Ventricular Contracture and Compliance Changes with Global Ischemia and Reperfusion, and Their Effect on Coronary Resistance in the Rat

C. S. Apstein, M. Mueller, and W. B. Hood, Jr.

SUMMARY We measured ventricular contracture and compliance (passive distensibility) of the isolated rat heart during and after 30 minutes of complete global ischemia. The passive filling or distensibility curves of intra-left ventricular pressure vs. volume were linear between 0 and 30 mm Hg during the control, ischemic, and reperfusion periods. During ischemia the effective stiffness (distensibility slope) of the ventricle increased from the preischemic control of 0.229 ± 0.027 to 0.514 ± 0.083 mm Hg/µl (P < 0.005) with reperfusion, stiffness markedly increased to 1.743 ± 0.442 mm Hg/µl (P < 0.001 vs. control). The changes in passive distensibility were parallel to changes in contracture which occurred when the ventricle was held in the isovolumic condition; during ischemia, contracture increased the preischemic diastolic pressure of -2 ± 1 to 17 ± 3 mm Hg (P < 0.001 vs. preischemic control). After 30 minutes of reperfusion contracture pressure decreased to 34 ± 7 mm Hg, (P < 0.001 vs. control). Contracture during reperfusion was associated with a parallel increase in the coronary vascular resistance; postischemic contracture may thus contribute to the no reflow phenomenon observed with attempted reperfusion of ischemic myocardium. The severity of contracture which occurred during ischemia and reperfusion was inversely related to the amount of ischemic tissue lactate accumulation and did not correlate with changes in tissue water content. Thus, global ischemia and reperfusion markedly decreased passive ventricular compliance; in the intact animal ventricular filling could be impaired by this process and diastolic pressure measurements would reflect the change in compliance as well as in ventricular contractile function.

THE DIASTOLIC properties of the myocardium are a major determinant of ventricular filling and overall hemodynamic function. A decrease in ventricular distensibility also has the effect of increasing the diastolic pressure at a

From the Cardiology Section of the Thorndike Memorial Laboratory, Department of Medicine, Boston City Hospital and Boston University School of Medicine, Boston, Massachusetts.

Supported in part by Grants-in-Aid Award Number 1222 from the American Heart Association, Western Massachusetts Chapter and the


Dr. Apstein was a Research Fellow of the Medical Foundation, Inc., Boston, Massachusetts, while this work was performed.

Part of this work was presented at the 25th Annual Scientific Session of the American College of Cardiology, New Orleans, Louisiana, February 1976.

Address for reprints: Carl S. Apstein, M.D., Boston University School of Medicine, 80 E. Concord Street, Boston, Massachusetts 02118.

Received September 3, 1976; accepted for publication January 6, 1977.
given diastolic volume; an increase in ventricular diastolic pressure, therefore, may result from a decrease in diastolic ventricular compliance without implying ventricular failure in the sense of incomplete ventricular systolic emptying. Since diastolic ventricular pressures commonly increase in the clinical setting of ischemia, during both angina and myocardial infarction, the underlying mechanism of the increase in diastolic pressure is of considerable clinical importance.

A change in diastolic ventricular distensibility may be recorded as contracture when the muscle is maintained under isometric or isovolumic conditions; such an increase in diastolic contracture tension or pressure represents one point on the myocardial passive length-tension or volume-pressure curve because length or volume is held constant. During hypoxia, isolated rat heart muscle has been shown to develop contracture as manifested by an increase in resting tension and a parallel decrease in the series elastic compliance.2-4 Investigations of the time-course of the changes in diastolic compliance during ischemia have not been in complete agreement.4,5 The occurrence of contracture during ischemia and reperfusion has received relatively little study.

In the current investigation, we have used the isolated rat heart to study changes in contracture and compliance with severe global ischemia and reperfusion. When contracture occurred, passive pressure-volume curves of the ventricle were measured so that a comparison of these two indices of diastolic distensibility could be made. The coronary vascular resistance was monitored and correlated with the severity of contracture to determine whether contracture could compress the myocardial capillary bed and increase the resistance to coronary flow.

Preliminary reports of portions of this study have been reported in abstract form.10-12

**Methods**

The experimental protocol consisted of 30 minutes of complete global ventricular ischemia, followed by a period of 30 minutes of reperfusion. Contracture pressure and coronary vascular resistance were monitored continuously; at selected times the protocol was interrupted, passive ventricular filling curves were determined, and the dry-weight ratio of the myocardium was measured.

An isolated isovolumic working heart preparation was used for these studies.13 In this preparation, a small cannulated fluid-filled balloon is placed in the left ventricle of the isolated heart and attached to a pressure transducer to monitor intraventricular pressure (Fig. 1). Since the balloon is noncompressible, contraction is isovolumic. Because intraventricular volume is held constant, "preload," or diastolic fiber length, does not change; developed pressure and its first derivative (dP/dt) therefore reflect the contractile state of the myocardium.

**SURGICAL AND PERFUSION TECHNIQUE**

Albino Sprague-Dawley male rats weighing between 200-300 g were decapitated and the thorax rapidly opened. The aorta was dissected free, an incision made at the level of the right innominate artery, and a cannula was tied into the root of the aorta. Retrograde coronary perfusion was immediately started from a perfusate reservoir at a level above the heart equivalent to a pressure of 75 mm Hg. In this way, coronary perfusion was maintained while the heart was being removed from the animal and only a few seconds elapsed between the time of decapitation and the onset of coronary perfusion.

The perfusate consisted of modified Krebs-Henseleit buffer: 118 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 0.4 mM Na2EDTA, 5.5 mM glucose, and 1.0 mM lactate. Lactic acid was neutralized with NaOH before being added to the buffer. The lactate was added to the perfusate so that aerobic myocardial lactate extraction could be measured.

