Volume Loading Slows Left Ventricular Isovolumic Relaxation Rate

Evidence of Load-Dependent Relaxation in the Intact Dog Heart

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SUMMARY We studied the effects of volume loading on left ventricular isovolumic relaxation rate in 16 intact anesthetized dogs. End-diastolic pressure, mean aortic systolic pressure, dp/dtmax, and heart rate were measured at end expiration and end inspiration. Volume loading to approximately 5, 10, 15, and 20 mm Hg above initial end-diastolic pressure was performed. In nine dogs, simultaneous ventricular dimensions were measured with previously implanted tantalum screws using biplane cineangiography. Similar volume loading was done in open-chest and open-pericardium states. Relaxation rate was measured in 3413 beats using T, the time constant of exponential isovolumic pressure fall. T was calculated as reported previously by others and also from a linear regression of dp/dt against p, to eliminate the effects of extracavity pressure changes. T always increased significantly with volume loading, indicating slower relaxation. (For example, with the chest intact, mean T increased from 26 ± 2 (SEM) msec before volume loading to 41.5 ± 4 msec after volume loading.) Using multiple linear regression analysis, we found, in agreement with previous reports, that T decreased significantly as dp/dtmax and heart rate increased. In contrast to previous reports, we also found that T increased significantly as end-diastolic and mean aortic systolic pressure increased. These four variables taken together accurately predicted T [SEE (standard error of estimate) = 3.2 msec, R = 0.94, P < 0.001].

Studies of the hemodynamic determinants of left ventricular isovolumic relaxation grew out of interest in the effects of ischemia on diastolic pressure. Following the observation of Barry et al. (1974) that left ventricular diastolic pressure increased without significant increases in end-diastolic volume during angina pectoris, McLaurin et al. (1973) investigated the hypothesis, first suggested by Mitchell et al. (1960), that incomplete relaxation during diastole might cause an apparent acute stiffening of the left ventricle on the next beat. McLaurin et al. (1973) observed that angina produced a significant decrease in the magnitude of dp/dtmin (also called −dp/dtmax) and suggested that this response indicated impaired relaxation. However, the interpretation of this and similar clinical studies (Papapietro et al., 1979) is clouded by the complex interaction of many changes that occur during ischemia, including depressed inotropic state, increased preload, and increased afterload. Studies of the determinants of dp/dtmin in nonischemic preparations demonstrated it to be sensitive to inotropic state, end-systolic dimensions (Cohn et al., 1972), peak aortic pressure, stroke volume, and heart rate (Weisfeldt et al., 1974), all of which change during ischemia. In other words, the reported changes in dp/dtmin could have been due to changes in the load the heart faced rather than any direct effect on the relaxing system.

This difficulty stimulated efforts to find a measure of relaxation rate that was independent of preload and afterload. In 1976, Weiss et al. studied isolated isovolumic canine left ventricles whose aortic and mitral valves were occluded by steel discs and showed that isovolumic pressure fall closely followed a single exponential function of time. Therefore, they described how fast the pressure fell with the time constant, T, of that exponential. In their study, as well as in one using an ejecting right heart bypass preparation (Frederiksen et al., 1978), T appeared independent of end-diastolic pressure, peak left ventricular pressure, stroke volume, and end-systolic fiber length. T was independent of heart rate, except at very high rates, when it fell slightly. Both studies also found that the extent of systolic shortening and interventions thought to...

STUDIES of the hemodynamic determinants of left ventricular isovolumic relaxation grew out of interest in the effects of ischemia on diastolic pressure. Following the observation of Barry et al. (1974) that left ventricular diastolic pressure increased without significant increases in end-diastolic volume during angina pectoris, McLaurin et al. (1973) investigated the hypothesis, first suggested by Mitchell et al. (1960), that incomplete relaxation during diastole might cause an apparent acute stiffening of the left ventricle on the next beat. McLaurin et al. (1973) observed that angina produced a significant decrease in the magnitude of dp/dtmin (also called −dp/dtmax) and suggested that this response indicated impaired relaxation. However, the interpretation of this and similar clinical studies (Papapietro et al., 1979) is clouded by the complex interaction of many changes that occur...
influence active relaxation such as ischemia, Ca\textsuperscript{2+}, and norepinephrine also affected T. On the basis of this work, T, the time constant of exponential left ventricular pressure fall after dp/dt\textsubscript{min} has been accepted as an index of isovolumic relaxation rate that is independent of load.

In particular, T has been used to describe how various diseases or interventions, including angina (Mann et al., 1979; Sharma et al., 1979), left ventricular hypertrophy (Ring et al., 1979), postextrasystolic potentiation (Blaustein et al., 1979), sinus tachycardia (Fioretti et al., 1980), and vasodilators (Ludbrook et al., 1977, 1979), affect ventricular relaxation in intact animals or humans.

Substantial physiological differences exist between the isolated left ventricle Weiss et al. (1976) studied the right heart bypass preparation Frederiksen et al. (1978) studied, and the intact hearts in these clinical studies. To use T to help understand the process of relaxation, as well as to interpret the results of such clinical studies, the hemodynamic determinants of isovolumic relaxation in general and T in particular must be established in intact animals.

