions ATP breakdown varies directly and linearly with external work, while in isometric tetanus it follows the length-active tension relationship, and (2) almost all of the high energy phosphate is used during the time of contractile activity, rather than after relaxation is complete. Jøbsis and Duffield have supplemented these direct assays of rapidly frozen tissue with an independent method (fluorometric assay of NADH) which indirectly determines the energy utilization of working amphibian skeletal muscle and have confirmed the essential points of the direct biochemical studies: energy utilization is determined by the time integrals throughout a contraction of force and shortening and by mechanical work. These findings support the concept of an active state that is a description of the time course and kinetics of energy-dependent actin-myosin interactions. In fact, Taylor's mathematical model of an active state based on the amount of calcium available to troponin predicts (Equations 4 and 22) the experimental results that Jøbsis and Duffield had already reported.

An appropriate way to measure this energetic active state in heart muscle would be to quantitate the MVO₂ associated with contractile activity. This statement is based on the fact that myocardial metabolism is almost entirely aerobic, together with the finding of Challoner that at least 87% of myocardial respiration is phosphorylating, with 85% of this respiration producing ATP used for contractile activity. The enormous amount of research which has been done on myocardial oxygen consumption, based on this supposition that MVO₂ is a valid measurement of myocardial energy utilization, is reviewed elsewhere. This work has been based largely on measurements of the difference between resting MVO₂ and the MVO₂ associated with contractile activity; the results have been used to describe the mechanical and metabolic determinants of MVO₂ (Table 1 of Braunwald). However, the possibility that the basis for these determinants is an energetically defined active state, with the implication that variations in the intensity and duration of the myocardial active state determine extrabasal MVO₂, apparently has not been considered. Validation of this concept would require that the energetics of the myocardial active state, as defined above, be quantitated apart from associated external mechanical work and tension development. Clearly this is not possible, but the develop-
ment of myocardial tetanization has allowed the present study to approach this goal, as will be discussed below.

The data presented in Figures 1–4 demonstrate that when activation is maximum and constant during tetanus, isometric tension maintenance is associated with a continual energy-dependent active state. This is not surprising, since this finding only confirms for heart muscle what has long been known about skeletal muscle: Hill showed in 1913 that the heat output of skeletal muscle is constant throughout a tetanus; furthermore, in 1936 Nicolai found repetitive changes in light diffraction during tetanus of isometric skeletal muscle which may well have reflected continual sarcomere or subsarcomere motion, although more recent work suggests that any such changes probably represent cyclical cross-bridge activity producing sarcomere motion of not more than 50 Å. Indeed, these results add little to the relationship between the myocardial tension-time index and MVO₂ established by Sarnoff et al. in 1958 or to similar myocardial heat data from Gibbs et al. These data do, however, serve to answer in the affirmative the question posed by Braunwald and by Sonnenblick and Skelton of whether myocardial tension maintenance has a significant associated oxygen cost.

