Experimental Subendocardial Ischemia in Dogs with Normal Coronary Arteries

By Gerald D. Buckberg, David E. Fixler, Joseph P. Archie, and Julien I. E. Hoffman

ABSTRACT

Subendocardial ischemia without anatomic coronary artery obstruction may result from a discrepancy between metabolic needs and available blood supply. We studied this in open-chest anesthetized dogs and measured pressures in aorta and left ventricle (LV), phasic left coronary arterial blood flow (CBF) by electromagnetic flowmeter, total CBF and LV subendocardial (endo) and subepicardial (epi) flow with radioactive microspheres 8–10μ in diameter. Since LV subendocardial flow is mainly or entirely diastolic, it should depend on coronary driving pressure and duration of diastole (i.e., the area between aortic and left ventricular diastolic pressures). This diastolic pressure time index (DPTI) was varied by opening arteriovenous fistulas to lower aortic diastolic pressure, constricting the ascending aorta to raise LV diastolic pressure and pacing to shorten diastole. Myocardial oxygen needs were estimated from the tension time index (TTI). Normal endo-epi flow ratios per gram (1:1) fell to 0.1:1 with these procedures and paralleled a fall in diastolic flow fraction (often nearly zero) and postischemic coronary reactive hyperemic responses. These changes occurred despite normal or raised mean CBF and 300–500% increase in systolic CBF. The altered flow ratios were best predicted by relating them to the ratio of DPTI (supply) to TTI (demand).

KEY WORDS regional coronary blood flow arteriovenous fistula subepicardial flow aortic constriction radioactive microspheres ventricular pacing reactive hyperemia tension time index phasic coronary blood flow diastolic pressure time index

- Patchy necrosis and fibrosis of left ventricular subendocardial muscle occur in patients whose coronary arteries are normal or narrowed by atheroma (1-5). These changes could be due to a discrepancy between myocardial oxygen demand and available blood supply in subendocardial muscle, but this hypothesis has not yet been tested in human disease. However, lowering coronary driving pressure in normal animals results in reduced perfusion of left ventricular subendocardial muscle relative to that of subepicardial muscle (6-10) and in the electrocardiographic signs of ischemia (10).

Vulnerability of subendocardial muscle to ischemia might be due to inhomogeneity of intramyocardial compressive forces, which are reported to equal or exceed intracavitary pressures in the subendocardial muscle and fall to near atmospheric pressure in subepicardial muscle (11-14). Subendocardial flow should therefore be predominantly diastolic and, once maximal vasodilatation has occurred, should depend on coronary arterial diastolic pressure, the opposition to flow by diastolic intramyocardial or coronary venous pressure (whichever is higher) and the duration of diastole. Coronary arterial pressure equals that in the aorta as long as there is
We transected the descending thoracic aorta, giving supplemental doses as needed. The dogs were placed no. 40 Bardex tubes into each end and connected them to a Y tube to reconstitute flow through the aorta. The third arm of the Y was connected to the right atrium by a no. 28 Bardex tube with a cannulating electromagnetic flowmeter transducer (Biotronix model BL 612) in line; the amount of flow diverted to the right atrium was controlled by a screw clamp. We measured cardiac output in ten dogs with a cuff electromagnetic flowmeter transducer around the main pulmonary artery and calibrated this flowmeter with dye dilution curves.

Umbilical tape was passed around the ascending aorta for an occlusive snare. Polyethylene tubes (PE 190) were placed into left and right atria, the supravalvular aorta through the left subclavian artery, the left ventricle retrograde through the carotid artery, and the femoral artery. Pressures were measured with Statham P23Db transducers and recorded on a Beckman goniograph. In some dogs, electrodes were placed on the right ventricle or atrium and connected to a Grass stimulator. We placed an electromagnetic flowmeter transducer (Biotronix model BL 612) around the left anterior descending coronary artery; such an alteration could be used to predict subendocardial ischemia. To estimate myocardial oxygen demand we measured the time tension index (TTI) of Sarnoff et al (23). Finally, since pressure dependency can occur only after maximal vasodilatation, we obtained information about the site, degree and timing of maximal coronary vasodilatation by measuring the hyperemic response to short periods of coronary ischemia (21, 24).

**Methods**

**Experimental Preparation.**—We anesthetized 29 dogs weighing 20–25 kg with 30 mg/kg sodium pentobarbital intravenously and gave supplemental doses as needed. The dogs were ventilated with oxygen-rich gas mixtures by a Harvard respirator via an endotracheal tube; arterial oxygen tensions were 150–450 mm Hg and carbon dioxide tensions were 25–42 mm Hg throughout the study. Through a left thoracotomy we transected the descending thoracic aorta, placed no. 40 Bardex tubes into each end and connected them to a Y tube to reconstitute flow through the aorta. The third arm of the Y was connected to the right atrium by a no. 28 Bardex tube with a cannulating electromagnetic flowmeter transducer (Biotronix model BL 612) in line; the amount of flow diverted to the right atrium was controlled by a screw clamp. We measured cardiac output in ten dogs with a cuff electromagnetic flowmeter transducer around the main pulmonary artery and calibrated this flowmeter with dye dilution curves.