Our data, obtained from intact animals, indicate that T increases during volume loading. Specifically, as both end-diastolic pressure and mean aortic systolic pressure increase, T increases, indicating that the heart is relaxing more slowly. This result is opposite to what would be predicted from previous studies.

Methods

Preparation

We studied 16 mongrel dogs. Twelve of the dogs were instrumented with endocardial tantalum screws 2–6 weeks before the experiment day using a modification of Carlson and Milne's (1967) method (Davis et al. 1980). The screws, (1–× 2-mm helices of sterile tantalum wire, were implanted in the left ventricular endocardium under Nembutal anesthesia with an 8F MediTech steerable catheter introduced via a carotid artery under fluoroscopic guidance. The chest wall, pleura, and pericardium remained intact. Two or three screws were positioned in the aortic valve ring, one at the left ventricular apex, and four to define the ventricle's anterior-posterior and medial-lateral diameters. This method permitted us to record left ventricular dimensions by filming the heart with a biplane cineradiographic system without opening the chest or injecting contrast media that present a sudden volume load to one ventricle.

On the experiment day, the dogs were premedicated with morphine 2 mg/kg, injected sc, and then anesthetized with α-chloralose, by iv infusion. We supplemented this anesthesia with a 10-mg iv injection of morphine every hour. This combination of chloralose and morphine maintained a normal resting heart rate (70–90 beats/min) and a physiological response to volume loading similar to that reported in conscious dogs. Specifically, the dogs responded to the volume load by increasing heart rate with little or no change in stroke volume (Arfors et al., 1971; Vatner and Boetticher, 1978).

We placed Millar microtip transducers in the left ventricle via a carotid artery, the aorta via the left femoral artery, and the right ventricle via an external jugular vein. The transducers were immersed in a 37°C water bath overnight and balanced and calibrated in the bath immediately before they were placed in the dog.

Pressure data were measured with an Electronics for Medicine DR12 amplifier and, along with ECG and cine mark synchronization channels, recorded on a Honeywell 5600C FM tape recorder. Before each recording, the Millar transducers were checked for baseline drift by comparison to a Statham P23Db transducer connected to the Millar transducer's fluid-filled lumen. The fluid-filled system was zeroed at the midpoint of the heart determined with the x-ray system. When necessary, we rebalanced the Millar transducers, either by matching the high gain pressure tracing to the lumen pressures or by removing the catheter and rebalancing it in the constant temperature bath.

Protocol

Data were recorded with the respirator stopped at end expiration. Simultaneous pressures and ECG were recorded for approximately 15 seconds. In the 12 dogs instrumented with tantalum screws, we recorded synchronized biplane x-ray cineradiographs at 30 frames/sec with the x-ray equipment in biplane-alternating mode. In some experiments, we also recorded data with the respirator stopped at end inspiration to investigate the effects of changing pleural pressure.

After obtaining control measurements, we infused (100 ml/min) a 1:1 mixture of saline and blood taken from a donor dog. This mixture maintained hematocrit in the experiment dog at approximately its original level. The donor dog was anesthetized with sodium pentobarbital, and saline was infused as the dog was bled to maintain intravascular volume. The blood was stored in a collection pack and rewarmed to 37°C with a Hemocool in a constant temperature bath before it was infused into the experiment dog. We did not see any evidence of a transfusion reaction. We measured arterial blood gases every 30–60 minutes and maintained them in the physiological range. We recorded data when end-diastolic pressure was approximately 5, 10, 15 and 20 mm Hg above the initial value from 2.0 to 9.5 mm Hg [4.9 ± 2.4 (sd)]. We recorded data approximately every 15 minutes. We denote this as the "closed-chest" condition.

After recording data with the chest intact, we inserted bilateral chest tubes to release pleural pressure. We denote this as the "open-chest" condition. If necessary, we bled the dog via the femoral artery.
cannula to return end-diastolic pressure to the physiological range (5-10 mm Hg). We saved the blood for autotransfusion. We recorded data under this new baseline condition and after volume loading as before.

The same protocol was performed again after a median sternotomy and pericardiectomy. We denote this as the "open-pericardium" condition.

The recorded data were digitized every 5 msec (200 Hz) with a 13-bit analog-to-digital converter, using a digitizing software package developed at our institution (Horowitz and Glantz, 1979) running on a PDP 11/70 computer.

We computed left ventricular dp/dt from the digitized pressure signal with the symmetric first difference:

$$\dot{p}_i = (p_{i+1} - p_{i-1}) \cdot 100.$$  

The resulting dp/dt was filtered once with the moving average lowpass digital filter:

$$\dot{x}_i = \frac{1}{4}(x_{i+1} + 2x_i + x_{i-1}).$$

We excluded all ectopic beats and postectopic beats from our analysis, leaving 3413 acceptable beats.

Computation of $T$

The isovolumic relaxation period was defined as the period from the time of dp/dt min to the time when left ventricular pressures fell to 5 mm Hg above end-diastolic pressure of the following beat. We chose this cutoff point to ensure that it would occur before the mitral valve opened. We computed $T$ in two different ways.