While the coronary bed was being perfused from the fixed pressure reservoir, the heart was dissected from the thorax. The left ventricle was immediately decompressed by an apical puncture so that a minimum amount of left ventricular contractile work was done during the dissection process. A drain was placed in the apex of the left ventricle so that it remained free of intra-left ventricular fluid from Thesbian drainage. The left atrium was removed and a collapsed latex balloon (manufacturered in our laboratory) was inserted into the left ventricular chamber.

The heart was then removed from the thorax and placed in a water-jacketed, constant temperature chamber which was kept at 37°C with a circulating pump. (The intraventricular temperature was monitored when control coronary flow rates were present and during complete ischemia and it was constant at 37 ± 1°C in this apparatus). Coronary perfusion was then switched from the fixed pressure reservoir to a constant flow pump (Harvard Apparatus...
Model No. 1203 or Technicon Instruments Proportioning Pump Model No. 1). A pacemaker wire was inserted into the right ventricle via a right atrial incision. A polyethylene cannula was inserted into the pulmonary artery or body of the right ventricle to collect the right ventricular ejectate which consisted of coronary sinus drainage, since there was no other flow through the right side of the heart. Some of the efflux drained via the cut vena cavae; this was collected and pooled with the pulmonary artery efflux for measurement of lactate concentration. Only samples drawn from the cannulated pulmonary artery or body of the right ventricle were used for the measurement of coronary venous Po2. The perfusate efflux from the heart was collected completely after one passage through the heart and was not recirculated.

MEASUREMENT OF MECHANICAL FUNCTION

Ventricular pressures were recorded via a 30-cm length of polyethylene tubing with an internal diameter of 0.045 inch and outside diameter of 0.062 inch (Intramedic Polyethylene Tube PE 160, Clay Adams, Inc., New York, N.Y.).

The frequency response of the pressure recording system was determined by the method of Fry14 with the balloon attached to the catheter and filled with a volume in the range used during the experiments. The wave-form produced by a sudden distortion and release of the balloon resulted in a 16% overshoot with after-vibrations of 47 Hz. The damping ratio was 0.54, and calculated natural resonant frequency was 75 Hz. Thus, the system was critically damped and the amplitude of the recorded pressure should accurately reflect the amplitude of the true pressure at frequencies between 0 and the natural resonant frequency of 75 Hz. All hearts were paced at 300/min (5 Hz) and it is possible that a minor frequency component of the pressure curve may have been greater than 75 Hz; in this case our recording system would tend to underestimate it. This would be reflected in an underestimated ventricular maximum dP/dt measurement because the most rapid rise of the pressure curve would reflect the highest frequency components. However, even though the absolute maximal ventricular dP/dt may be slightly underestimated by this recording system, since the same technique was used for all hearts, comparisons before and after ischemia in the same heart, and between groups of hearts, should be valid. Furthermore, because the pacing rate was maintained constant at 5 Hz throughout all experiments, the major frequency components of the ventricular pressure trace should not have changed relative to the natural resonance of the recording system.

The polyethylene tubing from the ventricular balloon was attached to a Statham P23Db pressure transducer. A photographic recorder with a high frequency response was used (Electronics for Medicine Model DR8 or Hewlett Packard Model 4560). Left ventricular dP/dt was obtained from the differentiator output circuit of the Electronics for Medicine SGM Strain Gauge Meter/Amplifier or with an RC differentiator circuit made in our laboratory for use with the Hewlett Packard pressure carrier amplifier Model No. 760-3000.

The collapsed intraventricular balloon was slowly filled with fluid from a precision micro-pump (Harvard Apparatus Model No. 1100) while left ventricular pressures were recorded. The volume of the balloon was adjusted to give a peak left ventricular systolic pressure of 60–80 mm Hg, with a diastolic pressure less than 12 mm Hg. Hearts which could not achieve this level of performance were discarded (approximately 10% of the preparations). Left ventricular pressure and dP/dt were monitored continuously throughout each experiment.

The relationship between balloon size and left ventricular size is critical in this perfusion technique. The balloon must be slightly more capacious than the ventricle, or else, as the balloon is filled, a rise in intraballoon pressure will be recorded due to increasing balloon wall tension rather than to ventricular wall tension. A series of balloons of slightly different size was manufactured so that in each experiment the volume of the ventricular cavity was always slightly less than the balloon capacity. The capacity of each balloon was measured by recording the pressure volume filling curve of the isolated balloon; the experiments were always performed on the flat portion of the balloon's pressure volume curve.

EXPERIMENTAL PROTOCOL

A control coronary flow rate of 8 ml/min (or approximately 16 ml/min per g of left ventricle) was used. This high rate of coronary flow is necessary to deliver adequate oxygen since the perfusate is hemoglobin free. Comparable coronary flow rates have been used with similar buffer-perfused experimental preparations. In order to test the stability of the preparation, six consecutive hearts were perfused for 120 minutes under well oxygenated conditions (95% O2-5% CO2 gassing, perfusate Po2 = 542 ± 6 mm Hg) at a constant coronary flow rate of 8 ml/min. Performance was stable under these conditions (see Results).

For the experimental protocol, the isolated heart was perfused with well oxygenated buffer at 8 ml/min for an initial 30 minutes, the time required for mechanical performance to become stable. Intraventricular balloon volume was adjusted to maintain a systolic pressure of 60–80 mm Hg during the stabilization period and was then held constant for the duration of the experiment. After 30 minutes of control conditions, i.e., well oxygenated perfusion at 8 ml/min, complete global ischemia was produced by turning a stopcock which shunted all aortic cannula flow back to the perfusate reservoir and eliminated all coronary perfusion. After 30 minutes of ischemia, the stopcock was opened and reperfusion occurred at the control coronary flow rate of 8 ml/min. Intraventricular and aortic pressures were monitored during the experimental protocol. Since retrograde aortic flow (coronary flow) was held constant at 8 ml/min during the control and reperfusion periods, the mean aortic pressure (coronary perfusion pressure) was a direct measure of coronary vascular resistance.