Before carrying this discussion of the oxygen cost of myocardial tetanus past the above description of the MVO₂ of tension maintenance based on an energetic active state to a consideration of active state MVO₂ apart from associated external mechanical events, one must be satisfied that the MVO₂ during tetanus measures primarily myofibrillar ATP hydrolysis rather than that occurring elsewhere in the cell and that the unusual conditions required for myocardial tetanization (high calcium concentration and caffeine) are not altering the normal pattern of energy utilization. The first potential problem is that these conditions might augment the ATP-dependent uptake of calcium by the sarcoplasmic reticulum, as is proposed to be the case during potassium contracture of myocardium. However, caffeine either does not affect or inhibits calcium uptake by various preparations of sarcoplasmic reticulum, and caffeine releases a labile fraction of previously bound calcium. In proportion to drug concentration. Since this effect of caffeine on the sarcoplasmic reticulum should be an increase in myoplasmic calcium, another potential source of altered ATP utilization is energy-dependent mitochondrial calcium accumulation. However, caffeine itself should prevent this because it substantially reduces energy-dependent calcium accumulation by heart mitochondria and enhances its release. A third potential source of altered energy utilization under these experimental conditions is the ATP required to maintain ionic equilibrium. However, it has been shown that skeletal muscle contracture induced by caffeine is not associated with changes in resting potential or ionic permeability of the muscle membrane. Nevertheless, while the conditions required for myocardial tetanization apparently should not increase nonmyofilibrillar ATP consumption, the extrabasal MVO₂ measured in these experiments must necessarily reflect overall ATP hydrolysis during a contraction. In the frog semitendinosus muscle 20–30% of the energy liberated during a twitch or tetanus is apparently related to noncontractile events, specifically calcium turnover by the sarcoplasmic reticulum. The decreased quantity and activity of cardiac sarcoplasmic reticulum as compared to that of skeletal muscle would suggest that this contribution should be less in cardiac muscle, and data from this preparation of cat myocardium demonstrate that the MVO₂/contraction for events not related to significant external work and tension generation (i.e., calcium turnover, shortening against only internal load, and intrasarcomere tension generation) is not more than 5.6% of the total MVO₂ of an isotonic twitch contraction when unloaded isotonic twitch contractions are compared to isotonic twitch contractions at Lmax in a physiological solution at 29°C. This basic relationship is not abrogated by the special conditions required for tetanization; in one experiment lightly preloaded isotonic twitch contractions in the tetanizing solution at 23°C produced an oxygen consumption too small to be accurately quantitated but clearly far smaller than that associated with isotonic twitch contractions at Lmax.

The above evidence strongly suggests that under these experimental conditions the MVO₂ during mechanical events is indeed associated very largely with myosin ATPase activity and that one would not expect caffeine to greatly alter myocardial energetic relationships. Furthermore, Table 3 and the left panel of Figure 9 confirm the mechanical responses to caffeine and increased calcium reported by Blinks et al. (Fig. 2, tracing 2 vs. 5), but Figure 10 demonstrates that this tetanizing bathing solution produces no basic alteration in myocardial energetic relationships either at rest or with activity.

This evidence validates the concept of extrabasal MVO₂ being a measure of an energetic active state for these experimental conditions. In this respect the energetic active states of fully activated, tetanized skeletal and cardiac muscle are very similar: Hartree and Hill observed in 1924 that heat production during tetanus and caffeine contracture were similar in skeletal muscle. There are data suggesting that the increased active tension and heat output of skeletal muscle in caffeine are the result of prolonging the active state. In cardiac muscle full activation is produced by caffeine, and this drug markedly increases the inward current during voltage clamps of cardiac muscle. Since during tetanus there is an equilibrium of the intracellular calcium concentration, there is an expected parallel between the maximum and constant rate of heat output when the stimulus frequency is sufficiently high in skeletal muscle and the constant rate of increase in MVO₂ during fully fused tetanus (Figs. 3 and 7) of heart muscle.