First, we used the method Weiss et al. (1976) originally suggested to compute $T$. If left ventricular pressure falls exponentially during isovolumic relaxation, then

$$p(t) = p_0 e^{-t/T} + p_B.$$  

Differentiate this equation with respect to $t$, to obtain

$$\frac{dp}{dt} = - \frac{p_0}{T} e^{-t/T}.$$  

and then use the equation for $p$ to eliminate $e^{-t/T}$ from this equation:

$$\frac{dp}{dt} = - \frac{1}{T}(p - p_B) = - \frac{1}{T} p + \frac{p_B}{T}.$$  

Therefore, dp/dt is a linear function of $p$ with slope $-1/T$ regardless of the magnitude of the baseline shift, $p_B$. ($p_B$ does not affect the intercept, but we do not use the intercept to compute $T$.) Thus, we can estimate $T$ by computing a linear regression of dp/dt against $p$ using the digitized data points from the period of isovolumic pressure fall. $T$ calculated from the derivative of pressure is denoted as $T_D$.

In addition to the values of $T_L$ and $T_D$ for each beat, we recorded the correlation coefficient from the linear regressions used to compute them.

The appendix examines how precisely the pressure fall during isovolumic relaxation follows an exponential function in two different ways.
Other Hemodynamic Measurements

In addition to T, we computed \( p_{A0} = \) mean aortic systolic pressure, \( p_{PSY} = \) mean left ventricular systolic pressure, \( p_{PED} = \) left ventricular end-diastolic pressure (left ventricular pressure at the peak of the ECG R wave), \( dp/dt_{min}, dp/dt_{max}, \) and \( HR = \) instantaneous heart rate (computed from the R-R interval). Mean systolic pressures were computed by averaging the pressures between the times of maximum and minimum left ventricular \( dp/dt. \)

Dimension Data

Nine of the 12 dogs instrumented with tantalum screws were technically suitable to obtain data on dimensions. Pressures and film data were synchronized by a cine mark channel produced by the cameras and recorded with the pressure signal. Two or three beats were analyzed in each experimental condition, with an average of 20 experimental conditions/experiment. The film data was analyzed by projecting each frame from the frontal and lateral cameras on a digitizing tablet connected to a Hewlett-Packard 9820A desk-top computer and entering each tantalum screw’s position by hand in both projections. Each screw’s three-dimensional coordinates in a system defined by the x-ray beams were determined and stored on magnetic tape (Davis et al., 1980) and later transferred to a disk file on the PDP 11/70.

The distance between any two screws was computed by

\[
d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2}
\]

where \((x_1, y_1, z_1)\) and \((x_2, y_2, z_2)\) are the coordinates of the two screws.

The apical and aortic screws defined the left ventricle’s major axis, the septal and free wall screws, one minor equatorial axis, and the anterior and posterior screws, the other minor axis. The volume was then computed assuming the heart to be a general ellipsoid with these three axes. We computed circumference in the equatorial plane with

\[
C = 2\pi \sqrt{a^2 + b^2 - b^2}
\]

in which \(a\) and \(b\) are the two equatorial semiaxes. We computed minimum and maximum equatorial circumference \((C_{min} \text{ and } C_{max})\) and volume \((V_{min} \text{ and } V_{max})\) and used these data to compute circumferential shortening fraction, \(S,\) and volume ejection fraction, \(E.\)

Statistical Analysis

Our null hypothesis was that \(T\) is not dependent on end-diastolic pressure, mean aortic systolic pressure, heart rate, \(dp/dt_{max},\) ejection fraction, or ventricular dimensions.

Since we sought to study a preparation that was as intact as possible, we did not vary independently each of the potential determinants of \(T,\) as has been done in excised hearts or right heart bypass preparations. We used forward-backward stepwise multiple linear regression to identify the important determinants of \(T\) (Draper and Smith, 1966; Neter and Wasserman, 1974; Kleinbaum and Kupper, 1978). The computer enters potential independent variables into the regression equation one at a time, according to their ability to reduce the residual variance in the dependent variable, \(T.\) After entering each variable, those already in the equation are tested to see whether, because of multicollinearity, any of them can be removed without significantly \((P > 0.05)\) increasing the residual variance in \(T.\) If a potential independent variable is covariant with one or more of the independent variables and this covariation accounts for an apparent correlation with \(T,\) only the more powerful variable will stay in the equation. It is important to emphasize that, while we specify potential independent variables, they will appear in the final regression equation only if they exert a statistically significant independent effect on \(T.\)

All multiple linear regression computations were done with the 2.0 version of BMDP2R (Dixon and Brown, 1977). F-to-enter and F-to-remove were set to 4.0 to force variables to the equation when \(P < 0.05\) for that variable and out of the equation when \(P > 0.05.\) We used METHOD = F to obtain a \(P > 0.05\). We used METHOD = F to obtain a forward-backward stepwise regression.

We used TL and TD as the dependent variable in two separate sets of computer runs.