At selected times the protocol was interrupted and passive ventricular pressure volume curves were determined, after which the dry-wet weight ratio of the left ventricle was measured. When these measurements were performed the rest of the protocol could not be completed.
and therefore different groups of hearts were compared for the passive pressure volume curves and dry-wet weight ratios which were obtained (1) after 30 minutes of control, well oxygenated perfusion, (2) after 90 minutes of control, well oxygenated perfusion, (3) after 30 minutes of complete global ischemia, (4) after 2 minutes of reperfusion, and (5) after 30 minutes of reperfusion. An additional group of hearts was used for sequential determinations of the passive pressure volume curves at 5, 10, and 20 minutes of ischemia.

The passive ventricular pressure volume curves were determined by clamping the mitral and aortic orifices and infusing volume into the ventricle at a known rate while simultaneously recording intraventricular pressure via a "Y" connector attached to the infusion cannula. In order to make this measurement during the control, well oxygenated period or the reperfusion period it was necessary to arrest the beating ventricle. Arrest was accomplished by instituting complete global ischemia which resulted in asystole within 2 minutes; at this time the passive pressure volume curve was determined; contracture did not occur during this relatively short period of ischemic arrest. The group which had been ischemic for 30 minutes was asystolic and the pressure volume curves were determined without any additional intervention.

A regulated infusion into the arrested ventricle was accomplished via an apical cannula attached to an airtight syringe (Hamilton Gastight Model No. 1725, Hamilton Co., Inc., Whittier, Calif.), which was driven by a Gilford infusion pump (Model No. 105-S) or Harvard infusion pump (Model No. 1100) so that the rate of volume delivery was constant. In order to avoid differences in measured distensibility resulting from stress-relaxation, all hearts were infused at the same range of rates of 6-8 μl/sec. At this relatively slow rate of flow the apical cannula bore was large enough so that there was no measurable resistance or pressure buildup in the infusing syringe. The intraventricular pressure was continuously recorded and the infusion was stopped when the intraventricular pressure reached 30 mm Hg in order to avoid overstretching the ventricle. The ventricle was then completely emptied and a duplicate filling curve was determined. This technique yielded excellent reproducibility despite the small volumes employed; the duplicate filling curves were usually superimposable or varied at most by 1 mm Hg at a given intraventricular volume.

In order to measure left ventricular water content, the heart was removed from the perfusion apparatus, the free wall of the right ventricle, the atria and the great vessels were removed, and the left ventricle (including the septum) was opened on its long axis, lightly blotted, and the wet weight determined. The ventricle was allowed to dry to constant weight at room temperature and the dry-wet weight ratio was determined.

**MEASUREMENTS OF METABOLISM**

Arterial and venous perfusate samples were collected and analyzed for lactate concentration by a specific enzymatic method.19 Effluent samples could not be collected during the period of complete ischemia or when pressure volume curves were being determined. Lactate samples were collected every 15 or 30 seconds during the early reperfusion period from 10 hearts for which a pressure volume curve was not determined until 30 minutes of reperfusion. Lactate washout was complete within 3 minutes of reperfusion; the area under the lactate washout curve was graphically integrated to calculate the total lactate production which had occurred during the period of ischemia. All perfusate samples were immediately mixed with iced 5% trichloroacetic acid solution and kept under refrigeration until chemical analysis.

Oxygen tension of arterial and pulmonary artery (or right ventricular) samples was determined with an oxygen electrode (Instrumentation Laboratories, Inc., Model No. 125A). Metabolic data (oxygen consumption, lactate production) are expressed per unit of left ventricular weight. In this experimental preparation the right ventricle performs very little contractile work because it pumps only a portion of the coronary sinus drainage to the cut pulmonary artery. Furthermore, the free wall of the right ventricle contributed only 10 ± 2% of the total heart weight. Therefore, the metabolic processes were considered to be due to the working left ventricle and metabolic data were related to the weight of this chamber.

**PACING TECHNIQUE**

Pacing was accomplished via a right ventricular bipolar pacemaker wire attached to a Grass Model S4 stimulator. The region of the sinus node was removed from the preparation; a stimulator rate of 300/min was used in all experiments since it exceeded the frequency of the remaining endogenous pacemakers and thus provided a stable, constant heart rate. A 5-msec rectangular unipolar impulse was used. Threshold was determined by slowly increasing the stimulator voltage until a mechanical systole occurred. The stimulator voltage was adjusted to 0.5 V above the initial threshold. Under well oxygenated conditions capture was achieved with less than a 2.5 V impulse.

The pacing threshold was checked every 5 minutes during the experimental protocol. The threshold was stable during the aerobic control perfusion periods but with ischemia the threshold progressively increased. In order to avoid variations in mechanical work during the period of ischemia as a result of the change in pacing threshold, the stimulator voltage was progressively increased and maintained at 20% above threshold up to a value of 25 V. Preliminary experiments demonstrated that the well oxygenated rat heart could be paced with a 25-V stimulus for 2 hours without any deterioration of mechanical performance; this indicates that the pacing impulse per se did not affect our experimental parameters.

**DATA ANALYSIS**

Statistical analysis of the data was performed by the Student's t-test. All data are reported as the mean value ± SEM.

**Results**

**STABILITY OF THE WELL OXYGENATED PREPARATION**

The performance of six consecutive hearts perfused under well oxygenated conditions is shown in Table 1. Left
ventricular pressure development and dP/dt were constant over the 90-minute perfusion period. A slight decline in ventricular diastolic pressure was observed. Mean aortic pressure (coronary perfusion pressure) was held constant, indicating that the coronary vascular resistance was constant.

Myocardial oxygen consumption was measured every 10 minutes and was constant over the 90-minute period with a mean value of 150.6 ± 10.0 μl O2/g wet weight per min. The mean arterial perfusate Po2 was 542 ± 6 mm Hg and the mean effluent Po2 was 153 ± 9 mm Hg, indicating that the heart extracted only 72% of the available oxygen and suggesting that tissue oxygenation was adequate. When oxygen demand is increased with catecholamines in this preparation, the isolated heart can extract more oxygen than occurred in this control series (unpublished observation).

