Mechanical restitution of isolated perfused canine left ventricles

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BURKHOFF, DANIEL, DAVID T. YUE, MICHAEL R. FRANZ, WILLIAM C. HUNTER, AND KIICHI SAGAWA. *Mechanical restitution of isolated perfused canine left ventricles.* Am. J. Physiol. 246 (Heart Circ. Physiol. 15): H8–H16, 1984.—We measured cardiac mechanical restitution curves, which describe the time course of recovery of ventricular contractile strength following a steady-state beat. In the first series of experiments, we studied left ventricles that beat isovolumically throughout the experiment, allowing use of dP/dt_{max} as a reliable index of contractile strength independent of the influence of changing ventricular pre- and afterload. The commencement of mechanical restitution was found to be associated with the onset of electrical diastole; thereafter, contractile strength rose monoexponentially to a plateau that was maintained for test pulse intervals as long as 15 s. The time constant of restitution (typically 245 ms) was independent of priming frequency and ventricular volume. These findings were interpreted in terms of a model of intracellular calcium fluxes within the myocardial cells. In a second series of experiments, we measured mechanical restitution curves from isolated ventricles that ejected against a simulated arterial impedance system. Under this condition, we did not observe the monoexponential time course of mechanical restitution as was measured under isovolumic conditions. The differences between the mechanical restitution curves measured under isovolumic and ejecting conditions were attributed to the influences of changing hemodynamic conditions on dP/dt_{max} that caused it to be an unreliable index of contractile strength.

cardiac force-interval relationship; excitation-contraction coupling; myocardial contractility

THE FORCE-INTERVAL RELATIONSHIP of cardiac muscle has been interpreted in terms of variable amounts of activator calcium made available to the myofilaments as a function of the interval between beats (9, 18, 25, 36). Following the advancement of these hypotheses, there has been increased interest in the potential use of the force-interval relationship in human hearts as a clinical tool for evaluating cardiac contractile disorders (1, 2, 8, 15, 19, 27). One fundamental characteristic of the myocardial force-interval relationship is described by the mechanical restitution curve (MRC) (7). The MRC is a plot of the contractile strength of a test beat as a function of the time interval between a steady-state stimulus and a subsequent test stimulus. When the test interval is short, the test beat is of low contractile strength; as the interval is lengthened, the contractile strength rises in a monotonic manner (3, 36). Because contractile strength reflects the amount of calcium supplied to the myofilaments in a beat, the MRC is believed to parallel the time course by which intracellular calcium is made available for release up to the time of excitation of the cell (9, 35). If this is indeed the case, the characteristics of this curve may differ between normal and diseased ventricles.

The interpretations of the MRC in terms of intracellular calcium dynamics, however, are based primarily on investigations of isolated cardiac muscle, in which contractile strength was quantified using either peak isometric force or the maximal rate of rise of isometric force. To apply the concepts advanced through such research in the clinical setting, it is necessary that it be understood how the global ventricular MRC relates to the MRCs of myocardial cells. Recently, several investigators have examined MRCs of in situ ejecting left ventricles using dP/dt_{max} as the index of contractile strength (2, 27). However, dP/dt_{max} under ejecting conditions is potentially unreliable as an index of myocardial performance due to its variation with changing ventricular pre- and afterload. Because changes in the interval between beats influence ventricular pre- and afterload, an MRC measured using dP/dt_{max} under ejecting conditions may not represent the intrinsic restitution process of the ventricle.

The purpose of this investigation was twofold. First, it was to characterize the MRC of the ventricle so that a comparison could be made with those reported in the literature for isolated myocardial muscle. In contrast to earlier studies on in situ ventricles, we studied isolated canine ventricles that were beating isovolumically, a condition under which dP/dt_{max} is considered to be a reliable index of ventricular contractile strength. Second, in light of the possible influences of ventricular pre- and afterload on dP/dt_{max} measured in the ventricle in vivo, we investigated the limitations of the use of this index for the measurement of MRCs when the isolated ventricles were ejecting against a simulated arterial impedance load.

METHODS

Surgical preparation. The procedures used to isolate and support a canine heart were similar to those described by Suga and Sagawa (31). A pair of mongrel dogs was anesthetized with pentobarbital sodium (30 mg/kg

iv). The femoral arteries and veins of one dog (support dog) were cannulated and connected to a perfusion system used to supply oxygenated blood to the isolated heart. The chest of the second dog (donor dog) was opened under artificial respiration. The left subclavian artery was cannulated with the arterial line of the perfusion system. The brachiocephalic artery was cannulated to monitor the coronary perfusion pressure. The azygous vein, superior and inferior vena cavae, descending aorta, and lung hili were ligated. The heart was then removed from the donor dog. The left atrium was opened and all the chordae tendineae were freed from the mitral valve leaflets. A metal adapter that holds the isolated heart to the ventricular volume servo pump system (see below) was sutured to the mitral ring. When the surgical preparation was complete, the isolated heart was positioned so that a water-filled balloon (see below) was inside the left ventricular cavity.

In all experiments the coronary perfusion pressure was maintained between 80 and 100 mmHg by a pressure servo system. The temperature of the perfusate was maintained between 35 and 37°C.

Ventricular volume servo system. A servo system was used to control ventricular volume. Details of its design and performance have been reported by Suga and Sagawa (32) and Sunagawa et al. (34). A linear motor (Ling Electronics model 411) controls the position of the piston within a rolling-diaphragm cylinder (Bellofram SS-4-F-SM). A latex balloon is secured to a tube connected to the outflow tract of the Bellofram cylinder. The cylinder, connecting tube, and the balloon are all filled with water. A linear displacement transducer (Trans-Tek series 240) senses the position of the piston, thus producing a signal proportional to the balloon (and therefore ventricular) volume. This signal is used in a negative feedback loop for comparison with a volume command signal (see below) that represents the desired instantaneous volume. The error signal resulting from this comparison is supplied to a power amplifier (Crown DC-300), which in turn drives the linear motor.

Impedance loading system. The volume command signal for the volume servo system is generated by the interaction between the measured left ventricular pressure and a specially designed loading system. The details of this system were reported by Sunagawa et al. (34). Briefly, the left ventricular pressure, which is measured by a high-fidelity semiconductor pressure transducer (Millar 380) placed inside the balloon, serves as the input to a hybrid computer programmed to solve the differential equations describing ventricular preloading and afterloading circuits. The preloading circuit consists of a simulated constant-pressure source in series with a filling resistance. The afterloading circuit is the three-element windkessel model, which has been shown to be a reasonable representation of the aortic hydraulic input impedance (24). This system also calculated the simulated aortic pressure resulting from the interaction between the ventricle and the afterloading model. The values of the circuit's parameters are controlled from the computer keyboard (Radio Shack TRS-80).

