Effects of Various Inotropic Interventions on the Dynamic Properties of the Contractile Elements in Heart Muscle of the Cat

By Dirk L. Brutsaert, M.D., William W. Parmley, M.D., and Edmund H. Sonnenblick, M.D.

ABSTRACT
Force-velocity-length (FVL) relations were obtained by determining phase-plane tracings of velocity of shortening vs. length during isotonic contractions. These measurements were then replotted in a three-dimensional graph after correction for the series elastic extension during the isometric phase of the contractions as derived from a quick release contraction. On examining the influence of temperature (29° and 37°), preload, frequency of stimulation (12/min and 24-30/min), paired stimulation (PS), calcium (2.5 mM and 7.5 mM), and isoproterenol (10^-5M), it was shown that the surface created by the three-dimensional FVL relations of the contractile element (CE) is unique for a given state of contractility. Furthermore, the course of velocity vs. length is determined only by the instantaneous CE length, regardless of the contractile state of the CE, and is independent of the time after stimulation over a large portion of the shortening. From the intersection of the linear load-shortening relation observed for the CE with the load axis the maximum force (Po) development of the CE was derived. The highest values of this corrected Po (1.80 ± 0.13 kg/cm²) were seen following inotropic interventions such as PS, calcium, or isoproterenol. Using the corrected Po, truly hyperbolic force-velocity curves were constructed from which V_max and the Hill equation constants a and b were derived for the various inotropic interventions.

ADDITIONAL KEY WORDS
- cat papillary muscles
- force-velocity-length relations
- contractility

It has been shown that the surface created by the three-dimensional plot of the instantaneous force, velocity, and length of the contractile elements (CE) of the myocardium is unique for a given state of contractility (1). Thus the velocity-length relations of the CE for a given load during isotonic contractions are independent of initial muscle length and independent of the time after stimulation except at the terminal portions of shortening when the intensity of active state is falling.

Further, the load-shortening relation of the CE has been shown to be linear. This permits an estimation of the maximum isometric force (Po) which the CE could develop if there were no extension of the series elastic elements (SE) and hence no shortening of the CE during the development of force. Moreover, when corrected to this maximum isometric force, the force-velocity relation of heart muscle becomes hyperbolic in form.

A. V. Hill (2) has demonstrated in skeletal muscle that there is an inverse relation between velocity of shortening (V) and load (P) which is described by the hyperbolic function: \((P + a) (V + b) = (P_o + a)b\), where a and b are constants. This mechanical relation takes on added interest since the constant a can be related to the extent of energy release per unit of shortening and the constant b to the rate of energy release (3). Although such quantitative analysis has
TABLE 1

Dynamic Constants of the Cat Papillary Muscle as Derived from the Equation of Hill