The pattern of lactate metabolism also indicated adequate tissue oxygenation. Despite the relatively high coronary flow rates, the hearts extracted an average of 5 ± 2% of the 1.0 ml perfusate lactate content in one passage through the myocardium (i.e., without recirculation of the perfusate) for a mean rate of lactate utilization of 0.60 ± 0.26 μmol/g per min.

**EFFECT OF ISCHEMIA (TYPICAL EXPERIMENT)**

The left ventricular pressure tracing from a typical experiment is shown in Figure 2. Control developed pressure was 75 mm Hg. Ischemia was induced by reducing coronary flow to zero, and a rapid decrease in developed pressure occurred. Within 10 minutes diastolic pressure began to progressively increase, and by the end of 25 minutes it was 18 mm Hg. With balloon volume was kept constant, and there is no filling of the left ventricle in this preparation, the increase in diastolic pressure represents contracture of the left ventricle on the balloon. With reflow after 30 minutes of ischemia, there was a marked increase in diastolic pressure which was characteristically seen with reflow after severe ischemia. In this experiment, at the end of the 30-minute reperfusion period, developed pressure had returned to 20% of its control value.

In the early reperfusion period we consistently observed an increase in the mean aortic (coronary perfusion) pressure which was parallel to the increase in contracture pressure. Since retrograde aortic and coronary flow was held constant, the increase in mean coronary perfusion pressure indicated an increase in coronary resistance. The intraventricular and aortic pressure tracings during the first moments of reperfusion in a typical experiment are shown in Figure 3. In the control period with a retrograde aortic and coronary flow rate of 8 ml/min, the left ventricular diastolic pressure was 8 mm Hg and mean coronary perfusion pressure was 116 mm Hg. Complete ischemia was induced for 30 minutes. In the immediate postischemic period, with reinitiation of the control coronary flow rate, the intraventricular diastolic pressure increased to 68 mm Hg indicating intense ventricular contracture; there was a parallel increase in the mean coronary perfusion pressure to 170 mm Hg, indicating a marked increase in coronary vascular resistance.

**GROUP RESULTS**

Figure 4 shows the time-course of changes in developed pressure, contracture pressure and mean coronary perfusion for the group of 13 hearts subjected to 30 minutes of complete global ischemia and 30 minutes of reperfusion. After ischemia occurred, developed pressure rapidly fell and after 10 minutes of ischemia 11 of the 13 hearts were asystolic, but 2 continued to have minimal systolic oscillations less than 1 mm Hg in amplitude. After 30 minutes of ischemia no hearts showed any contractile activity. Diastolic contracture pressure progressively increased during ischemia; maximum contracture occurred approximately midway in the ischemic period at 18 ± 1 min and was 35 ± 3 mm Hg.

After 6-8 seconds of reperfusion, contracture pressure increased to 75 ± 6 mm Hg. With the subsequent 30 seconds of reperfusion the contracture pressure decreased to 24 ± 6 mm Hg and then increased for the next 5 minutes of reperfusion, after which a moderate decrease occurred. At the end of the 30-minute reperfusion period the hearts remained in moderate contracture with a diastolic pressure of 34 ± 7 mm Hg.

The relationship between the mean coronary perfusion pressure and the diastolic contracture pressure is also shown in Figure 4. In this preparation the mean coronary perfusion pressure is a measure of total coronary vascular resistance since retrograde aortic (coronary) flow was held constant. The ischemic control mean coronary perfusion pressure was 64 ± 4 mm Hg. In the immediate reperfusion period the mean coronary perfusion pressure increased to 115 ± 4 mm Hg simultaneously with the development of intense ventricular contracture, indicating

---

**Table 1**

<table>
<thead>
<tr>
<th>Perfusion time (min)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular developed pressure (mm Hg)</td>
<td>68 ± 3</td>
<td>74 ± 5</td>
<td>74 ± 5</td>
<td>74 ± 6</td>
<td>71 ± 3</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Left ventricular dP/dt (mm Hg/sec)</td>
<td>1818 ± 106</td>
<td>2169 ± 109</td>
<td>2032 ± 137</td>
<td>2038 ± 151</td>
<td>2006 ± 111</td>
<td>2007 ± 100</td>
</tr>
<tr>
<td>Left ventricular diastolic pressure (mm Hg)</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>0 ± 2</td>
<td>-2 ± 2</td>
<td>-1 ± 2</td>
<td>-2 ± 2</td>
</tr>
<tr>
<td>Mean aortic (coronary perfusion) pressure (mm Hg)</td>
<td>74 ± 13</td>
<td>75 ± 13</td>
<td>78 ± 16</td>
<td>78 ± 16</td>
<td>75 ± 17</td>
<td>76 ± 17</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
CONTRACTURE AND COMPLIANCE WITH GLOBAL ISCHEMIA/Apstein et al.

?§, ioo

ISCHEMIA

CONTROL

REFLOW

20 SECS 45 SECS 10 MIN 25 MIN

15 SECS 15 MIN 30 MIN

FIGURE 2 Left ventricular pressure with ischemia and reflow. Segments of the left ventricular pressure tracing from a typical experiment are shown. The heart was paced at 300/min. During the 30-minute ischemic period coronary flow was reduced to zero. With reflow, coronary flow was returned to the 8 ml/min control level. Ischemia caused a rapid fall in developed pressure. After 10 minutes contractions had ceased and mild diastolic contracture or rigor had developed (as reflected by the sustained increased in intraventricular pressure), which increased with subsequent ischemia. Reflow caused a marked increase in contracture and return of contractile activity at a reduced level.

a doubling of the total coronary vascular resistance. In the subsequent reperfusion period the coronary resistance followed a time-course parallel to the diastolic contracture pressure and remained significantly increased after 30 minutes of reperfusion. During the reperfusion period, 99 separate measurements of simultaneous mean coronary perfusion pressure and diastolic contracture pressure were made at various times in the 13 hearts. The increase in contracture pressure above the control value correlated with the increase in coronary resistance, as assessed by the increase in mean coronary perfusion pressure above control ($r = 0.3559; P < 0.001 for 97 df$).