Isovolumic contractions can be produced by placing the analog integrator of the impedance loading system on "hold" so that the volume command output is constant. The computer can then be used to directly control the volume command signal through a digital-to-analog converter.

Electrical stimulation. The hearts were paced with bipolar electrodes from the region of the bundle of His. The digital computer used to control the impedance loading system also controlled a pacer that was used to stimulate the ventricles. The computer was programmed to produce the pattern of Fig. 1. The ventricles were paced during the "priming period" with a series of regularly timed stimuli at the "priming frequency." The priming period was long enough so that the response of the ventricle to stimulation reached a steady state. After the last steady-state beat in the series, a test stimulus was introduced at a time called the "test pulse interval" (TPI). This sequence was repeated for a wide range of TPIs (2 examples are shown in Fig. 1): the shortest TPI was taken as the smallest value at which the test beat had a positive dP/dt_{max} (i.e., the test beat and the last steady-state beat became "unfused"); the longest TPI was determined by the timing of ventricular escape beats.

Measurements. The measurements of ventricular pressure and volume and simulated aortic pressure have already been described. In addition, the left ventricular pressure signal was electronically differentiated (Gould model 13-4615-71, corner frequency 100 Hz) and recorded.

A cardiac surface electrogram was recorded by placing one electrode at the base of the right ventricle and another at the base of the left ventricle (Fig. 2). By examining the shape of this signal, we could identify ventricular escape and aberrantly conducted beats and exclude them from analysis.

To measure the durations of local myocardial depolarization (electrical systole) and repolarization (electrical diastole), we recorded monophasic action potentials (MAP) from the epicardial surface of each ventricle (16). MAPs were recorded using a modification of a newly designed contact-electrode recording technique (13). The electrophysiological principle underlying this technique is described elsewhere (16). The recording device consists of an L-shaped cantilever with two electrodes (sintered Ag-AgCl) mounted on its distal end. One electrode formed the tip of the cantilever, which was pressed against the epicardium by a springloading mechanism. The other electrode was positioned 5 mm away from the tip. Electrical contact between the proximal electrode and the heart was made through a small saline-soaked

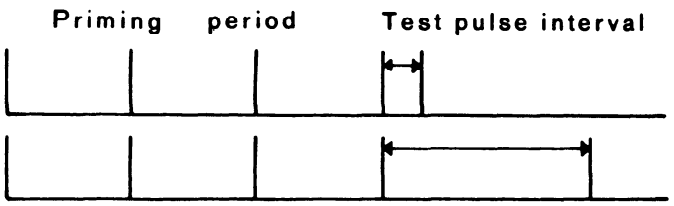

FIG. 1. Pacing protocol: priming period consists of approx 20 beats at priming frequency after which a test stimulus is delivered at test pulse interval (TPI). To measure mechanical restitution curve, this sequence is repeated for a wide range of TPIs. Two examples are shown; at *top*, test stimulus is introduced at a relatively short interval; at *bottom*, test stimulus is introduced at a relatively long interval.

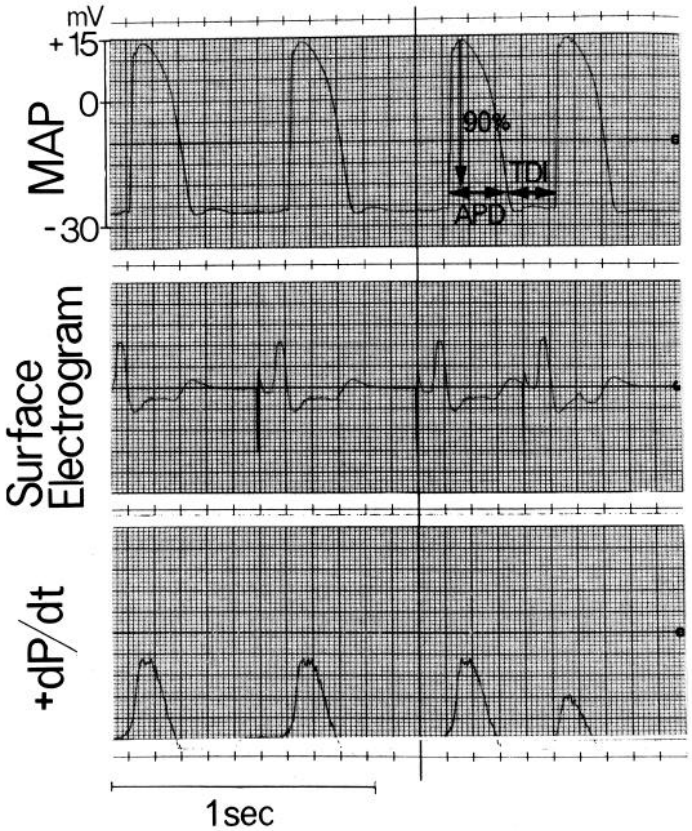

FIG. 2. Original recordings from isolated canine ventricle of monophasic action potential (MAP), surface electrogram, and positive dP/dt for a test pulse interval (TPI) of 400 ms. *Arrows*, durations of steady-state monophasic action potential at 90% repolarization (APD) and test diastolic interval (TDI). Note that APD + TDI = TPI.

piece of foam rubber. This bipolar electrode arrangement in conjunction with differential amplification minimized the effect of remote electrical activity on the MAP.

The time from the onset of the MAP to 90% repolarization and the time from 90% repolarization to the onset of the subsequent MAP were taken as the duration of local electrical systole and diastole, respectively. The electrical diastolic interval preceding a test beat was defined as the test diastolic interval (TDI) (Fig. 2). MAPs were recorded continuously and from a single epicardial site from the base of the left ventricle throughout the entire experiment. Since action potential durations vary from site to site in the heart, we only considered changes in systolic and diastolic durations produced on the test beats and assumed that these changes were approximately uniform over the heart.

The signals were recorded on an eight-channel pen recorder (Gould-Brush model 2800). Figure 2 shows a recording of a MAP, the corresponding surface electrogram, and mechanical response for a TPI of 400 ms. Figure 3 shows typical original mechanical recordings (at slower speed) obtained with the pacing pattern of Fig. 1 and several test pulse intervals. The recordings in Fig. 3*A* were obtained while the ventricle was beating isovolumically at a volume of 35 ml, and those in Fig. 3*B* while the heart ejected into the simulated physiological loading system from a preload of 35 ml. Mechanical restitution curves (MRC) were produced by plotting the dP/dt_{max} response of the ventricle, measured from the chart recordings, as a function of either the TPI or the duration of electrical diastole preceding the test beat (TDI).

PROTOCOLS

Effect of priming frequency. In a series of experiments, isolated ventricles were caused to beat isovolumically at a volume of approximately 25 ml. At this volume the preload pressure was relatively low (approx 0 mmHg) and therefore the dP/dt_{max} values of the steady-state priming beats were low (<1,000 mmHg/s); the low volume was used because fewer arrhythmias occurred than at higher volumes, thus providing more stable experimental conditions. In each heart we measured MRCs with four different priming frequencies ranging from 60 to 180 beats/min.