In our first analysis, we included only the hemodynamic variables measured for each beat, \(p_{D}, p_{AO} \text{ (or } p_{PSY}, dp/dt_{max}, \text{ and } HR, \) as the potential independent variables and analyzed the data from each dog separately. To account for any changes in \(T\) that may have occurred purely because of opening the chest, opening the pericardium, or taking the data at end inspiration instead of end expiration, we defined three dummy variables:

\[
D_{C} = \begin{cases} 
0 & \text{if chest intact} \\
1 & \text{if chest open} 
\end{cases}
\]

\[
D_{P} = \begin{cases} 
0 & \text{if pericardium intact} \\
1 & \text{if pericardium open} 
\end{cases}
\]

\[
D_{I} = \begin{cases} 
0 & \text{if data recorded at end expiration} \\
1 & \text{if data recorded at end inspiration} 
\end{cases}
\]

allowing the full potential regression equation to be:

\[
T = b_{0} + b_{ED}p_{ED} + b_{AO}p_{AO} + b_{dp/dt_{max}}dp/dt_{max} + b_{HR}HR + b_{DC}D_{C} + b_{DP}D_{P} + b_{DI}D_{I}
\]

We analyzed 3413 beats. Examination of the bi-variate scatter plots and the residuals from the multiple regression equation did not suggest any nonlinear components of the relationships between \(T\) and the other variables. Although the independent variables were allowed to vary simultaneously...
with volume loading, it was possible to isolate the influence of preload, afterload, contractile state, and opening the chest and pericardium, with a high degree of confidence \((P < 0.001\) for all variables in the final equation.)

In our second analysis, we pooled data for the nine dogs with dimension data to see whether changes in \(T\) associated with changes in \(P_{\text{ED}}\) and \(P_{\text{AO}}\) were really secondary manifestations of changes in ventricular size. As mentioned previously, we analyzed the tantalum screw data for 2-3 typical beats under each experimental condition. The values for the hemodynamic variables \((T_L, T_D, P_{\text{ED}}, P_{\text{AO}}, \text{HR, } \text{dp/dt}_{\text{max}})\) were averaged for all the beats under a given experimental condition. Together with the values of the dimension variables \((C_{\text{min}}, C_{\text{max}}, S, V_{\text{min}}, V_{\text{max}}, E)\) from the typical beats under the same experimental conditions, they formed the input to a single multiple regression analysis using the same equation as before, with terms added for either the circumferential or volumetric dimension data. The data file included 132 points for the nine dogs. To allow for between-dog differences in mean values of \(T\), we also included nine more dummy variables defined by

\[
D_n = \begin{cases} 
1 & \text{if data for dog } n \\
0 & \text{otherwise} 
\end{cases}
\]

and added these variables, together with the dimension variables, to the original potential regression equation. The analysis was performed separately for volume and circumference, and the complete analysis was done twice, once for \(T_L\) and once for \(T_D\).

### Results

#### Volume Loading

Figure 2 shows that volume loading always prolonged \(T_L\). Each line represents the results for a single dog in the baseline state and at maximum volume loading. Opening the pleura and pericardium did not affect the response to volume loading. (Statistical aspects of this observation will be discussed below.) Note that since volume loading is associated with increases in heart rate and \(\text{dp/dt}_{\text{max}}\), previous work would lead to the prediction that \(T\) should decrease, not increase.

#### Hemodynamic Variables

Figure 3 shows the relationship between \(T_L, P_{\text{ED}}, P_{\text{AO}}, \text{dp/dt}_{\text{max}}\), and \(\text{HR}\) for one dog. These graphs show the simple bivariate correlations without controlling for covariance between the independent variables. They suggest that \(T_L\) increases linearly as \(P_{\text{ED}}\) and \(P_{\text{AO}}\) increase, that \(T_L\) decreases as \(\text{dp/dt}_{\text{max}}\) increases, and that \(T_L\) is relatively insensitive to heart rate, except above about 170 beats/min, when it decreases. These results suggest that the heart relaxes more slowly as preload \((P_{\text{ED}})\) and afterload \((P_{\text{AO}})\) increase and faster as inotropic state (measured as \(\text{dp/dt}_{\text{max}}\)) and heart rate increase. Our results regarding \(\text{dp/dt}_{\text{max}}\) and heart rate agree with previous studies. On the other hand, our results regarding end-diastolic and aortic pressure differ from previous studies done using isolated and right heart bypass left ventricles (that concluded that \(T_L\) is independent of load).

To investigate whether or not the apparent load dependence of \(T_L\) was due to collinearity of preload or afterload with other variables, like \(\text{dp/dt}_{\text{max}}\), that have already been shown to affect \(T_L\), we completed the stepwise multiple linear regression analysis described above. Table 1 gives the regression coefficients for each of the 16 experiments, analyzed separately, together with the multiple correlation coefficient, \(R\), the standard error of the estimate of \(T_L\), and the number of beats analyzed for each dog. The results demonstrate that the relationships in Figure 3 reflect independent effects of each of the variables on \(T_L\).

The statistically significant variables accurately predict \(T_L\) over a wide range of loading conditions. The standard errors of the estimate were of the order of 2-4 msec in all dogs, indicating that, given
Figure 3  Bivariate correlations of TTAU (msec) with various hemodynamic variables for a typical dog. These plots suggest that isovolumic relaxation slows as preload and afterload increase and speeds up as left ventricular (LV) dp/dt max and heart rate increase. Each symbol represents a single beat. Data obtained with closed chest (inspiration), closed chest (expiration), open chest, and open pericardium all fall along the same lines.

the variables in the equation, one could predict T to within about 4–8 msec for any given beat. pED was a highly significant determinant of T in 15 of 16 experiments, pAO (or pPSYS for the five experiments in which we did not measure aortic pressure) was significant in 12 of 16, and dp/dt max in 13 of 16. T consistently increased with increasing preload and afterload and decreased with increasing dp/dt max. HR was a significant variable in 10 of 16 experiments.