Umbilical tape was passed around the ascending aorta for an occlusive snare. Polyethylene tubes (PE 190) were placed into left and right atria, the supravalvular aorta through the left subclavian artery, the left ventricle retrograde through the carotid artery, and the femoral artery. Pressures were measured with Statham P23Db transducers and recorded on a Beckman goniograph. In some dogs, electrodes were placed on the right ventricle or atrium and connected to a Grass stimulator. We placed an electromagnetic flowmeter transducer (Biotronix model BL 612) around the left anterior descending coronary artery; such an alteration could be used to predict subendocardial ischemia. To estimate myocardial oxygen demand we measured the time tension index (TTI) of Sarnoff et al (23). Finally, since pressure dependency can occur only after maximal vasodilatation, we obtained information about the site, degree and timing of maximal coronary vasodilatation by measuring the hyperemic response to short periods of coronary ischemia (21, 24).

In the experiments we altered each of the variables determining the DPTI. In six dogs we constricted the descending aorta. In nine other dogs the supravalvular aorta was constricted by progressively tightening the tape around it, with 3–5 minutes left for stabilization between each level of constriction; left ventricular diastolic pressure rose with aortic constriction. In two sets of experiments the diastolic duration was reduced; we paced the right ventricle at 210–300 beats/min in three dogs and in two other dogs crushed the sinusoidal node, moderately constricted the supravalvular aorta and paced the right
at increasing rates without changing the amount of aortic constriction. Finally, in nine dogs aortic diastolic pressure was lowered by progressively opening the aortic-atrial fistula with recorded phasic flow from the left anterior descending coronary artery as well as hyperemic responses with each experimental change.

Measurement of Total and Regional Coronary Blood Flow.—Microspheres with mean diameters of 14|\mu|, to 81|\mu| appear not to give correct values for flow within different layers of the heart (25). Therefore, we used microspheres 8–10|\mu| in diameter and found by comparisons with diffusible isotope data that they were able to measure local flow. We prepared radioactive microspheres 8–10|\mu| in diameter (3 M Company) labeled with 141Ce, 51Cr, and 82Sr by filtering microspheres 1–10|\mu| in diameter through 8|\mu| Nuclepore filters (General Electric Company). With a separate filter for each nuclide we washed the microspheres repeatedly with jets of normal saline containing 0.5% Tween 80; this mechanical agitation helped to break up clumps. After washing, the filters were shaken into a solution and the microspheres came off easily. When examined with a microscope, at least 95% of the microspheres were over 8|\mu| in diameter and they were usually not clumped. However, if allowed to stand for 10–15 minutes they tended to aggregate. To avoid clumping, the vial with the microspheres was placed in an ultrasonic bath for 15 minutes immediately before injection. In some of the later experiments, clumping was inhibited by suspending the spheres in 0.05% benzalkonium for 30 minutes and then placing them in heparin (100 units/ml). In addition to these microspheres, we also used those of 12 ± 2.5|\mu| (mean ± SD) labeled with 111In and 15 ± 2.5|\mu| labeled with 125I in some experiments to obtain measurements when only one type of 8–10|\mu| microspheres was available.

At selected times during the experiments we injected 600,000–800,000 microspheres into the left atrium from a small vial and flushed them in with 10 ml of warm saline over about 20 seconds. These injections usually produced no changes in heart rate, arterial blood pressure or coronary flow but we discarded a few studies (under 8% of the total) in which any of these variables changed. Immediately before the microspheres were injected, blood was allowed to drip at a rate of 15–25 ml/min into collecting vials from a catheter tied into a femoral or carotid artery. After preliminary experiments showed that 98% or more of the spheres emerged in the reference sample within 60 seconds, we collected all reference flows in two vials for 30 seconds each; the reference sample was discarded if the volumes of blood in the two vials differed by over 15%.

At the end of the experiment the dogs were killed with pentobarbital and the heart was removed. The atria were cut off and the ventricles divided into right and left ventricular free walls and septum as described previously (25). The left ventricular free wall was divided into subendocardial, middle and subepicardial layers of about equal thickness, but because of variability in this division the subendocardial and subepicardial muscle formed 16–30% and 17–38%, respectively, of the total weight of the free wall. The septum was divided into three layers and the right ventricle into two layers. The heart and blood samples were placed in vials and counted in a well scintillation detector connected to a 400 channel pulse height analyzer. The total activity of each isotope was calculated by modifying the method of Rudolph and Heymann (26). Finally, the total and regional coronary blood flows were calculated as flow to heart (or region) equals flow in reference sample times counts in heart (or region) divided by counts in reference sample (25).