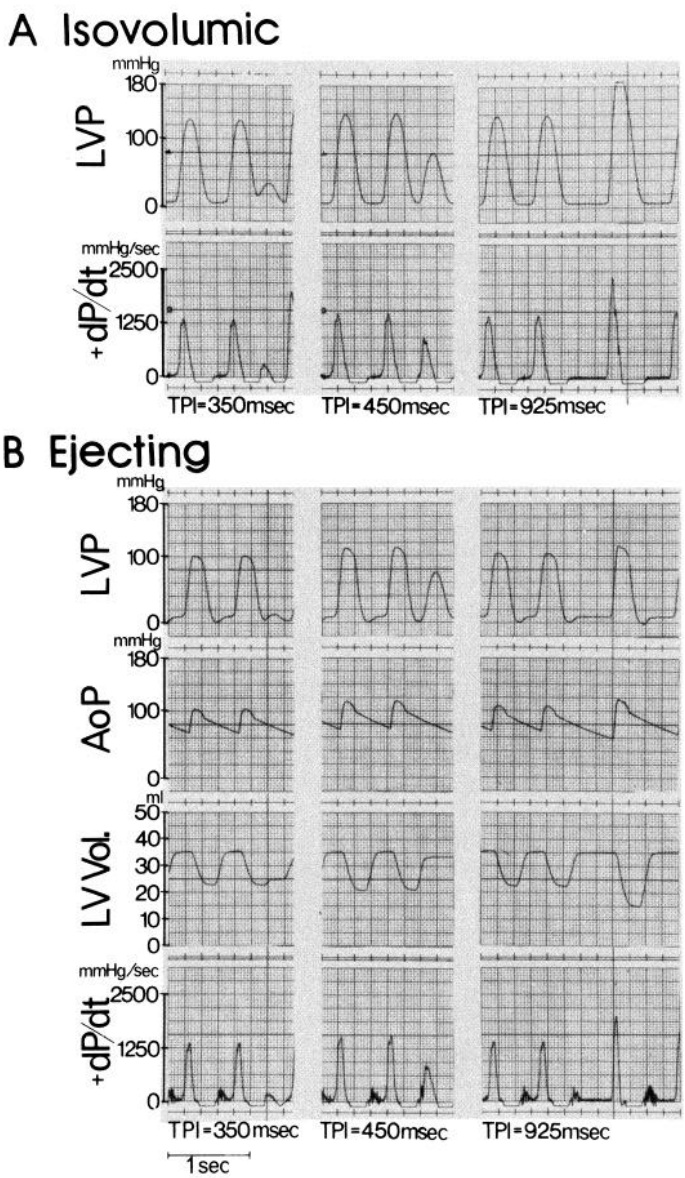

FIG. 3. Original recordings from isolated heart; each frame shows 2 steady-state responses and 1 test response at various test pulse intervals (TPIs). *A*: ventricle was beating isovolumically at 35 ml; *B*: ventricle ejected into simulated arterial system from a preload of 35 ml; note in *B* that ventricular preload volume and simulated aortic (i.e., afterload) pressure on test beats varied with TPI (see text).

Effect of ventricular volume. In another series of experiments, we measured MRCs in isovolumically beating isolated hearts with a single priming frequency of 100 beats/min. For each TPI the ventricular response was measured at volumes of 15, 25, and 35 ml. A wide range of TPIs was investigated.

Effect of hemodynamics on mechanical restitution curves. In a third series, we allowed the ventricles to eject against the simulated arterial impedance (see above) and obtained MRCs as follows. The parameters of the loading system were set at what are considered normal values for the canine arterial system (peripheral resistance of 3 $mmHg \cdot s \cdot ml^{-1}$, total arterial capacitance of 0.4 ml/mmHg, and characteristic impedance of 0.2 $mmHg \cdot s \cdot ml^{-1}$), and the ventricular preload pressure was adjusted so that the steady-state mean simulated aortic pressure was between 85 and 100 mmHg. The priming frequency was chosen so as to be slightly higher than the rate of spontaneous ventricular escape beats; it was always between 80 and 100 beats/min. Each MRC thus obtained in ejecting conditions was compared with one measured in the same ventricle under isovolumic conditions at the same priming frequency and at a volume equal to the steady-state end-diastolic volume from which the ventricle originally ejected.

RESULTS

Effect of priming frequency on mechanical restitution curves. Six hearts were studied in this series. A typical result is shown in Fig. 4; all dP/dt_{max} values were normalized to the steady-state dP/dt_{max} of the priming frequency being investigated. MRCs were obtained with four priming frequencies in the same ventricle. All curves increased monotonically to a plateau level, the maximum contractile response (CR_{max}). Once the plateau was reached, this CR_{max} was maintained up to the longest test pulse interval (TPI) attainable. The longest TPI in the examples of Fig. 4 was 2 s; in two other ventricles we were able to attain TPIs up to 10 s, and in one ventricle a TPI of 15 s was attained. Even after these long pauses, the ventricular CR_{max} was maintained at the plateau level.

Two trends were observed in the MRCs as the priming frequency was increased. First, there was a steady increase in the amplitude of the plateau (i.e., CR_{max}), as exemplified in the plot in Fig. 5 in which selected points from Fig. 4 are replotted without normalization. Figure 5 also shows that, for the same ventricle, the steady-state dP/dt_{max} varied only slightly with priming frequency. Table 1 summarizes that data on how priming frequency influenced the normalized plateau level (CR_{max}) in all hearts investigated. Because priming frequencies were not identical in all hearts, only frequencies tested in more than one heart were included.

Second, in addition to the increase in the plateau level, there was always a leftward shift in the MRCs as the priming frequency was increased (i.e., test beats could be elicited at shorter TPIs). Table 1 summarizes how the TPI-axis intercept of the MRC (t_0) varied with priming frequency. The leftward shift was quantified by the shift of the origin of the MRCs on the TPI axis, as denoted by the arrow in Fig. 4. To determine to what extent this leftward shift was associated with the shortening of the