The increase in coronary resistance during the development of contracture might have depended on the isovolumic condition imposed by the presence of the intraventricular balloon. This possibility was evaluated by collapsing the intraventricular balloon during the early reperfusion period (Fig. 5). During the intense contracture of the early reperfusion period, the intraventricular balloon was collapsed; there was no significant effect on the mean coronary perfusion pressure, indicating that the observed increase in coronary resistance which occurred with contracture does not require maintenance of the isovolumic state.

RECOVERY OF CONTRACTILITY WITH REPERFUSION

In this series of hearts contractile function, as measured by developed ventricular pressure, recovered to 19 ± 7 mm Hg or 27 ± 9% of the preischemic control value, at the end of the 30-minute reperfusion period. No increase in contractility occurred in the final 10 minutes of the reperfusion period (Fig. 4) suggesting that further recovery of contractile function with a longer period of reperfusion would be unlikely.

TIME-COURSE OF PASSIVE VENTRICULAR DISTENSIBILITY

Since the ventricle was held in the isovolumic state in these experiments, the contracture pressure represents one point on the passive pressure-volume filling curve. In order to measure passive ventricular compliance over a range of intraventricular volumes, passive filling curves were determined at selected times, as described above. The results are presented in Table 2.

The curves were analyzed over the physiological diastolic pressure range of 0-30 mm Hg. In this range of filling pressure, the pressure-volume curves were all linear. Linear regression analysis was performed for each filling curve; pressure measurements were read from the

![Figure 3](http://circres.ahajournals.org/)

FIGURE 3 Postischemic contracture and coronary resistance. The typical response of the mean aortic (coronary perfusion) pressure and intra-left ventricular pressure in the immediate reflow period is shown. The increase in aortic (coronary perfusion) pressure indicates an increase in coronary resistance which is parallel to the intraventricular contracture pressure.)
Continuous pressure tracing at each 5–10 μl increments of ventricular volume, so that an average of 8 points on each filling curve could be used for a least squares fit to the equation \( y = a + bx \) where \( y \) was the intraventricular pressure in mm Hg and \( x \) was the intraventricular volume in μl. The correlation coefficients in Table 2 demonstrate the linear nature of the passive pressure-volume relationship under control, ischemic, and reperfusion conditions in the range studied.

The slope of the linear filling curves, in mm Hg/μl of intraventricular volume, is a measure of the effective stiffness of the ventricle. At the end of the 30-minute preischemic control period the distensibility slope was 0.240 ± 0.036 mm Hg/μl. After a subsequent 60 minutes of oxygenated perfusion the ventricle became slightly more compliant as demonstrated by a distensibility slope of 0.198 ± 0.015 mm Hg/μl; however, this difference was not statistically significant (\( t = 0.682, P = ns \)). The distensibility slopes of these hearts, perfused for 30 and 90 minutes under oxygenated conditions were, therefore, considered to be a single control group with a mean value of 0.229 ± 0.027 mm Hg/μl, against which differences in distensibility at other experimental times between 30 and 90 minutes could be assessed for statistical significance.

After the onset of complete global ischemia, the ventricle became progressively stiffer as demonstrated by an increase in the distensibility slope (Table 2); after 5 minutes of ischemia the increase in distensibility slope was statistically significant at \( P < 0.05 \). During the period of ischemia, the ventricle was stiffest after 20 minutes of ischemia had elapsed (distensibility slope = 0.625 ± 0.177 mm Hg/μl). At the end of 30 minutes of ischemia, the distensibility slope decreased slightly to 0.514 ± 0.083 mm Hg/μl (\( P = ns \) vs. the 20-minute value).

After 2 minutes of reperfusion, the earliest time during reperfusion that distensibility could be measured by our technique, the ventricle was severely noncompliant. With subsequent reperfusion, for a total of 30 minutes, there was a slight increase in effective stiffness as indicated by an increase in the distensibility slope from 1.390 ± 0.119 to 1.743 ± 0.442 mm Hg/μl (\( P = ns \) for 2-minute reflow vs. 30-minute reflow).

In 36 hearts, at various time points in the experimental protocol, passive filling curves were compared to the measured intraventricular contracture pressure. There was a strong correlation between the distensibility slope and the increase in contracture pressure above the control level (\( r = 0.678, P < 0.001 \) for 34 df). The mean values for the groups measured at selected points in the protocol are shown in Figure 6; the mean values for the distensibility slopes and increase in contracture pressure are also highly correlated (\( r = 0.926, P < 0.05 \)).

**MYOCARDIAL EDEMA**

Since no colloid was added to the perfusate, tissue edema was expected to occur. Dry-wet weight ratios were obtained to evaluate the extent and time-course of the edema. The dry-wet weight ratio of the myocardium immediately after opening the animals' thorax prior to any perfusion with Krebs-Henseleit buffer was 0.247 ± 0.004 (\( n = 8 \)). After 30 minutes of oxygenated perfusion the dry-wet weight ratio was 0.214 ± 0.007 (\( n = 9 \)), a significant decrease (\( P < 0.01 \)). After 2 hours of oxygenated perfusion the dry-wet weight ratio was 0.211 ± 0.006 (\( n = 12 \)). Thus, there was an initial increase in myocardial water content soon after the start of perfusion with the noncolloidal buffer, but under oxygenated conditions the degree of tissue edema was constant over the subsequent 2-hour experimental period. The dry-wet weight ratio after 30 minutes of ischemia was 0.209 ± 0.005 (\( n = 6 \)), after 2 minutes of reperfusion it was 0.215 ± 0.004 (\( n = 6 \)) and after 30 minutes of reperfusion it was 0.202 ±
CONTRACTURE AND COMPLIANCE WITH GLOBAL ISCHEMIA/Apstein et al.