Calculations of Indexes.—Tension time index (TTI) of Sarnoff et al. (23) was obtained by planimetry of the area under the aortic systolic pressure curve and equals mean aortic systolic pressure times the duration of systole. The diastolic pressure time index (DPTI) is $\int_{t_1}^{t_2} (P_{ao} - P_{lv}) dt$, where $t_1$ is the time of the beginning of diastole, $t_2$ the time of the end of diastole, and $P_{ao}$ and $P_{lv}$ are the instantaneous aortic and left ventricular pressures, respectively. We estimated DPTI by planimetry of the area under the diastolic aortic pressure curve and subtracting from it the mean left atrial pressure (assumed equal to left ventricular diastolic pressure); right atrial mean pressure was used if it was above left atrial pressure. We used the mean atrial pressure to avoid the artifacts which occur when ventricular pressure is measured with undamped catheters. DPTI times heart rate is the DPTI per minute.

The DPTI as measured overestimates true DPTI by about 5–10% because our measurement included the small areas of ventricular isometric contraction and relaxation. For the same reason, TTI is about 5–10% less than integrated left ventricular systolic pressure. The mean left atrial pressure which we used in place of left ventricular diastolic pressure should be very similar to it in the absence of mitral stenosis: the difference is only about 1 mm Hg.
<table>
<thead>
<tr>
<th>Dog no.</th>
<th>HR (beats/min)</th>
<th>LA (mm Hg)</th>
<th>Aortic BP Peak syst. (mm Hg)</th>
<th>Duration (beats)</th>
<th>CBF</th>
<th>DPTI (mm Hg - sec)</th>
<th>TTI</th>
<th>Subendo flow (ml/100 g min)</th>
<th>Percent change from control</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>167</td>
<td>4</td>
<td>125</td>
<td>108</td>
<td>65</td>
<td>80</td>
<td>395</td>
<td>3950</td>
<td>2470</td>
<td>1.65</td>
</tr>
<tr>
<td>SD</td>
<td>21</td>
<td>2</td>
<td>24</td>
<td>18</td>
<td>3</td>
<td>3</td>
<td>150</td>
<td>670</td>
<td>633</td>
<td>0.32</td>
</tr>
</tbody>
</table>

**Control (n = 12)**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>HR (beats/min)</th>
<th>LA (mm Hg)</th>
<th>Aortic BP Peak syst. (mm Hg)</th>
<th>Duration (beats)</th>
<th>CBF</th>
<th>DPTI (mm Hg - sec)</th>
<th>TTI</th>
<th>Subendo flow (ml/100 g min)</th>
<th>Percent change from control</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>155</td>
<td>7</td>
<td>182*</td>
<td>147*</td>
<td>57*</td>
<td>75*</td>
<td>374</td>
<td>4626</td>
<td>4341†</td>
<td>1.10</td>
</tr>
<tr>
<td>SD</td>
<td>15</td>
<td>3</td>
<td>27</td>
<td>21</td>
<td>4</td>
<td>4</td>
<td>160</td>
<td>719</td>
<td>726</td>
<td>0.27</td>
</tr>
</tbody>
</table>

**Dilatation Aortic Constriction (n = 10)**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>HR (beats/min)</th>
<th>LA (mm Hg)</th>
<th>Aortic BP Peak syst. (mm Hg)</th>
<th>Duration (beats)</th>
<th>CBF</th>
<th>DPTI (mm Hg - sec)</th>
<th>TTI</th>
<th>Subendo flow (ml/100 g min)</th>
<th>Percent change from control</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>195</td>
<td>55</td>
<td>245</td>
<td>125</td>
<td>40</td>
<td>40</td>
<td>0</td>
<td>1060</td>
<td>8280</td>
<td>0.17</td>
</tr>
<tr>
<td>SD</td>
<td>10</td>
<td>8</td>
<td>20</td>
<td>125</td>
<td>65</td>
<td>36</td>
<td>10</td>
<td>660</td>
<td>4200</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Proximal Aortic Constriction (n = 9)**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>HR (beats/min)</th>
<th>LA (mm Hg)</th>
<th>Aortic BP Peak syst. (mm Hg)</th>
<th>Duration (beats)</th>
<th>CBF</th>
<th>DPTI (mm Hg - sec)</th>
<th>TTI</th>
<th>Subendo flow (ml/100 g min)</th>
<th>Percent change from control</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>206*</td>
<td>39*</td>
<td>211*</td>
<td>102</td>
<td>38*</td>
<td>34*</td>
<td>25*</td>
<td>1766*</td>
<td>6540*</td>
<td>0.27*</td>
</tr>
<tr>
<td>SD</td>
<td>4</td>
<td>11</td>
<td>43</td>
<td>25</td>
<td>7</td>
<td>12</td>
<td>32</td>
<td>574</td>
<td>1576</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Ventricular Pacing (n = 7)**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>HR (beats/min)</th>
<th>LA (mm Hg)</th>
<th>Aortic BP Peak syst. (mm Hg)</th>
<th>Duration (beats)</th>
<th>CBF</th>
<th>DPTI (mm Hg - sec)</th>
<th>TTI</th>
<th>Subendo flow (ml/100 g min)</th>
<th>Percent change from control</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>250*</td>
<td>10*</td>
<td>110</td>
<td>74*</td>
<td>57*</td>
<td>67*</td>
<td>106*</td>
<td>2588*</td>
<td>2520</td>
<td>1.05</td>
</tr>
<tr>
<td>SD</td>
<td>16</td>
<td>6</td>
<td>42</td>
<td>17</td>
<td>3</td>
<td>7</td>
<td>32</td>
<td>125</td>
<td>1052</td>
<td>0.18</td>
</tr>
</tbody>
</table>