We also wanted to compare the relative predictive power of the independent variables, both within each dog and between dogs. We could not simply compare the regression coefficients (the b1's in the regression equation) because a change of 1 mm Hg in end-diastolic pressure does not mean the same thing as a 1 mm Hg/sec change in dp/dt max or even a 1 mm Hg change in mean aortic pressure. In addition, the range of the variables we measured varied from dog to dog, so a 1 mm Hg change in pressure in one dog may not mean the same thing as a 1 mm Hg pressure change in another dog. To permit such comparisons, we computed the standardized regression coefficients for each significant variable. The standardized regression coefficient is a dimensionless number defined by

$$
\beta_i = \frac{b_i s_i}{s_T}
$$

where b_i is the coefficient of variable i in the un-normalized regression equation, s_i is the standard deviation of variable i, and s_T is the standard deviation of T. \beta_i says that for each standard deviation of change in independent variable i, T changes by \beta_i standard deviations. For example, if the standard deviation of T in a given experiment is 20 msec and that of pED is 10 mm Hg, a standardized regression coefficient of 0.5 means that a 1-mm Hg rise in pED would result in an average increase in T of 10 msec.

Table 2 shows that, in terms of the standardized regression coefficients, T is about as sensitive to changes in preload, afterload, and dp/dt max, with a much lower sensitivity to changes in heart rate. (The dummy variables were excluded because their standard deviations do not have a physical meaning.)

We consistently found these results, whether or not the chest was intact or the pericardium was opened. Similar regression coefficients were computed for each variable as a determinant of T whether the chest was open or closed. Dc was significant in 10 of 16 experiments, but its regression coefficient varied in magnitude and sign, showing no consistent pattern. Dp and Dv were not, in general, important determinants of T. In sum, the results of the three dummy variables suggest that pressure changes outside the left ventricle are not physiologically important determinants of T and do not explain the slowing in relaxation rate observed with volume loading.

A similar analysis of T computed from a plot of dp/dt vs. p yielded results consistent with those obtained from T. The results showed somewhat greater variability, described by a standard error of the estimate of TD of 11 msec and an average R of
Dashes under D indicate no data recorded during inspiration.

Table 1  Regression Coefficients for T

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<tr>
<th>Dog</th>
<th>n*</th>
<th>Y intercept</th>
<th>Dc</th>
<th>Dp</th>
<th>dp/dtmax</th>
<th>HR</th>
<th>Dc</th>
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Mean values

| 0.48| 0.22| -0.007| -0.05 |

Results are expressed as means ± SE of regression coefficients; P < 0.001 for all significant variables; P > 0.1 for all nonsignificant (NS) variables. Dashes under D indicate no data recorded during inspiration.

* Number of beats.
† Defined to be √ΣSE/n – p) where p is the number of variables in the regression equation [Dixon and Brown, 1977 (p. 403)].

0.85. This increased variability is probably due to two factors. First, as described in the appendix, isovolumic pressure fall is not really a single exponential, so the mathematical manipulation and substitutions needed to define T from dp/dt vs. p are not strictly correct. Second, differentiation increases the noise in any signal, and the noise in dp/dt decreases the certainty with which T can be estimated. (This lower certainty of the estimate is also reflected in the smaller correlation coefficient.) Nonetheless, ped remains significant in 12 of 16 experiments, pao (or psys) in 13 of 16, and dp/dtmax in 14 of 16. Dc remained significant in 10 of 16 experiments, with variable coefficients.

Hemodynamic and Geometric Variables

Combined

So far, we have been discussing only hemodynamic variables, not direct measures of heart size or muscle fiber length. Weigner and Bing (1978) suggested that relaxation is a length-dependent rather than a load-dependent process in isolated papillary muscle. Therefore, it could be argued that the pressure dependence of T that we detected was really an indirect manifestation of length dependence because volume loading by increasing heart size increased both the pressures and muscle fiber length, and so T. To examine this possibility, two typical heart beats from each experimental condition were examined. Biplane film data were analyzed using frame-by-frame analysis at 30 frames/sec to determine volume and circumference. Corresponding values for T, ped, pao, dp/dtmax, and HR were obtained. A total of 132 heartbeats were analyzed from the nine dogs, using a single stepwise regression analysis.

If the phenomenon we detected was due to length rather than load dependence, we expected one or
ventricular end-diastolic pressure and peak systolic output results from reflex increases in heart rate and chronotropic effects of stimulated catecholamine release. Increased cardiac output by increasing stroke volume with little change in heart rate. Our dogs (anhæsthetized with chloralose) had normal resting heart rates (70–90 beats/min) and responded to volume loading like Vatner’s conscious dogs: stroke volume remained relatively unchanged and heart rate increased.

According to the previous studies of Weiss et al. (1976) and Frederiksen et al. (1978), the net result of these complex changes should be a decrease of T with volume loading, i.e., faster relaxation. The increased end-diastolic pressure and increased aortic pressure should not directly affect T; increased heart rate should minimally but significantly decrease it. Increased contractility and perhaps increased systolic shortening should substantially decrease T. In contrast to this predicted result, our results demonstrate that volume loading produces a marked slowing of isovolumic relaxation rate, as measured by T, in intact dogs.