The control coronary flow rate of 8 ml/min resulted in an aortic (coronary perfusion) pressure of 50 mm Hg. The lower panel shows the characteristic development of contracture and increased coronary resistance during the early reperfusion period; with reperfusion at the 8 ml/min coronary flow rate, the aortic (coronary perfusion) pressure increased to 100 mm Hg after 15 seconds of reperfusion. After 20 seconds of reperfusion, the coronary resistance was significantly higher than control as manifested by the aortic (coronary perfusion) pressure of 80 mm Hg. The left ventricular balloon was collapsed as indicated; no change in aortic (coronary perfusion) pressure occurred, indicating that the increase in coronary resistance in the reperfusion period was independent of the intra-left ventricular balloon.

0.006 (n = 6). None of the values during ischemia or reperfusion was significantly different from values for the hearts which were perfused under oxygenated conditions for a similar period of time. Thus, there were no significant differences in tissue water content from the well oxygenated control group at the time that marked changes in distensibility, contracture, and coronary resistance occurred in the experimental groups.

LACTATE METABOLISM

During the control, oxygenated preischemic period the hearts extracted an average of 5% of the arterial perfusate lactate. In the early reperfusion period the lactate which had accumulated in the myocardium was rapidly washed out; after 3 minutes of reflow, the venous effluent concentration had fallen below the arterial level indicating myocardial lactate extraction and aerobic metabolism. The total mount of tissue lactate which accumulated during the period of ischemia was calculated by graphically integrating the area under the lactate washout curve of the arteriovenous concentration difference vs. time in 10 hearts where this measurement was made. The average total lactate accumulation during the 30-minute period of ischemia was 69.0 ± 6.8 μmol/g wet weight, with a range of 24.9–85.0 μmol/g due to spontaneous variation among the individual preparations.

The amount of ischemic lactate accumulation was inversely related to the severity of contracture which occurred during ischemia and reperfusion. Figure 7 shows the inverse correlation between the amount of tissue lactate accumulation and the severity of contracture after 30 minutes of ischemia and after 30 minutes of reflow. A similar least squares linear regression analysis for the maximum increase in contracture in the early reflow period vs. ischemic lactate accumulation also showed a high degree of correlation (r = 0.7088, P < 0.05).

Discussion

These results demonstrate a progressive decrease in the diastolic compliance of the isolated rat left ventricle over a 30-minute period of complete global ischemia. When the heart was held in the isovolumic state the decreased dis-
tensibility was registered as an increase in intraventricular pressure or contracture. In the first seconds of reflow after ischemia, the severity of the isovolumic contracture was transiently but markedly intensified and apparently compressed the myocardial capillary bed so that the resistance of the coronary circulation increased in a manner similar to the increase in coronary resistance which occurs in normal systole.

In this experimental model, the intense phase of contracture with reperfusion was relatively short-lived. In our preparation, the increased coronary resistance was overcome by the coronary perfusion pump so that reflow was obligatory; in the in vivo condition of regional ischemia where coronary perfusion is dependent upon the aortic pressure head (which may be abnormally low), intense contracture of the ischemic region of the myocardium during attempted reperfusion could shunt flow away from that area and sustain the ischemic state. In our experiments, the contracture pressure, the distensibility slope, and the coronary resistance remained significantly increased above control throughout the 30-minute reperfusion period.

A no reflow state has been described when reperfusion of ischemic myocardium has been attempted and has been attributed to tissue edema and intramyocardial hemorrhage. In our experiments there was no increase in edema at the time that marked changes in contracture, distensibility, and coronary resistance occurred; our hearts had increased in water content by 25% during the preischemic control perfusion period and further edema formation with ischemia or reperfusion may have been precluded. Although edema may be an important mechanism which impairs reflow in vivo, our experiments demonstrate that myocardial contracture may also play a significant role in the no reflow state.

In addition to being held responsible for the no reflow phenomenon, myocardial edema has also been postulated to play an important pathogenetic role in the causation of ischemic tissue injury. In our experiments, the control

### Table 2 Passive Ventricular Distensibility

<table>
<thead>
<tr>
<th>Distensibility slope (mm Hg/μl)</th>
<th>Oxygenated control perfusion (min)</th>
<th>Ischemia (min)</th>
<th>Reflow (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 (n = 11)</td>
<td>90 (n = 4)</td>
<td>5 (n = 4)</td>
</tr>
<tr>
<td></td>
<td>10 (n = 5)</td>
<td>20 (n = 4)</td>
<td>30 (n = 13)</td>
</tr>
<tr>
<td></td>
<td>2 (n = 7)</td>
<td>30 (n = 6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.036*</td>
<td>±0.015*</td>
<td>±0.112†</td>
</tr>
<tr>
<td></td>
<td>±0.0024</td>
<td>±0.0023</td>
<td>±0.0025</td>
</tr>
<tr>
<td>Correlation coefficient for linear regression of pressure-volume curve</td>
<td>0.9899</td>
<td>0.9906</td>
<td>0.9917</td>
</tr>
<tr>
<td></td>
<td>0.9961</td>
<td>0.9985</td>
<td>0.9939</td>
</tr>
<tr>
<td></td>
<td>0.9985</td>
<td>0.9949</td>
<td>0.9839</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* P = ns. Oxygenated control values were pooled and used as a single control group to test significance of other groups.
† P = 0.05 vs. oxygenated controls.
‡ P < 0.005 vs. oxygenated controls.
§ P < 0.001 vs. oxygenated controls.

A no reflow state has been described when reperfusion of ischemic myocardium has been attempted and has been attributed to tissue edema and intramyocardial hemorrhage. In our experiments there was no increase in edema at the time that marked changes in contracture, distensibility, and coronary resistance occurred; our hearts had increased in water content by 25% during the preischemic control perfusion period and further edema formation with ischemia or reperfusion may have been precluded. Although edema may be an important mechanism which impairs reflow in vivo, our experiments demonstrate that myocardial contracture may also play a significant role in the no reflow state.