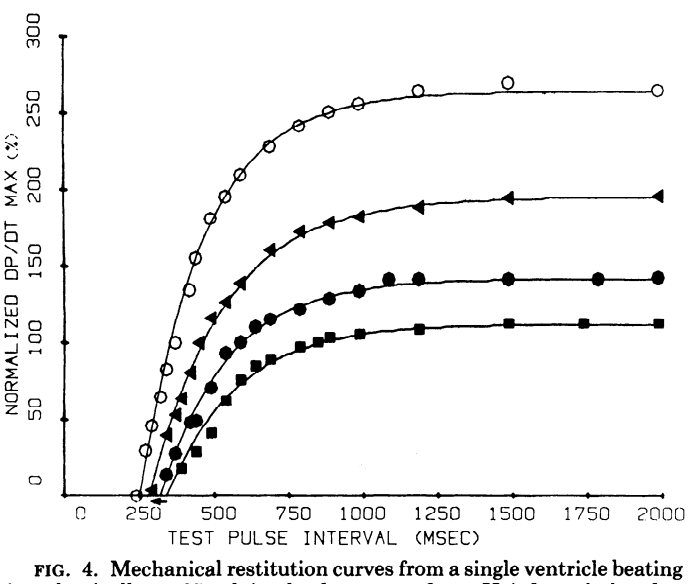

FIG. 4. Mechanical restitution curves from a single ventricle beating isovolumically at 25 ml (preload pressure 0 mmHg) for priming frequencies of 70 (■), 100 (●), 130 (▲), and 160 (○) beats/min. dP/dt_{max} values were normalized to steady-state dP/dt_{max} at frequency being investigated. *Curves* were fit to data points by linear regression as described in text. *Arrow*, example of how leftward shift was quantified for a priming frequency of 130 beats/min.

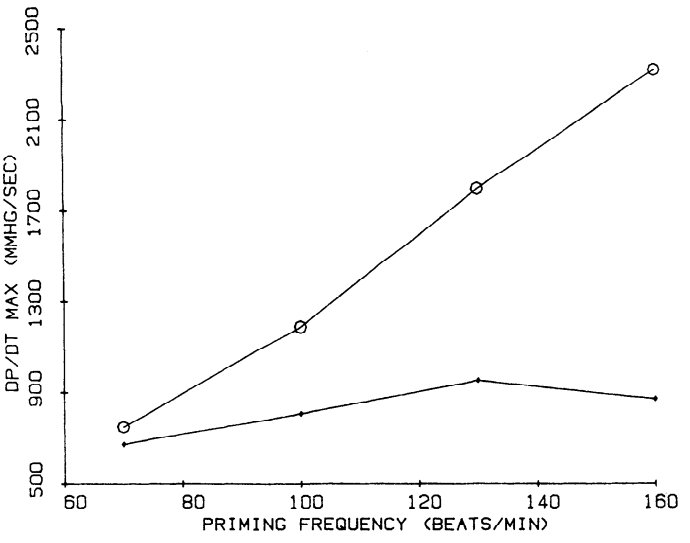

FIG. 5. Dependence of steady state (♦) and plateau (○) dP/dt_{max} values on priming frequency from a single ventricle beating isovolumically at 25 ml (preload pressure 0 mmHg). These are selected data points from Fig. 4, replotted without normalization.

TABLE 1. *Effect of priming frequency on CR_{max} and t_0*

Priming Frequency	n	CR_{max}, %	t_0, ms
60	4	107.0 ± 5.4	384 ± 58
80	2	118.3 ± 2.4	366 ± 35
100	6	141.5 ± 8.0	345 ± 22
130	4	177.0 ± 13.0	296 ± 3
160	5	233.0 ± 25.0	260 ± 9

Values are means ± SD. n, No. of ventricles in which a particular priming frequency (beats/min) was tested; maximum contractile response (CR_{max}), normalized plateau level of the mechanical restitution curve (MRC); t_0, test pulse interval-axis intercept of the MRC.

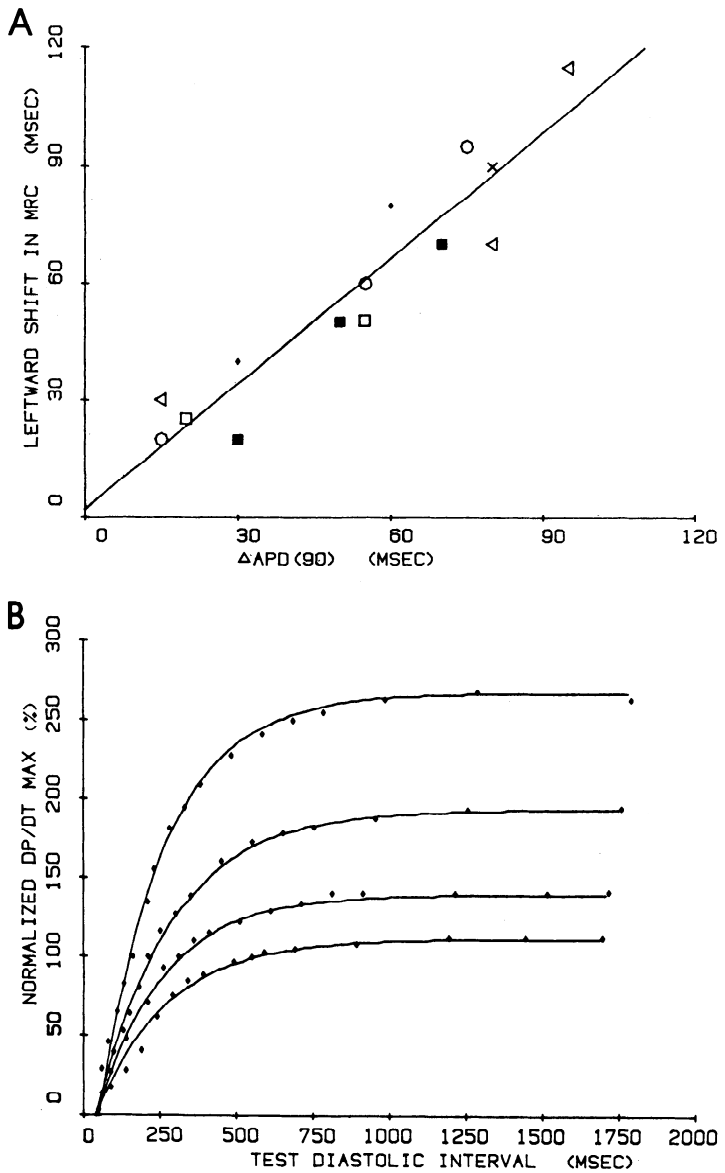

FIG. 6. *A*: summary of results from 6 ventricles of correlation between leftward shift in mechanical restitution curve (MRC) with increased priming frequency and change in steady-state action potential duration at 90% repolarization (APD_{90}); different symbols represent data from different hearts ($r = 0.88$). *B*: data from Fig. 4 were replotted as a function of test diastolic interval (TDI) defined as TPI minus measured steady-state action potential duration at 90% repolarization. When plotted this way leftward shift was abolished.

steady-state action potential at increased priming frequency, we plotted the change in steady-state action potential durations versus the amount of leftward shift in the MRC (i.e., the changes from their values at the lowest priming frequency investigated). Figure 6*A* summarizes the results from the six hearts studied. We found a high correlation between these quantities (regression slope, 1.06; intercept, 2.81 ms; and correlation coefficient, 0.88), indicating that the onset of mechanical restitution is associated with and may be keyed to the commencement of electrical diastole. To test this point further, we replotted in Fig. 6*B* the normalized MRCs of Fig. 4 as a function of the electrical diastolic interval (TDI defined in METHODS); plotting the data this way abolished the leftward shift shown in Fig. 4. However, these curves did not intersect the TDI-axis at 0 ms, but rather at a slightly positive value. This observation most likely derives from the differences in steady-state action potential duration between various sites within the myocardium.