### Results

For most of the experiments, only one or two sets of 8-10 μm microspheres were available so that we did not use these microspheres for control measurements in every dog once we had noted that normally the subendocardial-subepicardial ratio of flow per gram varied very little. In 12 dogs this ratio had a mean of 1.01 and a standard deviation of 0.07. Therefore the control data in Table 1 show the mean results for these 12 dogs, some of which were in each experimental group. Heart rates, pressures, and flows were measured in all 29 dogs in the control state (except for 2 dogs with no flows), and the mean values did not differ significantly from the mean values for the 12 dogs used to provide the control values in Table 1. Statistical comparisons were usually made by paired t-test (27) between control measurements and measurements in the same dog, after an induced change and immediately before the injection of microspheres. The exceptions were that subendocardial-subepicardial flow ratios and subendocardial flows for each experimental group were compared with the normal values for 12 dogs by unpaired t-test (27).

The subendocardial flows and subendocardial-subepicardial flow ratios in Table 1 are for the free wall of the left ventricle; the data for the left, middle, and right layers of the septum were similar to those for the layers of the free wall and will not be presented. Furthermore, when there was a difference in the flow per gram of the subendocardial and subepicardial muscle, the flow per gram of muscle in the middle layer was always intermediate in amount. For brevity, the data for the middle layer will not be given.

With distal aortic constriction (Table 1), the systolic and diastolic hypertension was associated with a 60% rise in TTI (P<0.01) and a 13% rise in DPTI. Mean coronary flow increased 54% (P<0.01) and the proportion of diastolic flow fell 5% (P<0.01). Although reactive hyperemic responses were 22% below control values (P<0.01), the flow debt was always repaid by over 150%.

### Table 1

<table>
<thead>
<tr>
<th>Dog</th>
<th>Heart Rate</th>
<th>BP</th>
<th>Systolic</th>
<th>Diastolic</th>
<th>TTI</th>
<th>DPTI</th>
<th>Control</th>
<th>Aortic Constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>195</td>
<td>7</td>
<td>105</td>
<td>60</td>
<td>50</td>
<td>0.91</td>
<td>78</td>
<td>0.87</td>
</tr>
<tr>
<td>21</td>
<td>195</td>
<td>7</td>
<td>120</td>
<td>70</td>
<td>78</td>
<td>0.87</td>
<td>78</td>
<td>0.87</td>
</tr>
<tr>
<td>22</td>
<td>220</td>
<td>5</td>
<td>100</td>
<td>40</td>
<td>36</td>
<td>0.31</td>
<td>20</td>
<td>-20</td>
</tr>
<tr>
<td>23</td>
<td>216</td>
<td>10</td>
<td>120</td>
<td>50</td>
<td>42</td>
<td>0.31</td>
<td>30</td>
<td>-30</td>
</tr>
<tr>
<td>24</td>
<td>190</td>
<td>6</td>
<td>80</td>
<td>35</td>
<td>41</td>
<td>0.29</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>25</td>
<td>184</td>
<td>4</td>
<td>80</td>
<td>30</td>
<td>37</td>
<td>0.36</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>26</td>
<td>180</td>
<td>6</td>
<td>120</td>
<td>45</td>
<td>41</td>
<td>0.38</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>192</td>
<td>9</td>
<td>115</td>
<td>40</td>
<td>39</td>
<td>0.23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>180</td>
<td>6</td>
<td>105</td>
<td>60</td>
<td>44</td>
<td>0.45</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Mean</td>
<td>193*</td>
<td>7*</td>
<td>100*</td>
<td>44*</td>
<td>39*</td>
<td>0.29</td>
<td>38*</td>
<td>38*</td>
</tr>
</tbody>
</table>

11H = heart rate; LA = mean left atrial blood pressure; BP = blood pressure; syst = systolic; diast = diastolic; CBF = left anterior descending coronary artery blood flow; RH = reactive hyperemia (percent payback of flow debt); DPTI = diastolic pressure time index; TTI = tension time index; LV = left ventricle; subendo flow = subendocardial muscle blood flow; Endo/epi = subendocardial-subepicardial blood flow ratio.