In addition to being held responsible for the no reflow phenomenon, myocardial edema has also been postulated to play an important pathogenetic role in the causation of ischemic tissue injury. In our experiments, the control

**Figure 6** Relationship between contracture pressure and distensibility slope. The mean values ± SEM are plotted for the distensibility slope vs. the increase in contracture pressure above the preischemic control value. The symbols refer to the measurements made in different groups at different times in the experimental protocol: (O) = after 30 minutes of oxygenated perfusion (preischemic control value), n = 11; (●) = after 90 minutes of oxygenated perfusion, n = 4; (△) = after 30 minutes of ischemia, n = 8; (△) = after 2 minutes of reperfusion, n = 7; (■) = after 30 minutes of reperfusion, n = 6. These points were subjected to a least squares fit linear regression analysis which yielded r = 0.926, P < 0.05 and the line shown in the figure.

**Figure 7** Lactate production and ischemic contracture. A significant spontaneous variation in the degree of contracture and the amount of tissue lactate accumulation occurred among the individual experiments. The amount of tissue lactate which accumulated during the 30-minute ischemic period in individual hearts is plotted against the severity of contracture (increase above control, preischemic diastolic pressure) which was present after 30 minutes of ischemia (O) and after 30 minutes of reperfusion (●) in each heart. A least squares linear regression analysis demonstrated a significant correlation at both times: after 30 minutes of ischemia, r = 0.7128, P < 0.05; after 30 minutes of reperfusion, r = 0.8564, P < 0.005.
well oxygenated preparation performed at a stable level of mechanical and metabolic function for 2 hours, despite a 25% increase in water content, a value similar to that obtained for the ischemia and reperfusion groups. Thus, tissue edema did not appear to be deleterious per se.

The degree of injury, induced by our protocol of 30 minutes of complete global ischemia at normothermia probably was relatively severe. Maintaining the pacemaker rate at 300/min and progressively increasing the pacing stimulus to overcome the increase in threshold maintained myocardial oxygen demand at a time of no oxygen availability. Thus, the imbalance between tissue oxygen demand and supply was probably greater than that which occurred in isolated rat papillary muscles which were stimulated at a rate of 6-12/min under hypothermic conditions; in these papillary muscle preparations a greater return of contractile function and resolution of hypoxic contracture generally occurred with reoxygenation than occurred in our protocol of ischemia and reperfusion.

The development of contracture during hypoxia or ischemia has recently been recognized in several species. Rat heart muscle appears to develop hypoxic contracture relatively easily. For example, cat papillary muscles did not develop contracture under conditions which have consistently produced contracture in rat heart muscle. No contracture or change in passive distensibility occurred in isolated canine hearts subjected to mild global ischemia (37% reduction in total coronary flow for 30-120 minutes) although left ventricular end-diastolic pressure increased within the normal range due to ventricular failure. However, reperfusion after ischemia induced by coronary artery occlusion in the canine heart has produced an immediate marked increase in effective stiffness in the reperfused segment of the ventricle. A marked increase in diastolic wall thickness occurred with postischemic reperfusion in the pig heart with only a 4% increase in tissue water content, suggesting that impaired relaxation or contracture had occurred. Complete global ischemia has recently been reported to produce contracture in the isovolumic (balloon in left ventricle) dog heart after 60 minutes of severe normothermic ischemia. Recent work in our own laboratory (unpublished observations) has shown that the isolated, isovolumic (balloon in left ventricle), normothermic rabbit heart develops contracture during severe ischemia; the contracture intensified with reperfusion and followed a time-course similar to our observations on the isolated rat heart reported herein. In man, severe cardiac contracture may be the basis of the "stone heart," a condition which occasionally occurs during open heart surgery.

Thus, the contracture response to ischemia or hypoxia appears to occur in several species other than the rat, but the occurrence of contracture in a given experimental protocol may depend on the severity and duration of the ischemic or hypoxic state. Rat heart muscle has a higher contractile velocity and rat heart myosin has a relatively high level of intrinsic ATPase activity. The rat heart, therefore, probably has a higher metabolic demand than cat heart muscle; a given degree of oxygen deprivation may thus cause more severe "relative" hypoxia and induce contracture in the rat while not causing it in the cat. Likewise, mild ischemia did not induce contracture in the dog, but severe ischemia did. The factors responsible for causing the "stone heart" in man are not understood, but this phenomenon occurs most commonly in markedly hypertrophied ventricles where the muscle mass may exceed coronary arterial supply capabilities; hence, ischemia may be the underlying basis for this condition also.

The measurement of passive ventricular distensibility correlated closely with the increase in the isovolumic contracture pressure at all times throughout the experimental protocol (Fig. 6); this relationship is not surprising, since the contracture pressure represents one point on the passive ventricular filling curve. The linear nature of the filling curves during the control, ischemic, and reperfusion periods differs from the exponential filling curves of larger ventricles, for reasons which are not readily apparent.

Although contracture and compliance may represent the same underlying phenomenon, they are measured in a different manner and have been studied independently. Ventricular compliance progressively decreased over the 30-minute period of ischemia in our experiments, and the heart became markedly stiffer with reperfusion. Previous studies of the occurrence and time course of changes in compliance during and after ischemia are not wholly consistent. Some workers report an early increase in the compliance of an infarcting region of the canine ventricle with stiffening not occurring until 6 hours after coronary occlusion. Others have reported that stiffening of the ischemic segment occurs within 5 minutes. In these settings of regional ischemia after coronary artery occlusion, the diastolic properties of the ischemic segment may be influenced by the systolic tension and stress resulting from the contraction of the surrounding nonischemic myocardium. As noted above, a recent report of global ventricular ischemia failed to demonstrate any change in the passive pressure-volume characteristics of the canine heart during or after ischemia of relatively mild severity; the failure to observe a decrease in compliance in these experiments probably was due to the relatively mild ischemia which was imposed. In man, a decrease in left ventricular compliance with transient ischemia has been reported.