The MRCs appeared to follow a monoexponential time course. Hence we plotted $\log_e[CR_{max} - dP/dt_{max}(TDI)]$ versus TDI for each MRC. A monoexponential time course was confirmed by the linearity of these plots; the average linear regression correlation coefficient, r, was 0.992 (SD = 0.007) for all 32 curves measured from 6 ventricles. The time constant of restitution is equal to the reciprocal of the slope of the regression line with its sign inverted. For each ventricle we determined the line of regression between the time constant of mechanical restitution and priming frequency. The average slope of this regression from all six ventricles was 0.008 ms/frequency (SD = 0.280); there was no significance to the difference of this regression coefficient value from zero, indicating that the time constant of mechanical restitution was independent of priming frequency. Therefore, the MRC can be described by the equation

$$DP(TDI) = CR_{max} \times [1 - e^{-(TDI/T)}] \qquad (1)$$

where DP(TDI) is dP/dt_{max} of a test beat introduced at a TDI, T the time constant of mechanical restitution, and CR_{max} an increasing function of the priming frequency (Fig. 5). The average T from all hearts studied

was 245 ms (SD = 21 ms).

Effect of volume on mechanical restitution curves. In each of six isolated hearts beating isovolumically, we measured MRCs at three different volumes. Figure 7*A* shows the increase in the magnitude of the curves with increasing volume, demonstrating the preload dependence of dP/dt_{max}. We canceled this preload dependence by normalizing the test beat dP/dt_{max} values with respect to the dP/dt_{max} in the steady-state beats at the same volume; Fig. 7*B* shows that these normalized points of the mechanical restitution curves closely superimpose on each other.

We determined the lines of regression both between volume and time constant of mechanical restitution and between volume and normalized plateau level (i.e., CR_{max}) for each ventricle. The average slopes of these lines from the six ventricles studied were 0.099 ms/ml (SD = 1.390) and −0.005 normalized dP/dt_{max} units/ml (SD = 0.006), respectively. The differences in value of these slopes from zero were insignificant, indicating that ventricular volume did not influence either the time constant of mechanical restitution or the normalized plateau level.

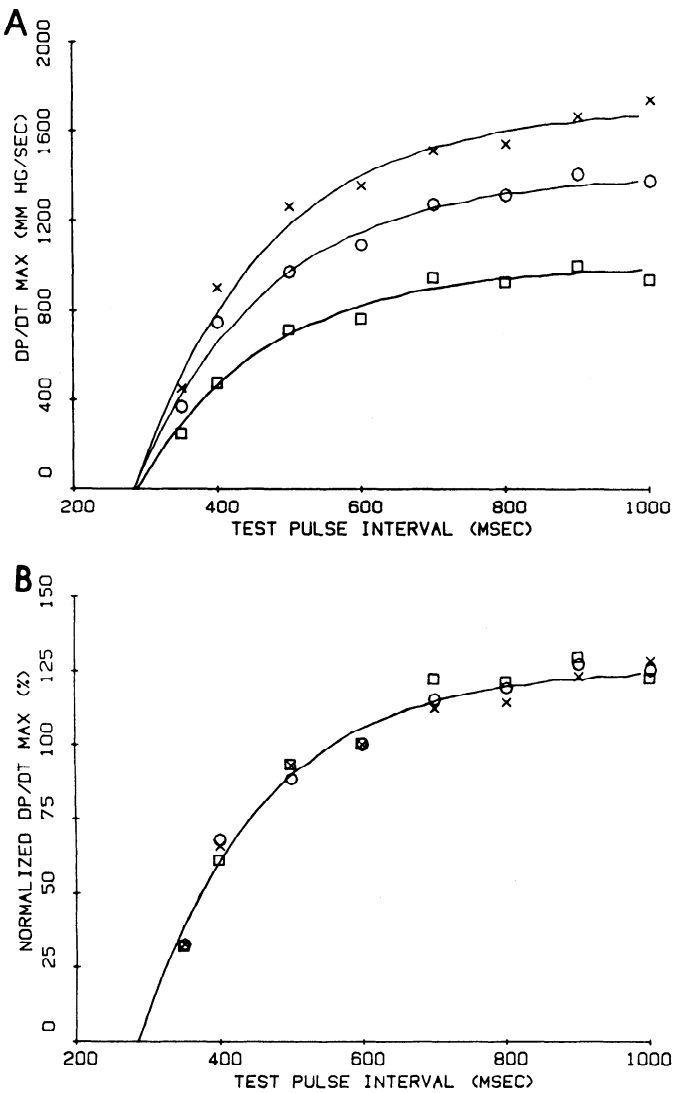

FIG. 7. Mechanical restitution curves from a single ventricle beating isovolumically at 15 (□), 25 (○), and 35 (×) ml. *A*: nonnormalized test beat dP/dt_{max} values are given. *B*: same responses were normalized to steady-state dP/dt_{max} at volume being investigated. Normalized points were nearly superimposable indicating that volume did not affect either normalized plateau (i.e., CR_{max}) or time constant of mechanical restitution.

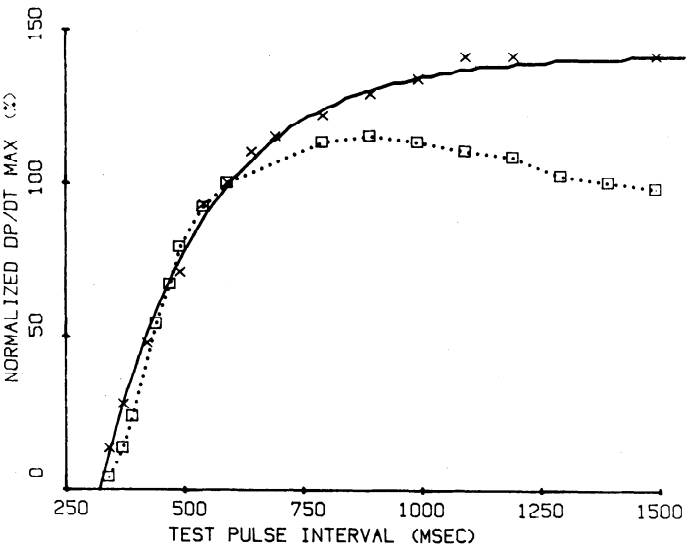

FIG. 8. Mechanical restitution curves from a single ventricle that first beat isovolumically (×, *solid line*) and was then allowed to eject (□, *dotted line*) into simulated arterial system; differences between these curves were attributable to effects of changing hemodynamic factors on dP/dt_{max} (see text).