<table>
<thead>
<tr>
<th>Preload</th>
<th>No.</th>
<th>P ′ /P o</th>
<th>P ′ /area (g/mm²)</th>
<th>a (g)</th>
<th>u /P o</th>
<th>V a/e /l l</th>
<th>b/l l</th>
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<tr>
<td>&lt; 0.25 g/mm²</td>
<td>10</td>
<td>0.55 ± 0.02</td>
<td>7.14 ± 1.17</td>
<td>1.01 ± 0.20</td>
<td>0.14 ± 0.02</td>
<td>1.55 ± 0.10</td>
<td>0.21 ± 0.03</td>
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<tr>
<td>(av. length 5.70 mm)</td>
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<tr>
<td>&gt; 0.25 g &lt; 0.5 g</td>
<td>10</td>
<td>0.60 ± 0.02</td>
<td>9.50 ± 1.02</td>
<td>1.64 ± 0.12</td>
<td>0.17 ± 0.02</td>
<td>1.50 ± 0.09</td>
<td>0.25 ± 0.02</td>
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<tr>
<td>(av. length 6.05 mm)</td>
<td></td>
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<tr>
<td>Temperature</td>
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</tr>
<tr>
<td>29°C</td>
<td>9</td>
<td>0.58 ± 0.03</td>
<td>10.43 ± 1.54</td>
<td>1.26 ± 0.16</td>
<td>0.13 ± 0.01</td>
<td>1.77 ± 0.07</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>37°C</td>
<td>9</td>
<td>0.53 ± 0.02</td>
<td>9.00 ± 1.24</td>
<td>1.21 ± 0.11</td>
<td>0.15 ± 0.02</td>
<td>2.59 ± 0.20</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>12/min</td>
<td>9</td>
<td>0.56 ± 0.01</td>
<td>10.60 ± 0.89</td>
<td>1.26 ± 0.12</td>
<td>0.12 ± 0.01</td>
<td>1.65 ± 0.11</td>
<td>0.19 ± 0.02</td>
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<tr>
<td>24-30/min</td>
<td>9</td>
<td>0.58 ± 0.02</td>
<td>13.00 ± 1.09</td>
<td>1.33 ± 0.15</td>
<td>0.10 ± 0.01</td>
<td>2.25 ± 0.23</td>
<td>0.22 ± 0.02</td>
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<td>Stimulation</td>
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<tr>
<td>SS 12/min</td>
<td>10</td>
<td>0.58 ± 0.02</td>
<td>8.40 ± 1.01</td>
<td>1.29 ± 0.13</td>
<td>0.16 ± 0.01</td>
<td>1.46 ± 0.10</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>PS 12/min</td>
<td>10</td>
<td>0.56 ± 0.01</td>
<td>17.00 ± 1.74</td>
<td>1.16 ± 0.11</td>
<td>0.06 ± 0.01</td>
<td>3.05 ± 0.17</td>
<td>0.19 ± 0.02</td>
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<tr>
<td>7.5 mM</td>
<td>NS</td>
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<tr>
<td>Isoproterenol</td>
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<td></td>
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<tr>
<td>prior</td>
<td>8</td>
<td>0.53 ± 0.02</td>
<td>9.20 ± 0.81</td>
<td>1.23 ± 0.16</td>
<td>0.12 ± 0.01</td>
<td>1.50 ± 0.16</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>10-5M</td>
<td>8</td>
<td>0.53 ± 0.01</td>
<td>18.00 ± 1.29</td>
<td>1.35 ± 0.20</td>
<td>0.07 ± 0.01</td>
<td>3.50 ± 0.22</td>
<td>0.22 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. P ′ o = experimental force; P o = force obtained after correcting for the SE; tO = initial muscle length; SS = single stimuli; PS = paired stimuli; a and b are constants in the Hill equation. NS (nonsignificant) = P > 0.05. *0.025 < P < 0.05; †P < 0.005; §0.01 < P < 0.025.

proved to be most useful in studies on the mechanical characteristics of skeletal muscle, their direct application to the myocardium has been limited, since the true P o in heart muscle could not directly be determined (4), with the result that uncorrected force-velocity curves may not appear hyperbolic (5).

Accordingly, the present study was designed to examine the influence of various inotropic interventions on the instantaneous three-dimensional force-velocity-length relations of the CE relative to time after stimulation. Furthermore, using the true P o of the CE, further information was obtained concerning the dynamic constants in heart muscle as calculated from the force-velocity equation of Hill.

Methods

Twenty-nine papillary muscles obtained from the right ventricles of cats anesthetized with sodium pentobarbital (25 mg/kg) were used in this study. The experiments were performed at a bath temperature of 29°C, and a stimulation frequency of 12/min was employed throughout, except when the effects of changes in temperature (29°-37°C) or frequency (12, 24-30/min) were specifically studied.

Initial muscle length was always set by a small preload (<0.25 g/mm²), except in the experiments in which the effects of higher preloads (0.25-0.5 g/mm²) were examined. Three additional inotropic interventions were studied in these muscles: (a) paired stimulation, (b) isoproterenol (10-5M), and (c) an increase in calcium concentration from 2.5 to 7.5 mM.

In all instances, the influence of these interventions was examined on the instantaneous force-velocity-length relations of the CE relative to time after stimulation during isotonic contractions. The technique to obtain these force-velocity-length relations has been described previously (1) and is briefly summarized in Figure 1.

Utilizing the corrected P o derived from these three-dimensional graphs by extrapolating the linear CE load-shortening relation to the load axis, force-velocity curves were then constructed.
Force-velocity-length relations of the contractile elements. At top left tension-length and velocity-length phase-plane tracings of the whole muscle during isotonic contractions at increasing afterloads. A quick release (QR) to zero afterload from peak tension is superimposed on all tracings. From these phase-plane tracings a three-dimensional graph of the velocity-length (VL) relations of the contractile elements at increasing loads is constructed after correction for the extension of the series elastic component, as derived from the QR (1). Since the VL relations of the CE have been derived from isotonic contractions, during the isometric portion of the contraction they are not represented on this graph. Times from stimulation to peak velocity are indicated on the left, and times to peak shortening and time to peak tension on the right. The delay from stimulus to onset of tension development averaged 25 msec. The extrapolation of the linear load-shortening relation of the CE permits an estimation of the maximum isometric force (P_max) which the CE could develop at that particular length if there were no extension of the SE. As shown in the panel at bottom right, the force-velocity relation of the CE becomes hyperbolic when corrected to this maximum isometric force. The Hill constants a and b can then be determined equally well either graphically (right) (7) or by plotting \( \frac{P}{V} \) against P (left). Preload 0.3 g; muscle length (L0) 4.82 mm; temperature 29°C; stimulation frequency 12/min.