Six subendocardial flows/100 g were not calculated because of inadequate reference samples.

*P < 0.01; †P < 0.05. With 12 μm microspheres. With supravalvular constriction as well.
Phasic aortic blood pressure and mean and phasic blood flow (left anterior descending coronary artery) during distal aortic constriction. The cross-hatching in the pressure curve indicates the area used to calculate TTI (see text) and the clear area between two cross-hatched systolic intervals shows the area from which DPTl is calculated. The broken line in the upper tracings is drawn at the level of mean left atrial pressure, and the blackened area shows how left atrial pressure affects DPTl. The cross-hatched area in the flow recording indicates systolic flow.

Phasic aortic pressure and mean and phasic blood flow in the left anterior descending coronary artery with increasing supravalvular aortic constriction. The last panel shows that DPTl is reduced in four ways: (1) increased left atrial pressure, (2) decreased aortic diastolic pressure, (3) increased systolic ejection time, and (4) decreased diastolic time per beat (because of increasing heart rate). Markings as in Figure 1.

Circulation Research, Vol. XXX, January 1972
After constricting the ascending aorta, systolic pressure rose 62% \((P < 0.01)\) above control values (Table 1, Fig. 2), but mean aortic diastolic pressure varied from 75 to 140 mm Hg and was not significantly different from control levels. The duration of systole increased markedly, especially with tachycardia. Left atrial pressure rose progressively as the aorta was constricted more tightly and reached an average of 39 mm Hg \((P < 0.01)\); in two dogs it exceeded 50 mm Hg. There was no evidence of pulmonary edema in these dogs and arterial oxygen tensions did not fall. Cardiac output decreased an average of 35% at the time of maximal constriction. TTI rose in all experiments \((P < 0.01)\) but DPTI fell progressively \((P < 0.01)\), especially when aortic diastolic pressure was low or tachycardia developed. Mean coronary blood flow increased to 45% above control values \((P < 0.01)\) due to a 253% \((P < 0.01)\) rise in systolic coronary flow. Diastolic coronary flow was unchanged at first but eventually fell 38% \((P < 0.01)\). Mean coronary flow fell only when the dogs could not maintain systolic hypertension, and when this occurred, left atrial pressure rose still further and the dogs deteriorated unless the constriction was relieved. Reactive hyperemia decreased \((P < 0.01)\) as the constriction was tightened and payback of the flow debt was under 100% when diastolic coronary flow fell below 30% of the total.

With ventricular pacing, aortic systolic and diastolic pressures were usually maintained until heart rate was above 220 beats/min. TTI/min was raised when these pressures were maintained but fell as arterial pressure was lowered. DPTI fell progressively \((P < 0.01)\) because diastolic time decreased. These indexes fell almost in parallel, and DPTI/TTI usually remained above 1 until heart rate exceeded 275 beats/min, when DPTI/TTI fell progressively. Mean coronary blood flow rose initially due to increasing proportions of systolic flow as the rate rose (Table 1) and then fell at rates which differed in different dogs. Reactive hyperemia fell progressively, and the payback of the flow debt was always less than 100% at rates above 275/min; in one dog it was under 100% at 210/min. In the two dogs with atrial pacing and a supravalvular aortic constriction, DPTI/TTI was below 0.5 and the reactive
hyperemic response was under 100% at a rate of 150/min. When we increased heart rate in increments of 30 beats/min, aortic systolic and diastolic pressures fell progressively, diastolic duration decreased and left atrial pressure rose an average of 5 mm Hg with each increment of heart rate. The decreased aortic pressures were preceded by a fall in mean and diastolic coronary flow. DPTI fell more than TTI because of the shortened diastole, higher left atrial pressure and lower aortic diastolic pressures. Reactive hyperemic responses remained below 100% in each dog.

When the fistulas were opened progressively until 2000 ml/min passed through them, aortic systolic pressure was unchanged until near the maximal fistula flow but aortic diastolic pressure fell progressively \((P < 0.01)\) (Table 1, Fig. 3). Left atrial pressure did not rise until systolic hypotension was seen. Systolic ejection time was prolonged absolutely and relative to cycle length \((P < 0.01)\) so that TTI rose to 31% above control at first and then with larger fistulas gradually returned to base-line levels. DPTI fell progressively \((P < 0.01)\). Mean coronary blood flow was initially stable but fell an average of 17% below control values with the biggest fistulas; this mean flow was maintained by progressive increases in systolic coronary flow \((P < 0.01)\) until almost all the flow was systolic. When diastolic flow fell below 30% of the stroke flow, all dogs developed hypotension and bradycardia or ventricular fibrillation unless the fistula was closed. The reactive hyperemic responses fell progressively as the fistulas were opened wider \((P < 0.01)\) and payback of the flow debt was under 100% when the diastolic flow was under 30% of the total.