Effect of hemodynamics on the measurement of mechanical restitution curves. In each of six isolated ventricles, we measured MRCs under two conditions: first, while the ventricle beat isovolumically ("isovolumic" MRC), and second while it ejected against the simulated impedance loading system ("ejecting" MRC). For each pair of MRCs the same priming frequency (typically between 80 and 100 beats/min) was used. Figure 8 shows a typical result. The characteristics of the isovolumic MRC have already been discussed. In comparison, the ejecting MRC started out lower and rose slightly more steeply, reached a maximum, and thereafter exhibited a steady decline over longer TPIs.

DISCUSSION

The first objective of this study was to characterize the MRCs of isolated whole ventricles beating isovolumically using dP/dt_{max} as the index of contractile strength. There were three major results. First, the ventricular MRC rose monoexponentially to a plateau level from which there was no discernible decay, even after TPIs as long as 10–15 s. Second, as the priming frequency was increased the MRC shifted to the left and increased in magnitude but rose with the same time constant (typically 250 ms). Third, both the time constant and relative magnitude (normalized to the steady-state dP/dt_{max}) of the MRC were independent of ventricular volume within the range of volumes studied (15–35 ml).

The general characteristics of the MRCs measured in isovolumically beating ventricles were strikingly similar to those previously measured in isometrically beating isolated canine ventricular muscle (3), which demonstrated a monotonic rise and plateau. This suggests that

despite the complex geometry, activation sequence, and architecture of the heart, the ventricular MRC is representative of the time course of mechanical restitution of the muscles that comprise the ventricle.

The second result of our study is consistent with those of Edman and Johannsson (9), who found that, in rabbit papillary muscle, increases in priming frequency caused the MRC to shift leftward and increase in magnitude. They found, as did we, that the plateau magnitude increased nearly linearly with priming frequency. They attributed this increase to an augmentation in the circulating pool of "activator" calcium in the cardiac cells. This observation is therefore believed to be related to the previously noted positive effect of activation (20).

The leftward shift of the MRC was closely correlated with the shortening of the action potential that occurs at higher priming frequencies (Fig. 6*A*). The shift disappeared when we replotted the contractile response of the ventricle to test stimulations as a function of the test electrical diastolic interval rather than the TPI (Fig. 6*B*). This is consistent with the hypothesis that the processes of mechanical restitution are voltage dependent and proceed at appreciable rates only after the membrane has repolarized past a certain potential (14, 21, 35). This theory predicts the leftward shift with increased priming frequency, since a shortened action potential duration would allow mechanical restitution to begin earlier.

The finite time required for mechanical restitution (9) suggests a mechanism contributing to the negative inotropic effect of activation (20). For a given time constant of mechanical restitution, a shorter duration of electrical diastole allows only a smaller fraction of the "potential" contractile strength (i.e., CR_{max}) to become available on excitation of the ventricle (*Eq. 1*). For example, as the pacing frequency is increased, CR_{max} increases, but the steady-state diastolic interval decreases, allowing less time for restitution of contractility between steady-state beats; despite the increased potential strength (i.e., CR_{max}), this shortened diastolic interval results in a decrease in steady-state contractile strength at high heart rates (Fig. 5).

Our third result, the volume independence of the time course of mechanical restitution, is consistent with those of Anderson et al. (1, 3) who have shown that, in isolated canine ventricular muscle, the ratio between dF/dt_{max} of a test beat and dF/dt_{max} of the preceding steady-state beat was unaffected by the length of the muscle. They found similar results in the in situ canine ventricle using dP/dt_{max} as the index of contactile strength (3). In our experiments, the ventricular end-diastolic pressures were always in the physiological-to-subphysiological range (between approx 0 and 15 mmHg), and the volumes were always within the range of 15–35 ml. It is possible that this result of volume independence of the MRC may not hold at extreme volumes.

In the previous discussions we have compared our observations on the MRC with those measured in isolated myocardium from various mammals. However, species-related differences have been observed. For example, MRCs from kitten myocardium demonstrate a sharp rise, a peaking, and a subsequent quick decline (22). In contrast, adult cat myocardium has an MRC that rises in an exponential manner and reaches a plateau from which it declines relatively slowly (6, 22). MRCs from rat myocardium have been found to rise in an exponential manner with a very long time constant and reach a plateau from which there is no decay for test interval longer than 120 s (28, 29). Rabbit myocardium exhibits an MRC that differs only slightly from that of canine myocardium in that it declines from its plateau level with a relatively long time constant (9, 17). Therefore, properties of myocardial MRCs (e.g., response to increased priming frequency, effect of volume, etc.) measured in one type of myocardium do not necessarily hold for other types.

The second objective of our study was to investigate whether reliable MRCs (in terms of dP/dt_{max}) could be measured during ejecting contractions. We used the MRC measured under isovolumic conditions as the standard for comparison. To this end, we first determined the preload dependence of test beat dP/dt_{max} values (Fig. 7) and found it qualitatively similar to the results of several studies of steady-state ventricular dP/dt_{max} preload dependence (23, 30). Our finding conflicts, however, with those of Pidgeon et al. (26, 27). These investigators changed ventricular preload by volume infusion in intact dogs and by leg raising in human patients and found no statistically significant change in steady-state left ventricular dP/dt_{max}. In those studies, however, both the dogs and human patients had intact baroreceptor reflexes. It is likely that reflex-mediated changes in ventricular contractility and vascular tone would have obscured the true preload dependence of dP/dt_{max}. Our study with isolated hearts was free from this problem.

The preload dependence of dP/dt_{max} can explain the difference we observed between the early rising portion of the MRC (TPIs less than about 1 s) measured under isovolumic and ejecting conditions. The duration of ventricular filling is determined by the TPI, since on stimulation ventricular pressure rises and the mitral valve closes. Therefore, as the TPI increases, so does the ventricular end-diastolic volume at which dP/dt_{max} of the test beat is measured; this is demonstrated in the volume tracings in Fig. 3*B* with TPIs of 350 and 450 ms. This causes the ejecting MRC to start out below and rise more steeply than the isovolumic MRC. At longer TPIs, the extra increment of diastolic filling becomes small (diastasis); this makes the influence of changing preload most prominent for small values of the TPI. We expect this preload effect to be greater in the in situ ventricle because the steady-state stroke volume (approx 20 ml at a heart rate of 100 beats/min) is likely to be larger than could be produced by our ventricles (13–15 ml), which had slightly depressed contractilities due to their isolation.

For a test beat with a TPI greater than the steady-state priming interval, the aortic pressure falls during the preceding diastole. This low diastolic aortic pressure results in an early onset of ejection in the test beat, which in turn causes the dP/dt_{max} of the test beat to be less than the true isovolumic dP/dt_{max}. This is demonstrated in the dP/dt_{max} tracings of Fig. 3, *A* and *B*, with the TPI set equal to 950 ms.