We divided the known effects of volume loading into the following components: end-diastolic pressure, aortic systolic pressure, inotropic state (measured by dp/dtmax and ejection fraction), heart rate, and ventricular dimensions.

Since the dimension variables did not prove to be statistically significant, the resulting values for the regression coefficients of the hemodynamic variables represent a summary of the pooled results of all experiments. These results are entirely consistent with those of the individual experiments in Tables 1 and 2.

Discussion

Vatner et al. (1978) described the effects of volume loading in conscious dogs. Increased cardiac output results from reflex increases in heart rate (Bainbridge reflex) and the positive inotropic and chronotropic effects of stimulated catecholamine release. Increased intravascular volume causes left ventricular end-diastolic pressure and peak systolic pressure to increase. There is some increase in ventricular dimensions and systolic shortening. These changes contrast with what is observed in dogs anesthetized with some agents (e.g., pentobarbital) that produce high resting heart rates (120–160 beats/min). In these dogs, cardiac output increases by increasing stroke volume with little change in heart rate. Our dogs (anhæsthetized with chloralose) had normal resting heart rates (70–90 beats/min) and responded to volume loading like Vatner’s conscious dogs: stroke volume remained relatively unchanged and heart rate increased.

We chose T as the dependent variable in the regression equation, because in this experiment the increase in T is clearly the result of volume loading. Therefore, the physiological components of volume loading can be hypothesized to cause the increase in T and were chosen as the independent variables in the equation.

The original studies of Weiss et al. (1976) and Frederiksen et al. (1978) support the inclusion of heart rate, systolic circumferential shortening, and dp/dtmax as important determinants of T. Karliner et al. (1977) studied T in intact conscious dogs and observed a significant increase in T with increasing aortic pressure. Similarly, Gausch et al. (1980) reported the hemodynamic determinants of T in open-chest anesthetized dogs and also found that T changed with changes in aortic pressure. Do any studies support the inclusion of end-diastolic pressure as a determinant of T? Karliner et al. (1977) did not detect a statistically significant prolongation of T with increasing end-diastolic pressure pro-

![Table 2](image)

<table>
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<tr>
<th>Dog</th>
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<th>dp/dtmax</th>
<th>HR</th>
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<td>−0.52</td>
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**Mean values**

0.54 0.42 −0.44 0.28

more of the geometric variables to appear in the final regression equation, whereas the pressure variables would not. If there was an element of length dependence and an element of load dependence, we expected both geometric and pressure variables to appear in the final regression equation. Table 3 lists the raw and standardized regression coefficients for clarity, since they represent merely the random effects component of this mixed effects regression model. No dimension variable had a statistically significant influence both geometric and pressure variables to appear in the final regression equation. Therefore, the physiological components of volume loading can be hypothesized to cause the increase in T and were chosen as the independent variables in the equation.

The original studies of Weiss et al. (1976) and Frederiksen et al. (1978) support the inclusion of heart rate, systolic circumferential shortening, and dp/dtmax as important determinants of T. Karliner et al. (1977) studied T in intact conscious dogs and observed a significant increase in T with increasing aortic pressure. Similarly, Gausch et al. (1980) reported the hemodynamic determinants of T in open-chest anesthetized dogs and also found that T changed with changes in aortic pressure. Do any studies support the inclusion of end-diastolic pressure as a determinant of T? Karliner et al. (1977) did not detect a statistically significant prolongation of T with increasing end-diastolic pressure pro-

![Table 3](image)

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<tr>
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* Not significant: minimum circumference, maximum circumference, minimum volume, maximum volume, fractional shortening, and ejection fraction.

† Mean ± SE of regression coefficients.
duced by volume infusion. T did increase from 19 to 22 msec with a relatively small change in end-diastolic pressure (5–13 mm Hg). This difference might not have reached statistical significance because of the small number of animals and beats studied (n = 13). However, Karliner et al. (1977) report data from several interventions that are associated with a wider range of end-diastolic pressures (pED = 1–27 mm Hg). Figure 4 shows the values of T plotted against pED for all these interventions; it shows that T increases significantly (P < 0.001) with pED. This result is very similar to the one we found (cf. Fig. 2). Likewise, Gaasch et al. (1980) found that volume infusion that changed pED from a mean of 2.7 ± 1.3 (sd) to 10.3 ± 1.9 mm Hg resulted in an increase in T from 31 ± 5.1 to 40 ± 8.9 msec (P < 0.001). Because they did not use multivariate analysis, neither Karliner et al. (1977) nor Gaasch et al. (1980) could isolate increases in end-diastolic pressure from coincident increases in systolic pressure and ventricular dimension.

Based on these results, we included end-diastolic pressure, mean aortic systolic pressure, heart rate, and ventricular dimensions as potential determinants of T in our analysis. Our results indicate that the hemodynamic load, quantified with end-diastolic pressure and aortic systolic pressure, was a significant determinant of T, but fractional shortening and systolic dimensions were not.