**Distribution of Coronary Blood Flow.**—In the control period, the percent of diastolic flow was 75–85% and subendocardial flow ranged from 58 to 106 ml/min 100 g⁻¹ (Fig. 4, left). There were significantly lower subendocardial flows in the dogs with fistulas, most values with distal aortic constriction were above normal \((P < 0.01)\) and with proximal aortic constriction or ventricular pacing the

---

**FIGURE 4**

Percent diastolic blood flow recorded by an electromagnetic flowmeter transducer on the left anterior descending coronary artery when the microspheres were injected is plotted against (left) left ventricular subendocardial blood flow (ml/100 g min⁻¹) measured by microspheres and (right) the ratio per gram of left ventricular subendocardial to subepicardial blood flow measured by microspheres.
Flows could be above or below the normal range. More consistency was noted when the ratio subendocardial:subepicardial flow was plotted against the percent of diastolic flow (Fig. 4, right). The inner and outer layers of the left ventricle each received approximately equivalent flows during the control period, with distal aortic constriction and with pacing as long as the diastolic flow was above 65% of the total flow. All the flow ratios fell below control levels in proportion to the reduction of diastolic flow with more rapid pacing, lower aortic diastolic pressures due to fistulas, or raised left atrial pressures from proximal aortic constriction (correlation coefficient 0.80, \( P < 0.01 \)). All correlation coefficients will refer to these three types of experiments alone since the data for controls and distal aortic constriction show no major changes, i.e., they fall on the horizontal parts of the curves.

There was considerable variability when the subendocardial-subepicardial flow ratios were related separately to mean aortic diastolic pressure, left ventricular diastolic pressure, and diastolic duration, the correlation coefficients being respectively 0.22, 0.49 (\( P < 0.05 \)), and 0.73 (\( P < 0.01 \)). Since the design of the experiment did not allow these variables to be changed independently, the partial correlation coefficients were calculated (27) and were respectively -0.09, 0.31, and 0.70 (\( P < 0.05 \)). (The probabilities given for the correlation coefficients indicate a significant difference from zero correlation.)

If all these variables were combined to form the DPTI (Fig. 5, left), the correlation of DPTI with the subendocardial-subepicardial flow ratio was 0.65 (\( P < 0.05 \)) with a standard deviation from regression of 0.28. This correlation was not better than for diastolic duration alone. A higher correlation coefficient of 0.85 (\( P < 0.01 \)) with a smaller standard deviation from regression of 0.18 was found when we related the flow ratio to the ratio DPTI/TTI (Fig. 5, right). Flow ratios below 0.8 always occurred whenever DPTI/TTI was less than
Left ventricular subendocardial-subepicardial blood flow ratios per gram are plotted against the reactive coronary hyperemic responses recorded 3 minutes before each microsphere injection. The aortic and left atrial pressure, heart rate, and phasic and mean coronary flow had returned to preocclusion levels when the microspheres were injected.

0.8. (Similar correlations were obtained by plotting the flow ratio against the ratio DPTI/peak systolic pressure; here \( r = 0.83 \) \( (P < 0.01) \), standard deviation from regression 0.19.) Thus when DPTI/TTI rose in parallel with distal aortic constriction or fell in parallel with pacing, homogeneity of myocardial perfusion was maintained. DPTI was less than TTI in three dogs with ventricular pacing, and the subendocardial-subepicardial flow ratios in two of them were 0.58 and 0.79; both of these abnormal ratios occurred with the 12\( \mu \) Nb microspheres and would be slightly lower with the 8-10\( \mu \) microspheres.

Reactive Hyperemia.—In control studies and with distal aortic constriction the payback of the flow debt was always over 175% and the myocardium was homogeneously perfused (Fig. 6). Whenever the debt was repaid by less than 100%, the subendocardial-subepicardial flow ratios were always below 0.80; DPTI/TTI was always below 0.40 at these times. The repayment was slightly higher in dogs with proximal aortic constriction than with fistulas when hyperemic responses were compared at equivalent flow and DPTI-TTI ratios. Mean coronary blood flow decreased after the 8-second occlusion in several dogs only when mean arterial pressures decreased during and after occlusion; many of these dogs developed arrhythmias within 10-30 seconds after release of the occlusion.

Discussion

Critique of the Methods.—The microspheres can measure coronary blood flow within 20% of its true value and so can detect changes above 20% in single experiments and of smaller amounts if groups of data are pooled. Also, as long as more than 400 microspheres are present in the subendocardial and subepicardial layers (total 800) the ratio of subendocardial to subepicardial microspheres per gram will vary less than 20% for a single value; smaller differences can be detected if groups of ratios are compared (28).

The control subendocardial-subepicardial flow ratios which we obtained with 8-10\( \mu \) microspheres are similar to those obtained with diffusible indicators (7, 8, 29, 30); the slight differences noted could be due to differences in the techniques used or the physiology of the preparations. In addition, the way the heart has been divided to provide these ratios is not uniform in different studies. Some have separated the wall into three layers, some into two, and usually the variability of the division is not mentioned. The variability of division that we found might explain some of the variability in flow ratios whenever there was inhomogeneous perfusion.