These hemodynamic findings, which we obtained by imposing computer-generated physiological loading conditions on the ventricle, are consistent with those of

Yellin et al. (39), who measured changes in ventricular pre- and afterload of in situ hearts in response to extrasystoles. Therefore, our results concerning the hemodynamic effects on the MRC measured under ejecting conditions are applicable to the in situ ventricle.

MRCs have been measured in in situ canine ventricles by Anderson et al. (3), who called them graphical representations of the "first-stage experiments," and by Pidgeon et al. (26). These two groups of investigators have also measured MRCs in patients undergoing cardiac catheterization (2, 27). Anderson et al. (2, 3) only investigated TPIs in the range below 600 ms and were therefore only faced with changing preload conditions. Pidgeon et al. (26, 27) measured MRCs with TPIs greater than 1,200 ms in both their dog and human studies. The MRCs they measured were qualitatively similar to those measured in our ejecting isolated ventricles; each had a peak at a TPI between 500 and 700 ms and a subsequent descending limb. Our studies of isolated ventricles indicate that the descending limb reflects the influence of hemodynamic factors on dP/dt rather than the loss of activator calcium from intracellular stores as Pidgeon et al. (26) hypothesized.

Besides these hemodynamic considerations, there is an additional factor that may create a difference between the MRCs measured under isovolumic and ejecting conditions. Suga and Sagawa (33) have demonstrated in perfused canine papillary muscle that the history of the mode of contraction (isotonic or isometric) influences the contractile performance of the muscle. Priming periods with ejecting contractions may therefore create different steady-state conditions, potentially resulting in a real difference between contractile strengths of test beats with the same test pulse interval. Some of this effect may be canceled by normalizing the test response to the steady-state response, but a change in the parameters of restitution (e.g., CR_{max}, time constant, or TPI-axis intercept) might still be present. Based on their data, these influences may be quantitatively significant and should be investigated.

Although we only considered the contractile index dP/dt_{max}, other commonly used indexes (e.g., $dP/dt/P_{max}$) have been shown to demonstrate the same preload and/or afterload dependence under steady-state conditions and would therefore be expected to show the same limitations as dP/dt_{max} for the purpose of measuring the MRC.

It is possible to interpret the time course of mechanical restitution in terms of recently suggested models of calcium fluxes within the myocardial cell (9, 11, 12, 18, 25, 35, 38). These models include an intracellular calcium store, which functionally consists of two compartments. Calcium is sequestered from the myoplasm by an "uptake" compartment, which in turn gradually fills a "release" compartment. On excitation of the cell the release compartment empties its contents into the myoplasm; the calcium entering the cell on this same beat is not believed to contribute to its contractile strength (4, 5, 25). Therefore, a particular point on the MRC reflects the amount of calcium that had been transferred to the release store by the time the test stimulus was introduced. The MRC as a whole represents the time course of the transfer process. The plateau level of the MRC (CR_{max}) reflects the maximal releasable amount of activator calcium in the internal stores of the myocardial cell. When CR_{max} is normalized, this maximal releasable amount is expressed as a percentage of the activator released on each steady-state beat. Because mechanical restitution appears to begin at the onset of electrical diastole, the transfer of calcium from the uptake to the release compartment appears to occur only while the membrane is repolarized past a certain potential. Furthermore, the exponentiality of the MRC implies that the conversion from the uptake to the release compartment is governed by first-order kinetics

$$dR(t)/dt = (1/T)\ U(t) \qquad (2)$$

where $R(t)$ and $U(t)$ are the contents of the release and uptake compartments as functions of time, respectively, and T is the time constant of the MRC. Finally, the invariance of the time constant of mechanical restitution suggests, for the model, a kinetic rate constant $(1/T)$, which is also independent of priming frequency and ventricular volume.

Because it is believed that the mechanical restitution curve reflects intracellular processing of activator calcium, its characteristics (e.g., time constant, plateau level, response to increased priming frequency, etc.) may differ between normal and diseased ventricles. Such differences could possibly lead to increased understanding of the mechanisms underlying certain pathological states of the myocardium. The present study warns, however, that the influences of changing hemodynamic loading conditions on contractile indexes must be carefully considered when the results of clinical tests on ejecting hearts are interpreted in terms of intrinsic myocardial function.

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REFERENCES

1. ANDERSON, P. A., A. MANRING, C. E. ARENTZEN, J. S. RANKIN, AND E. A. JOHNSON. Pressure-induced hypertrophy of cat right ventricle. An evaluation with the force-interval relationship. *Circ. Res.* 41: 582–588, 1977.
2. ANDERSON, P. A. W., A. MANRING, G. A. SERWER, D. W. BENSON, S. B. EDWARDS, B. E. ARMSTRONG, R. J. STERBA, AND R. D. FLOYD. The force-interval relationship of the left ventricle. *Circulation.* 60: 334–348, 1979.
3. ANDERSON, P. A., J. S. RANKIN C. E. ARENTZEN, AND R. W. ANDERSON. Evaluation of the force-frequency relationship as a descriptor of the inotropic state of canine left ventricular myocardium. *Circ. Res.* 39: 832–839, 1976.
4. ANTONI, H. Elementary events in excitation-contraction coupling of the mammalian myocardium. *Basic Res. Cardiol.* 72: 140–146, 1977.
5. ANTONI, H., R. JACOB, AND R. KAUFMANN. Mekanische Reakti-