Reoxygenation following brief periods of hypoxia and ischemia is associated with altered diastolic properties of the heart which take the form of transient impaired relaxation or tension prolongation. The state of sustained contracture and change in passive distensibility characteristics with reflow after complete ischemia for 30 minutes may be a more severe manifestation of the same basic process which transiently impairs relaxation after a less severe ischemic insult.

The underlying biochemical processes responsible for the mechanical changes which we measured (decrease in contractility and compliance, development of contracture) were not investigated in our study. However, extrapolation from the work of others may provide some insights. Our results show that contractile performance decreases within seconds after the onset of ischemia, well before the onset of contracture. Thus, these two mechanical events probably have a different underlying biochemical basis. The precise mechanism for the immediate fall in contrac-
tility with ischemia is still not completely understood. A popular current hypothesis is that the intracellular acidosis induced by ischemia interferes with the contractile process by blocking the Ca++ interaction with troponin, 44, 45 or other sites in the excitation-contraction coupling mechanism. 44 An ischemia-induced reduction in the slow inward Ca++ current could theoretically rapidly decrease contractility; 44 changes in the plateau phase of the myocardial action potential concomitant with a fall in contractility have been reported with ischemia and hypoxia. 47, 48

The biochemical events responsible for the slow development of contracture and the decrease in passive compliance during the period of ischemia are also uncertain. Our data indicate that the development of tissue edema cannot account for these mechanical changes. Contracture might be caused by an abnormal increase in intracellular calcium accumulation which activates the contractile process, or by marked intracellular ATP depletion which leads to rigor or a state of contracture. 29 The mechanism by which intracellular calcium accumulation causes contracture may in part be due to the associated decrease in intracellular ATP which occurs as the excess calcium activates myofibrillar ATPases. 44

The intense contracture which we observed with reperfusion had a relatively dynamic time-course (Fig. 4), suggesting that rapidly changing underlying biochemical events were responsible. The known pathophysiological events which occur with postischemic reflow are complex and we cannot with certainty relate them to our observed time-course of reperfusion contracture. Reflow after transient ischemia in the dog heart was associated with the occurrence of contraction bands, mitochondrial swelling, sarcolemmal blebs, accumulation of intracellular edema (without enlargement of the extracellular space) and a progressive increase in dense mitochondrial bodies believed to be rich in calcium. 45 Reoxygenation after anoxia in the isolated rat heart was associated with similar ultrastructural changes and a dramatic release of intracellular enzyme. 44 One might postulate that in the early reperfusion period the cell is flooded with calcium as a result of the ischemia-induced inability of the sarcolemma to extrude calcium; the excessive calcium could trigger the intense contracture we have observed and also induce ATP depletion as suggested by Fleckenstein et al. 44 With subsequent reperfusion, the partial reversal of contracture might be due to the resynthesis of ATP and/or increased extrusion of intracellular calcium with recovery of membrane function. Obviously, further basic studies are required to answer these questions and to relate the underlying biochemical processes to the mechanical events which we have demonstrated in this study.

Some insight into the contracture mechanism may be provided by recent experiments on rat papillary muscle and the relationship which we observed between contracture and tissue lactate accumulation (Fig. 7). Hypoxic contracture in the rat heart can be prevented by acidosis, 45 or by exposing the hypoxic tissue to an increased substrate level of glucose. 2 In the current experiments, the severity of contracture during both ischemia and reperfusion was inversely proportional to the spontaneous variation in the amount of ischemic lactate production and tissue accumulation among the preparations; this suggests that either or both factors, increased tissue acidosis or a presumed increase in glycolytic ATP production (as reflected by the greater amount of lactate accumulation), might have been responsible for partially preventing contracture in some hearts. The reason for the spontaneous variation in ischemic lactate production and accumulation was not studied, but it probably was due to a difference in cardiac glycogen stores, since the perfusate substrate was glucose (100 mg/100 ml) for all hearts for 30 minutes prior to the onset of ischemia.

Our results may have some clinical implications, though extrapolation from the globally ischemic isolated rat heart to the regionally ischemic human ventricle can be made only with caution. Nonetheless, our observations suggest that contracture, (in particular, the intense contracture observed with reperfusion) may tend to perpetuate the ischemic condition by directly increasing the coronary resistance as we have demonstrated.

The effect of nonglobal contracture or decreased compliance on overall pump function is more difficult to predict. The net result of a decrease in the compliance of ischemic or postischemic myocardial tissue may depend on the distribution of the ischemic tissue in the ventricle. In the context of localized, regional ischemia a decrease in the distensibility of the ischemic region of the ventricle could serve to reduce the overall ventricular inefficiency which results from aneurysmal bulging, 46 however, under circumstances in which the ischemic state prevailed diffusely throughout the ventricle, such as might occur with subendocardial ischemia, impairment of ventricular filling, a decreased cardiac output, and arterial hypotension and shock might be the result.

Finally, our data indicate that ischemia and reperfusion markedly shift the passive pressure-volume relationship in the direction of decreased ventricular compliance. Accordingly, if the same phenomenon occurs in man, diastolic pressure measurements in the patient with acute myocardial ischemia, or recovery therefrom, may reflect changes in compliance as well as in ventricular function.

Acknowledgments

We gratefully acknowledge the technical assistance of Lillian Hagopian and the secretarial help of Deborah Walker.

References


41. Kuhlhardt M, Kübler M: The influence of metabolic inhibitors upon the transmembrane slow inward current in the mammalian ventricular myocardium. Naunyn-Schmiedebergs Arch Pharmacol 290: 265-274, 1975


Ventricular contracture and compliance changes with global ischemia and reperfusion, and their effect on coronary resistance in the rat.

C S Apstein, M Mueller and W B Hood, Jr

_Circ Res._ 1977;41:206-217
doi: 10.1161/01.RES.41.2.206

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1977 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/41/2/206.citation