During volume loading all these variables, as well as T, tend to change simultaneously. This fact clouds the interpretation of simple bivariate relations between the various hemodynamic variables and T. For example, Figure 3 shows that T increases as pED increases and that T increases as pAO increases. During volume loading, both pED and pAO increase, so how can one be confident that the relationship between T and pED is not an artifact due to the fact that pED and pAO increase together and that T increases with pAO? To answer this question we did a stepwise multiple linear regression analysis in which all the hemodynamic variables were permitted to enter the regression equation. The criterion for entry of (and for keeping previously entered) variables in the equation is that they provide a statistically significant (P < 0.05) reduction in the residual variance in T given the other variables that are already in the equation. If the apparent bivariate relationship between T and one hemodynamic variable (say pED) is due to the colinearity of that hemodynamic variable with one or more others (say pAO), then only the better predictor of T (in this case, pAO) will appear in the final regression equation. The fact that a variable is in the final regression equation means that it has a statistically significant independent effect on T, even in light of the other variables in the equation.

In addition to problems of interpretation due to possible colinearity between the potential independent variables, one need also be cautious when concluding that the statistically significant variables in a regression equation, or, for that matter, any statistical result, is due to a physiological meaningful cause-and-effect relationship (Kleinbaum and Kupper, 1978). There are two reasons why our statistical analysis supports our physiological conclusions. First, as discussed above, earlier studies using more conventional methods of analysis suggested that hemodynamic and geometric variables might affect isovolumic relaxation rate, described with T. Second, in each beat we analyzed, the potential independent variables (e.g., pED, dp/dtmax, E, etc.) took on their values before the start of isovolumic relaxation.

There are several reasons why our results differ from those of previous studies. Weiss et al. (1976) studied isolated isovolumic left ventricles with aortic and mitral values occluded by steel discs. The normal valve movement during isovolumic relaxation may affect the response to aortic pressure. In addition, the studies of both Weiss et al. (1976) and Frederiksen et al. (1978) of the ejecting left ventricles with the right heart bypassed yielded baseline values for T that were high, in the range of 35–40 msec. By contrast, we observed baseline values, in the range of 15–25 msec; Karliner et al. (1977) and Gaasch et al. (1980) found similar values. It may be that the surgery needed to create the isolated left ventricle or right heart bypass preparation depressed the heart to the point at which relaxation could not respond further to pressure changes.

Lack of dependence of T on systolic shortening and systolic dimensions contrasts with the findings of Weiss et al. (1976) and Gaasch et al. (1980). This difference probably is the result of our ability to
account for possible covariance between ventricular size and shortening with other variables such as end-diastolic pressure and $dp/dt_{max}$ in our analysis.

However, this difference also may be due to differences in the anesthetic agents used. Vatner et al. (1978) observed that the response to volume loading in conscious dogs is different from dogs anesthetized with pentobarbital. The increase in cardiac output observed in conscious dogs was due to an increase in heart rate (from $62 \pm 2$ (SD) to $155 \pm 6$ beats/min), while stroke volume did not change. In contrast, dogs anesthetized with pentobarbital began with a high heart rate ($163 \pm 14$ beats/min) and responded by increasing stroke volume ($13 \pm 2$ to $43 \pm 9$ ml). Gaasch et al. (1980) anesthetized their dogs with sodium pentobarbital, and so stroke volume may have increased substantially. In our studies, a combination of morphine and chloralose yielded a response to volume similar to that of conscious dogs. With the closed chest, volume loading did not consistently increase stroke volume (mean stroke volume was $31$ ml base-line and $28$ ml at maximum volume loading; $SSD$ (standard deviation of difference) = $11.7$ ml; $P > 0.20$ by paired $t$-test). In contrast, heart rate rose dramatically (61 beats/min to 131 beats/min, $SSD = 24$ beats/min, $P < 0.001$). Results with the chest and pericardium open were the same. Therefore, changes in ventricular size and shortening probably were less prominent in our experiments than in those employing barbiturate anesthesia.

The underlying mechanism for the change in relaxation rate with volume loading may be related to the findings of Brutsaert and co-workers (Brutsaert et al., 1978a, 1978b; LeCarpentier et al. 1979). They have shown a species-specific effect of changing afterload on the onset of relaxation in isolated cardiac muscle. As afterload increases, the onset of isometric relaxation occurs later in the contraction in cat papillary muscle, whereas it was unaffected in frog muscle. When the sarcoplasmic reticulum was stripped away with a detergent or inactivated with caffeine, the behavior of the cat muscle resembled that of the frog; it was no longer sensitive to load. The authors hypothesized that $Ca^{2+}$ sequestration in the well-developed cat sarcoplasmic reticulum is crucial to cat cardiac muscle’s load sensitivity. These results are not strictly analogous to our findings, since the authors describe a delay in the onset of relaxation, rather than a change in its rate. However, when the effect of such a delay on individual fibers is integrated (more precisely, convolved) over the entire ventricle, increases in the duration of contractions of individual fibers that were stimulated at different times could well be reflected as a lengthening of the time constant with which the pressure falls in the whole heart.

There have been many recent reports of the effects of experimental and clinical states on isovolumic relaxation, as measured by $T$ (Ludbrook et al., 1977; Ring et al., 1979; Lorell et al., 1979; Blau-stein et al., 1979; Sharma et al., 1979; Rousseau et al., 1979; Kay et al., 1979; Mann et al., 1979; Serizawa et al., 1980). In some of these studies, significant changes in loading conditions occurred which probably influenced $T$ in ways not anticipated by the authors, since $T$ is widely considered to be independent of load.