onen des Frosch und Saugetiermvokards bei Veranderung der Aktionspotential-Dauer durch konstante Gleichstromimpulse. *Pfluegers Arch.* 306: 33–57, 1969.
6. BASS, B. G. Enhanced contractility during relaxation of cat papillary muscle. *Am. J. Physiol.* 228: 1708–1716, 1975.
7. BRAVENY, P., AND V. KRUTA. Dissociaton de deux facteurs: restitution et potentiation dans l'action de l'amplitude de la contraction du myocarde. *Arch. Int. Physiol. Biochim.* 66: 633–652, 1958.
8. COHN, P. F. Editorial: Evaluation of inotropic contractile reserve in ischemic heart disease using postextrasystolic potentiation. *Circulation* 61: 1071–1075, 1980.
9. EDMAN, K. A. P., AND M. JOHANNSSON. The contractile state of rabbit papillary muscle in relation to stimulation frequency. *J. Physiol. London* 254: 565–581, 1976.
10. ELZINGA, G., M. J. LAB, M. I. M. NOBLE, D. E. PAPADOYANNIS, J. PIDGEON, A. SEED, AND B. WOHLFART. The action-potential duration and contractile response of the intact heart related to the preceding interval and the preceding beat in the dog and cat. *J. Physiol. London* 314: 481–500, 1981.
11. FABIATO, A., AND F. FABIATO. Calcium and cardiac excitation-contraction coupling. *Annu. Rev. Physiol.* 41: 473–484, 1979.
12. FOZZARD, H. A. Heart: excitation-contraction coupling. *Annu. Rev. Physiol.* 39: 201–220, 1977.
13. FRANZ, M. R., M. SCHOTTLER, J. SCHAEFER, AND W. A. SEED. Simultaneous recording of monophasic action potentials and contractile force from the human heart. *Klin. Wocheschr.* 58: 1357–1359, 1980.
14. GIBBONS, W. R., AND H. A. FOZZARD. Voltage dependence and time dependence of contraction in sheep cardiac purkinje fibers. *Circ. Res.* 28: 446–460, 1971.
15. HOFFMAN, B. F., H. J. BARTELSTONE, B. J. SCHERLAG, AND P. F. CRANEFIELD. Effects of postextrasystolic potentiation on normal and failing hearts. *Bull. NY Acad. Med.* 41: 498–534, 1965.
16. HOFFMAN, B. F., P. F. CRANEFIELD, E. LEPESCHKIN, B. SURAWICZ, AND H. C. HERRLICH. Comparison of cardiac monophasic action potentials recorded by intracellular and suction electrodes. *Am. J. Physiol.* 196: 1297–1301, 1959.
17. JOHNSON, E. A. Force-interval relationship of cardiac muscle. In: *Handbook of Physiology. The Cardiovascular System.* Bethesda, MD: Am. Physiol. Soc., 1979, sect. 2, vol. I, chapt. 13, p. 475–496.
18. KAUFMANN, R., R. BAYER, T. FUERNISS, H. KRAUSE, AND H. TRITTHART. Calcium-movement controlling cardiac contractility. II. Analog computation of cardiac excitation-contraction coupling on the basis of calcium kinetics in a multi-compartment model. *J. Mol. Cell. Cardiol.* 6: 543–544, 1974.
19. KILZ, U., W. NIEDERMAYER, K. J. NORDMANN, J. SCHAEFER, H. J. SCHWARZKOPF, AND P. A. VAN ZWIETEN. A comparison between frequency potentiation and post-extrasystolic potentiation in vitro and in situ. *Pfluegers Arch.* 311: 226–242, 1969.
20. KOCH-WESER, J., AND J. R. BLINKS. The influence of the interval between beats on myocardial contractility. *Pharmacol. Rev.* 15: 601–652, 1963.
21. LIPSIUS, S. L., H. A. FOZZARD, AND W. R. GIBBONS. Voltage and time dependence of restitution in heart. *Am. J. Physiol.* 243 (*Heart Circ. Physiol.* 12): H68–H76, 1982.
22. MAYLIE, J. G. Excitation-contraction coupling in neonatal and adult myocardium of cat. *Am. J. Physiol.* 242 (*Heart Circ. Physiol.* 11): H834–H843, 1982.
23. MASON, D. T. Usefulness and limitations of the rate of rise of intraventricular pressure (dP/d*t*) in the evaluation of myocardial contractility (Abstract). *Am. J. Cardiol.* 23: 516, 1969.
24. MILNOR, W. R. Arterial impedance as ventricular afterload. *Circ. Res.* 36: 565–570, 1975.
25. MORAD, M., AND Y. GOLDMAN. Excitation-contraction coupling in heart muscle: membrane control of development of tension. *Prog. Biophys. Mol. Biol.* 27: 257–313, 1973.
26. PIDGEON, J., M. LAB, A. SEED, G. ELZINGA, D. PAPADOYANNIS, AND M. I. M. NOBLE. The contractile state of cat and dog heart in relation to the interval between beats. *Circ. Res.* 47: 559–567, 1980.
27. PIDGEON, J., G. A. H. MILLER, M. I. M. NOBLE, D. PAPADOYANNIS, AND W. A. SEED. The relationship between the strength of the human heart beat and the interval between beats. *Circulation* 65: 1404–1410, 1982.
28. POSNER, C. J., AND D. A. BERMAN. Mathematical analysis of oscillatory and non-oscillatory recovery of contractility after a rested-state contraction and its modification by calcium. *Circ. Res.* 25: 725–733, 1969.
29. RAGNARSDOTTIR, K., B. WOHLFART, AND M. JOHANNSSON. Mechanical restitution of the rat papillary muscle. *Acta Physiol. Scand.* 115: 183–191, 1982.
30. SCHMIDT, H. D., AND H. HOPPE. Preload dependence of dP/dt_{max}, V_{CEmax}, and calculated V_{max} compared to the inotropic sensitivity of these indices of contractility. *Basic Res. Cardiol.* 73: 380, 1978.
31. SUGA, H., AND K. SAGAWA. Instantaneous pressure-volume relationships and their ratio in the excised, supported canine left ventricle. *Circ. Res.* 35: 117–126, 1974.
32. SUGA, H., AND K. SAGAWA. End-diastolic and end-systolic ventricular volume clamper for isolated canine heart. *Am. J. Physiol.* 233 (*Heart Circ. Physiol.* 2); H718–H722, 1977.
33. SUGA, H., AND K. SAGAWA. Transient force responses in blood-perfused papillary muscle after step changes in load. *Am. J. Physiol.* 235 (*Heart Circ. Physiol.* 4): H267–H275, 1978.
34. SUNAGAWA, K., D. BURKHOFF, K. O. LIM, AND K. SAGAWA. Impedance loading servo pump system for excised canine ventricle. *Am. J. Physiol.* 243 (*Heart Circ. Physiol.* 12): H346–H350, 1982.
35. TRITTHART, H., R. KAUFMANN, H.-P. VOLKMER, R. BAYER, AND H. KRAUSE. Ca-movement controlling myocardial contractility. I. Voltage, current and time dependence of mechanical activity under voltage clamp conditions (cat papillary muscles and trabeculae). *Pfluegers Arch.* 338: 207–231, 1973.
36. WOHLFART, B. Relationship between peak force, action potential duration and stimulus interval in rabbit myocardium. *Acta Physiol. Scand.* 106: 395–409, 1979.
37. WOHLFART, B. *Interval-Strength Relations of Mammalian Myocardium Interpreted as Altered Kinetics of Activator Calcium During the Cardiac Cycle* (thesis). Lund, Sweden: Univ. of Lund, 1982, p. 9–144.
38. WOOD, E. H., R. L. HEPPNER, AND S. WEIDMANN. Inotropic effects of electric currents. *Circ. Res.* 24: 409–445, 1969.
39. YELLIN, E. L., A. KENNISH, C. YORAN, S. LANIADO, N. BUCKLEY, AND R. FRATER. The influence of left ventricular filling on postextrasystolic potentiation in the dog heart. *Circ. Res.* 44: 712–722, 1979.