Myocardial Oxygen Needs.—Pressure loads on the left ventricle are accompanied by a proportional rise in myocardial oxygen uptake and coronary blood flow (31). Our studies with distal aortic constriction showed an increased coronary blood flow of 71% above control values and a rise of TTI of 60%. These figures are in quite good agreement, since Sarnoff et al. (23) showed that oxygen requirements of the heart were directly related to the area under the systolic pressure curve. Although they proposed the term “tension time index” and realized that tension might be involved, this area can more accurately be described as a systolic pressure time index. The area under the systolic pressure curve...
pressure curve bears a constant relationship to wall tension only if geometry remains constant; if the heart dilates, wall tension will increase at any given pressure. All of our experiments with supravalvular aortic constriction would be expected to increase ventricular volume, so that in them TTI would underestimate oxygen demand. In the other experiments, there was no or only slight change in left ventricular end-diastolic pressure and thus probably little change in ventricular volume. Despite this variability due to geometric changes, the study of McDonald et al. (32) did show that TTI correlated almost as well with myocardial oxygen consumption as did peak wall tension, so that errors in using this easily measured index are probably not great. Sonnenblick et al. (33) showed that during inotropic stimulation there could be an increased myocardial oxygen uptake despite a fall in TTI. In their studies, systolic ejection shortened and the rate of left ventricular ejection rose. In all of our experiments (except the pacing studies), systolic ejection was prolonged and the rate of left ventricular ejection was decreased; this potential error, therefore, did not seem to apply to our results.

Monroe (34) observed that myocardial oxygen uptake was almost maximal by the time the peak systolic pressure had been reached and that peak systolic pressure could also be used to predict myocardial oxygen uptake. The increased coronary flow following distal aortic constriction was more closely associated with change in TTI than with this pressure in our studies so that we used TTI to estimate myocardial oxygen needs.

Changes in Distribution of Myocardial Blood Flow.—After distal aortic constriction, the increased coronary blood flow was distributed almost equally to subendocardial and subepicardial muscle, as noted previously (8, 35). With proximal aortic constriction, coronary flow rose a similar amount (45% vs. 54%, 0.8 < P < 0.4) but was not evenly distributed, subendocardial flow averaging only 37% of epicardial flow. The increased coronary flow was predominantly diastolic with distal aortic constriction and the increased oxygen requirements of systolic hypertension were met since subendocardial flow rose because of diastolic hypertension. Conversely, the increased coronary flow was predominantly systolic with proximal constriction, as aortic diastolic pressure did not change significantly following this intervention. Myocardial oxygen requirements (TTI) were higher after proximal constriction (6540 vs. 4341 mm Hg sec/min, P < 0.01), but diastolic subendocardial flow became limited because left ventricular diastolic pressure rose and diastole was shortened by tachycardia and prolonged systolic ejection times. Actual flows per 100 g of subepicardial muscle averaged 205 ml/min for the proximal constriction and 110 ml/min for the distal constriction (P < 0.05), while the subendocardial flows were respectively 61 and 124 ml/min (0.1 > P > 0.05). Since for more cardiac work the subendocardial muscle was getting less flow during proximal than distal constriction it is likely that it was ischemic at that time. The conclusion is supported by the deterioration (fall in cardiac output, death) that occurred after severe proximal aortic constriction. The deterioration was not associated with clinical pulmonary edema or hypoxemia, the right atrial pressure did not rise and subepicardial muscle was sometimes receiving an increased amount of blood. In similar experiments, electrocardiographic evidence of ischemia has been noted (10). Further indirect evidence for subendocardial ischemia can be found in the occurrence of heart failure (fall in cardiac output, systemic hypotension, raised left atrial pressure) in dogs with a large arteriovenous fistula. At these times, they all showed a markedly reduced ratio of subendocardial to subepicardial flow and a reduced subendocardial flow averaging 38 ml/min 100 g⁻¹ muscle. Since the subepicardial flow was at control levels of 71 ml/min 100 g⁻¹ with these fistulas and since increasing cardiac output alone causes little increase in myocardial oxygen uptake (31), the absolute reduction of subendocardial flow probably produced ischemia of that muscle and impaired cardiac performance. We should emphasize that in
these dogs left atrial pressure rose and cardiac output fell only after the marked reduction in subendocardial flow. Similar findings have been reported with controlled reduction of coronary blood flow (7, 8, 36) and in one of those studies (36) ischemia was documented by a fall in the pH of coronary venous blood and by the myocardial production of lactate. We do not imply that any fall in the subendocardial-subepicardial flow ratio below normal indicates subendocardial ischemia, but that when the discrepancy is marked there is good reason to believe that ischemia is present. Like all ratios this should be interpreted with care since a change could be due to either of its components. Thus a rise in the ratio from 0.3 to 0.5 may not indicate increased subendocardial blood flow; it could in theory follow the administration of a vasoconstrictor that had no effect on maximally dilated arteries in ischemic subendocardial muscle but reduced subepicardial flow by constricting its arteries. If this occurred, subendocardial flow would not increase unless perfusing pressure rose; in fact, if perfusing pressure then fell for any reason it might be possible for the subendocardial-subepicardial ratio to rise while the subendocardial flow was actually falling. One advantage of the microsphere method is that it avoids misinterpretation of ratios because it allows measurement of changes in both the subendocardial-subepicardial flow ratio and absolute subendocardial flow if a reference sample is collected when the microspheres are injected.