For example, Mann et al. (1979) studied 26 patients during angina induced by rapid atrial pacing. They found statistically significant increases in end-diastolic pressure ($17 \pm 5$ to $30 \pm 5$ (SD mm Hg), mean arterial pressure ($101 \pm 15$ to $113 \pm 15$ mm Hg), and $T$ ($43 \pm 7$ to $58 \pm 15$ msec). In their discussion, they suggest that ischemia caused the impairment in ventricular relaxation evidenced by the prolongation of $T$. However, we have found that increasing end-diastolic pressure by volume loading is associated with a similar increase in $T$. Figure 5 illustrates the relationship between $T$ and $p_{DE}$ from the data of Mann et al. (1979). This plot is similar to our Figures 2 and 4 [based on the data of Karliner et al. (1977)] that contain data collected in nonischemic preparations. Thus, the increase in $T$ observed during these experiments may be due to increases in end-diastolic pressure and mean arterial pressure during ischemia. Ischemia may directly slow relaxation rate, but to prove this, it is necessary to isolate its effects from the effects of the associated pressure changes.

In summary, the present study demonstrates that volume loading increases $T$, the time constant of
isovolumic relaxation, in the intact dog heart. Further analysis shows that this change may well be due to the fact that relaxation rate depends on both preload (end-diastolic pressure) and afterload (increased aortic systolic pressure), as well as changes in inotropic state and heart rate, with the heart relaxing more slowly as load increases. Our results caution against study of the relaxation process without an awareness of its sensitivity to pressure loading.

### Appendix

#### Tests of Exponential Pressure Fall

**Methods**

We first examined the plots of ln p vs. t during isovolumic relaxation and looked for evidence of systematic deviations from linearity. This method has the advantage of being direct but the disadvantage of sensitivity to baseline shift, as discussed in the text. Therefore, we derived the following additional measure of exponentiality based only on derivatives so that it will be totally insensitive to baseline shifts.

As noted in the text, if the pressure fall follows a single exponential,  
\[
\frac{dp}{dt} = -\frac{p_0}{T} e^{-t/T}
\]
even when there is a baseline shift \(p_0\). Differentiate this equation again with respect to \(t\):  
\[
\frac{d^2p}{dt^2} = -\frac{p_0}{T^2} e^{-t/T}.
\]

Use the first of these equations to eliminate \(e^{-t/T}\) from the second, in which case  
\[
\frac{d^2p}{dt^2} = -\frac{1}{T} \frac{dp}{dt},
\]
so  
\[
T = -\frac{dp}{dt}/\frac{dp}{dt}
\]

where the dots indicate differentiation with respect to time. In other words, the ratio of the first and second derivatives of pressure should be a constant, equal to \(T\), during isovolumic relaxation. This ratio is not affected by baseline pressure shifts due to intrapleural pressure changes or incorrectly zeroed catheters. We will denote the ratio of derivatives defined by the equation as \(T_R\). We obtained the second derivative of left ventricular pressure by applying the digital differentiator twice and then filtering the result once with the digital filter \(T_R(t)\) was also filtered once with the digital filter.

**Results**

We examined the linear regression of ln p vs. time for all 3413 beats. As reported by others (Weiss et al., 1976; Karliner et al., 1977), the correlation coefficient was extremely high (mean \(r = 0.99\); range = 0.96–1.00). These high values of the correlation coefficient have been cited as evidence that the isovolumic pressure fall is exponential. However, a closer examination of the deviations between the data and regression line revealed small, though consistent, deviation from linearity (Fig. 1). As can be seen, left ventricular pressure fell faster than an exponential would predict during the latter portion of isovolumic relaxation. The \(dp/dt\) vs. p plots showed an analogous pattern, and this way of looking at relaxation rate is not affected by baseline shifts. Thus, regardless of how one chooses to look at the data, the latter part of the isovolumic period is characterized by a faster rate of fall than the single exponential that appears to be such a precise characterization of isolated left ventricles and right heart-bypassed hearts.

Systematic errors in pressure measurement could make the ln p vs. t plot deviate from linearity. Positive errors would make the line curve up and negative errors would make it curve down. In every beat the line curved down. Thus, if this observation was due to errors in the pressure baseline, due to intrapleural pressure or catheter zeroing and balancing errors, the error would not always have had to have been one that always led to erroneously low readings. Since this pattern of response was seen in every beat, including those recorded with the chest and pericardium open, we think this is very unlikely.

Finally, we examined \(T_R\) to assess further how close to a true exponential the pressure fall was, since this ratio is independent of changes in baseline pressure. This ratio of derivatives should be constant, equal to \(T\) during isovolumic relaxation, if the pressure fall follows a single exponential. In every beat we examined, \(T_R\) is not constant but falling during isovolumic relaxation.

These three different approaches all demonstrate that isovolumic left ventricular pressure does not follow a single exponential but falls faster than one late in relaxation, in the intact heart. As a result, it probably is not possible to relate changes in isovolumic relaxation rate, as described by \(T\), to single exponential biochemical and subcellular processes. On the other hand, even though a single exponential does not describe the functional form of isovolumic relaxation accurately enough to use it to make direct statements about the underlying physiology, \(T\) does provide a reasonable empirical index of how fast pressure is falling during isovolumic relaxation. Therefore, it can provide the basic for making general statements about how fast the heart is relaxing.

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