and the Hill equation constants calculated (Fig. 1). Since peak velocities of CE shortening after correction for the SE extension at increasing afterloads occur at almost the same CE length independent of a wide range of times after stimulation (1), only peak velocities were used for the construction of the curves.

To eliminate the effects of altered loading on CE performance at each load (6), only the first isotonic contraction immediately following a series of isometric beats was recorded. For the same reason, only stable QR beats were used for correction of the SE at each load.

Results

(1) Influence of Initial Length.—Table 1 summarizes the mean values of P_o corrected
FIGURE 2

Force-velocity-length relations of the contractile elements at 29°C and at 37°C and the derived dynamic constants (insert). In A (29°C) and B (37°C) velocity-length phase-plane tracings (lower) of the whole muscle were recorded at increasing afterloads, with a superimposed length-tension (upper) tracing of an isometric contraction which was quick-released at peak tension. In C, the phase-plane tracings from A and B have been replotted in a three-dimensional representation after correction for the internal shortening due to stretching of the SE, as determined for each afterload from the quick release contraction. The times from stimulus to peak velocity are indicated on the left, while the times to peak shortening and times to peak tension (TTP) are indicated on the right of the three-dimensional plot; the values found at 29°C are underlined to distinguish from those found at 37°C. The delay from stimulus to the onset of tension development averaged 20 msec at 37°C and 25 msec at 29°C. Preload, 0.2 g; muscle length, 6.3 mm; cross-sectional area, 0.7 mm²; stimulation frequency, 12/min.

<table>
<thead>
<tr>
<th></th>
<th>29°C 12/min</th>
<th>37°C 12/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₀/P₀</td>
<td>0.63</td>
<td>0.47</td>
</tr>
<tr>
<td>a/P₀</td>
<td>0.11</td>
<td>0.13</td>
</tr>
<tr>
<td>b/L₀</td>
<td>0.20</td>
<td>0.28</td>
</tr>
<tr>
<td>Vmax/L₀</td>
<td>1.82</td>
<td>2.20</td>
</tr>
</tbody>
</table>

for SE extension and of P₀/P₀ obtained in 10 muscles at two different ranges of preloads. Applying the Hill equation to the hyperbolic force-peak velocity relation, utilizing the corrected P₀, Vmax and the Hill constants have also been calculated. With increasing preload which increased initial muscle length (L₀) from 5.70 ± 0.08 mm to 6.05 ± 0.10 mm, the ratio of the experimentally obtained isometric force (P₀') to the force corrected for the series elastic component (P₀) was significantly augmented from 0.55 ± 0.02 to 0.60 ± 0.02. Vmax/L₀ was not significantly different, whereas P₀/area was increased as preload and muscle length was increased. The constant a of the Hill equation was altered to the same degree as the corrected P₀, so that no significant differences were noted in the values of a/P₀. Similarly, the constant b normalized for muscle length was unaltered by changes in preload.

(3) Influence of Temperature.—In Figure 2 are shown the effects of temperature (29° and 37°C) on the force-velocity-length relations of the CE.

As shown in C, the stress-strain relations of the SE were slightly more compliant at the higher temperature. This finding explains the
Influence of various inotropic interventions on the velocity-length phase-plane tracings of non-afterloaded isotonic contractions. Muscle 1 (A, B, C): preload, 0.35 g; initial length 9.35 mm; cross-sectional area, 1.38 mm². In A, the velocity-length relations are compared at 29° and 37°C. In B and C, the influence of different stimulation frequencies (12 and 30/min) is examined at 29° (B) and at 37°C (C). Muscle 2 (D, E, F): preload, 0.2 g; initial length, 6.3 mm; cross-sectional area, 0.71 mm²; stimulation frequency 12/min; temperature 29°C. The effects of paired stimulation (PS) (D), an augmented calcium concentration (7.5 mM) (E) and isoproterenol (10⁻⁵M) (F) are examined on the velocity-length relations and compared with control conditions. EB = the extra beat during paired stimulation; SS = single stimulation. The times from stimulus to peak velocity and to peak shortening are indicated on each tracing. The delay from stimulus to the onset of tension development was about identical in both muscles and averaged 20 msec at 37° and 25 msec at 29°C.

significant reduction of the ratio of the measured isometric force to the isometric force corrected for the shortening of the series elastic component (Pc/Po) from 0.58 to 0.53 (Table 1) and the almost identical corrected Po at both temperatures.