If the extrabasal MVO₂ reflects the ATP hydrolysis at the contractile sites during myocardial tetanus when the energetic active state is associated with external tension development (Fig. 4), an insight into the energetics of the active state in the absence of associated external mechanical events would, as alluded to before, be of greater interest. The data shown in Figures 5–7 and 11 provide this insight. First, the "worst case" assumption was made that tension maintenance accounted for all of the MVO₂ observed during the isotonic tetani, implying that active tension rather than the underlying energy-dependent actin-myosin interactions was somehow responsible for the MVO₂ of these tetani.
Since the value for $MV_{O_2}$/active tension as measured externally was known rather precisely for each muscle, a known $MV_{O_2}$ value for external tension generation could be subtracted from the overall $MV_{O_2}$ of a series of pre-loaded isotonic tetani of the same muscle. There might be some contribution to overall isotonic $MV_{O_2}$ from intrasarcomere tension generation in this situation of marked myocardial shortening, as Huxley, in his Figure 22, has shown compression of thick filaments near the Z lines and rather extensive thin filament overlap in the center of the A bands at short skeletal muscle lengths, and this has been seen in myocardium fixed in systole (Fig. 4 of Sonnenblick et al.). However, as noted above, externally unloaded twitch contractions of this preparation of heart muscle to very short lengths are associated with only a very small $MV_{O_2}$. Therefore, any residual $MV_{O_2}$ during ongoing tetanus after shortening is complete should reflect primarily the oxygen cost of the energetic active state, as no further work is being done, and the externally inconsistent assumption that there might be an $MV_{O_2}$ unique to tension maintenance has been allowed for. The y intercept of Figure 11 presumably reflects the oxygen cost of that part of the energetic active state when shortening is taking place. The increase in this corrected $MV_{O_2}$ with progressively longer tetani measures the $MV_{O_2}$ not accounted for by external mechanical events; i.e., this $MV_{O_2}$ reflects the energetic active state of ATP-dependent actin-myosin interactions apart from their external mechanical or myothermal expression. It should be noted again that while these data reflect the rate and time course of these interactions, they neither provide nor allow any explicit insight into what these interactions might be accomplishing within the sarcomere once shortening is complete. Figure 8 shows that this residual $MV_{O_2}$ is not trivial, since the bar on the right represents a value which is a bit more than two-thirds of that shown by the left bar for isometric tetani at $L_{max}$. Obviously, this division of the energetic active state as defined above into work and tension-related events and into myosin ATPase activity separate from mechanical events is artificial. The middle bar of Figure 8 represents a more internally consistent comparison with the left bar and shows that the absolute $MV_{O_2}$ value per unit time of preloaded isotonic tetanus is only slightly less than that for isometric tetanus at $L_{max}$. Actually, if actin-myosin interactions of the same rate and duration are proposed to occur in these two situations, and if it is further proposed that these interactions rather than their associated mechanical expressions determine $MV_{O_2}$, one would expect these two values to be equal. While no definite explanation for their significant difference is available in these data, one might speculate that during the lightly preloaded tetani enough sarcomere shortening occurred to allow significant thin filament overlap, and thus remove some of the potential actin-myosin interaction sites from further activity once shortening was complete.

The major conclusion to be drawn from these data is that in a situation in which active state intensity is maximum and its duration variable at will, independent of other factors thought to control myocardial oxygen consumption, active state maintenance is the major determinant of myocardial oxygen consumption. A point implied by this study, which is certainly more general in its implications, is that the mechanism whereby mechanical and other factors act as determinants of myocardial oxygen consumption in a more physiological setting may be through modification of this active state, i.e., by changing the rate and time course of energy-dependent actin-myosin interactions during the heartbeat in varying physiological and pathological states. The previous finding that the $MV_{O_2}$ of preloaded isotonic papillary muscle twitch contractions is only 43% of that for isometric contractions of the same muscle at $L_{max}$ contrasts sharply with the value of 83% shown in Figure 8 for tetani under the same two mechanical conditions of identical isotonic preload and isometric length when studied with the same apparatus. This is the same sort of phenomenon described by Hill in 1930 for relatively inextensible semimembranosus skeletal muscle, which is mechanically more nearly analogous to cardiac muscle than the commonly studied but very extensible sartorius: the heat generated during lightly preloaded isotonic twitches was much less than that for isometric twitches generating significant tension, but in isotonic tetani the heat was less only for tetani with very light preloads. The implication for both tissues is that active state autoregulation must occur during the course of a contraction, whether via load variability as suggested by Sträuer via mechanical effects on the action potential with consequent changes in the internal calcium modulators, as Kaufmann et al. suggest based partly on the detailed study of Wood et al. or via calcium modulation through length-dependent membrane resistance and potential changes. Ongoing studies are attempting to delineate this phenomenon by examining the time course and extent of oxygen consumption during isotonic and isometric twitch contractions of heart muscle.

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