At the higher temperature, both Vmax/Io and b/Io (Table 1) were substantially augmented, whereas the constant a and the ratio a/Po underwent no significant changes.

In Figure 3 (A, B, C) are shown the velocity-length relations of non-afterloaded isotonic contractions at 29° and at 37°C. Although the times at which peak velocity was reached were markedly different, the CE lengths at which this occurred were quite
similar. Thus the factor of time was not a dominant consideration during this portion of the shortening trace and does not vitiate the uniqueness of the velocity-length relation over its largest portion when the effect of various temperatures is considered.

(3) Influence of Changing Frequency of Stimulation.—Table 1 also summarizes the mean values obtained in nine muscles at 29°C when the frequency of stimulation was augmented from 12/min to either 24 or 30/min. No significant changes were seen in $P_o/P_e$, $a/P_e$, and $b/l_0$.

$V_{max}/l_0$ almost doubled at the higher frequency, while $P_o/area$ was also higher, but to a lesser degree. Also, $b/l_0$ was significantly higher when frequency was augmented.

Figure 3 shows the phase-plane velocity-length relations of non-afterloaded isotonic contractions at two stimulation frequencies (12 and 30/min) at 29°C (B) and 37°C (C). Again it is clearly demonstrated that peak velocity at different stimulation frequencies occurs at nearly identical CE lengths independent of the absolute value of this velocity and despite some differences in time at which these velocities are attained.

(4) Influence of Calcium, Paired Stimulation, and Isoproterenol.—A representative example of the effects of these inotropic interventions on the force-velocity-length relations of the CE is illustrated in Figure 4 (paired stimulation), Figure 5 (increased calcium concentration), and Figure 6 (isoproterenol). In addition, in Figure 3 the effects of paired stimulation (D), increased calcium (E), and isoproterenol (F) on the phase-plane velocity-length relations of the non-
afterloaded isotonic contractions are compared in the same muscle. In all three, the rise in velocity was initially the same as in control conditions, but it subsequently separated so that velocity of shortening was then augmented at any CE length and load. As they did with changes in temperature or in stimulation frequency, peak velocities occurred at almost identical CE lengths independent of the absolute values of these velocities and despite the differences in time at which they were attained. Moreover, this higher trajectory of velocity relative to length yielded a larger extent of shortening. Nevertheless, in the augmented inotropic state, the relation between extent of CE shortening and load was still linear. Due to the increased velocity of shortening, these events occurred in a shorter time interval.

As shown in Table 1, most changes in these three different potentiated states were quite similar. With all three inotropic interventions, no change was found in the relation of the experimental \( P_0 \) to the corrected \( P_0 \). There was equally no change in the compliance of the SE as derived from the quick release data. However, \( P_0/area \) and \( V_{max}/l_0 \) were doubled, and \( P_0/area \) reached the maximal values obtained in this study (17.6–17.9–18.0 g/mm²).

Further, due to a practically unchanged \( a \) constant, the \( a/P_0 \) was strikingly reduced to almost half its initial value.

When interpreted in terms of the Hill equation, a dissociation between these three types of potentiation was noted. Thus, the \( b/l_0 \), which was unchanged with an increase in calcium concentration or with paired...

**FIGURE 5**

Influence of two different calcium concentrations (2.5 and 7.5 mM) on the three-dimensional force-velocity-length relations of the contractile elements, and the dynamic constants as derived from the Hill equation (insert). Times from stimulation to peak velocities are indicated on the left, and times to peak shortening and time to peak isometric tension (TTP) are indicated on the right of the graph; the values found at the higher calcium concentration are underlined. Preload, 0.3 g; muscle length, 6.0 mm; cross-sectional area, 0.67 mm²; temperature, 29°C; stimulation frequency, 12/min.
stimulation, was significantly augmented after addition of isoproterenol.

Discussion
In the present study, the effects of various inotropic interventions on the relations between velocity of shortening, load, and instantaneous length of the CE of heart muscle were examined. It has been demonstrated that the surface created by the three-dimensional plot of instantaneous force, velocity, and length of the CE during isotonic contractions is unique for a given state of contractility, and that in all instances, the course of velocity vs. length is largely independent of the time after stimulation. Following inotropic interventions, such as addition of isoproterenol, increased calcium, or paired electrical stimulation, this surface is shifted upward so that the velocity of shortening of the CE is greater at any length and load.
The force-velocity-length relations of the CE thus provide a most valuable dynamic approach to the study of the effects of various inotropic interventions. Changes in the initial time course of active state do not alter these conclusions.

Since the load-shortening relation of the CE is linear, the intersection of this line on the load axis provides a measure of the maximum tension of the CE, if shortening against the SE did not occur (1). Thus, for a given contractile state of the myocardium, this tension (P₀) constitutes the theoretical maximum intensity of the active state, i.e., the maximum force development at a constant CE length as initially proposed by Gasser and Hill (8). The ratio of the experimentally determined P'₀ to this derived P₀ was quite constant on transition to a higher stimulation frequency or when the influence of calcium, paired stimulation, or isoproterenol was examined. This finding would hold if an important limitation to maximal force development is the compliance of the SE and the SE is unchanged by these interventions. Indeed, the latter has been shown to be the case in the present study. The increased P'₀/P₀, when initial muscle length is augmented, is consistent with the known increased stiffness of the SE at the higher preloads (9); on the other hand, the reduction of this value at a higher temperature can be explained by the increased compliance of the SE (10).

The highest values of the corrected isometric tension (P₀/area) in the present study were found following augmentation of the contractile state of the muscle by increased calcium, paired stimulation, or isoproterenol. These values of about 1.8 kg/cm² are within the lower range of P₀ values generally obtained in mammalian skeletal muscle (11-15). Using the highest mean value for the P'₀ (1.08 kg/cm²) as experimentally obtained in the heart muscle at the optimal length of the length-tension relation (15) and correcting this value for the SE extension by the factor 0.6 derived from the present study, a maximal isometric tension of 1.80 kg/cm² would be obtained in heart muscle. When an additional augmentation of 14% is taken into account for the correction of an altered loading factor as described by Parmley et al. (6), a value of 2.05 kg/cm² can be obtained. The presence of a more compliant SE in heart muscle and the fact that the experimental P₀ in skeletal muscle would not markedly be augmented by correcting for the SE, the extension of the SE can largely explain the lower experimental P₀ in the myocardium. Hence the force-generating capacity of these two forms of striated muscle is quite similar.

Over the range of muscle lengths studied, Vmax was independent of initial muscle length, as shown previously (4, 16). These results are consonant with those obtained recently with single skeletal muscle fibers (17). Experiments using quick release methods which show changes in Vmax associated with changing initial length (18) may be reinterpreted in a different way. Thus measurements of velocity obtained after large quick releases do not represent the contractile element velocity of the isometric muscle at the time of the release and may be reduced by the release itself (1, 5). Moreover, velocity measured after releases is not in steady state and is thus subject to error. Also, the course of the active state may be altered by the quick release itself. Furthermore, time and length of the contractile element are not kept constant simultaneously, and this in itself may alter interpretation. In addition, at very short CE lengths, as must be the case following a quick release from peak tension, restoring forces may be brought into play and reduce velocity of shortening, but these are not observed at physiologic initial lengths.

All other changes of P₀/area after correction for the SE and of the calculated Vmax as derived from the Hill equation were also in agreement with previous findings of force-velocity relations of heart muscle (4, 16). Thus, on increasing initial muscle length, P₀ was markedly augmented, while Vmax was unchanged. The values of Vmax were enhanced when either temperature or fre-
frequency was augmented, whereas the corrected $P_o$ was only slightly increased with stimulation frequency and little altered by temperature changes. Furthermore, both $V_{\text{max}}$ and $P_o$ were about equally augmented when the effects of paired stimulation, calcium, and isoproterenol were examined.

Utilizing the corrected $P_o$ of the CE and the peak velocities at the smallest afterloads, reasonably hyperbolic force-velocity curves could be constructed in all cases. As clearly shown in this and a previous study (1), the deviation of the force-velocity curve from a hyperbola can be attributed largely to the series elastic component and not to a basic difference in the contractile mechanisms, as others have suggested (18).

References
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