It is possible that a flow ratio below 1 may exaggerate the amount of relative subendocardial ischemia. This might occur if a vasodilator were released from the ischemic inner layer (37, 38) and caused the subepicardial vessels to dilate more than necessary to supply blood for the metabolic needs of subepicardial muscle.

The decrease in subendocardial and increase in subepicardial flow were often associated with normal or even elevated total coronary blood flows so that under the circumstances of these changes, methods that measure only total left ventricular flow (coronary sinus drainage, nitrous oxide washout, xenon washout) give limited information. Electromagnetic flowmeter recordings of phasic flow are more helpful, since phasic flow patterns can be used to predict distribution of coronary flow. A decreased proportion of diastolic flow correlated well with a fall in subendocardial-subepicardial flow ratio even when the absolute subendocardial flow was increased, as in the dogs with proximal aortic constriction. From these studies it appears that an increased proportion of systolic flow in the left coronary artery indicates preferential flow to the subepicardial muscle. Similar changes in phasic flow patterns have been shown in studies with proximal aortic constriction (22), experimental aortic incompetence (18, 19, 21) and hemorrhagic shock (20). This consistency helps to dispel the possibility that these changes were artifactual, especially since in one study (18) the flowmeter was not electromagnetic.

Although no single variable consistently predicted subendocardial underperfusion in our studies, diastolic duration seemed to correlate better with the distribution of blood flow than did any other variable. This might be expected since subendocardial perfusion is predominantly diastolic and tachycardia occurred with each intervention. A better prediction, however, was obtained when the ratio of DPTI to TTI was measured, since this takes account not only of factors responsible for supply (DPTI) but also for those regulating demand (TTI). This ratio is similar to the coronary-ventricular ratio of Griggs and Nakamura (7) but may be more useful because it identifies the events affecting subendocardial flow more clearly.

When the DPTI ratio was over 0.8, all areas of the left ventricle were evenly perfused. If this ratio was reduced, but remained above 0.8, the coronary arteries dilated to maintain the parity of flow in different layers. This vasodilatation was evident when we tested the reactive hyperemic responses. Since the proportion of flow to subendocardial and subepicardial muscle remained normal until the reactive hyperemic responses were less than
100–150%, vasodilatation probably occurred at the same rate in both areas. Once payback fell below this level, the subendocardium became relatively underperfused. Since there was still some payback at these times, the subepicardial arteries must still have been capable of dilating when the subendocardial arteries were maximally dilated. Some have inferred from tissue oxygen tensions and capillary densities that subendocardial vessels are normally maximally dilated (39, 40). Our findings indicate that subendocardial arteries are not normally maximally dilated, and this conclusion is supported by the pacing studies in which absolute subendocardial flow increased from control levels at a time when perfusion pressure had fallen.

Clinical Implications.—The importance of considering all factors contributing to the supply-demand ratio is shown in all of our studies. Although DPTI was progressively reduced when the ventricles were paced, subendocardial perfusion remained adequate until DPTI/TTI was below 1. The normal phasic coronary artery flow patterns during most of the pacing studies are thus explained, and confirm the results reported by Pitt and Gregg (16) and Cobb et al (17). If, however, DPTI-TTI ratios were already lowered by proximal aortic constriction, then pacing shortened diastole, lowered mean diastolic blood pressure and raised left atrial pressure. This caused a further fall in DPTI/TTI and in the proportion of flow going to subendocardial muscle. These relationships could explain the occurrence of anginal pain and electrocardiographic evidence of subendocardial ischemia caused by tachycardia in patients with aortic stenosis (41) in whom diastolic duration may be reduced at any given heart rate because left ventricular ejection is prolonged. Atrial pacing, which is reported to increase TTI, causes similar ischemic changes in patients with coronary atherosclerosis (42). Since coronary diastolic pressure is low beyond an area of significant stenosis, any reduction of diastolic time would lower DPTI further and might jeopardize subendocardial flow. At this time, any increase in left ventricular diastolic pressure, as can occur in ischemic heart disease, could further reduce subendocardial perfusion (43).

Although our studies show that shortened diastolic duration can reduce subendocardial blood flow, it should be emphasized that it is the effect of diastolic time on DPTI/TTI that determines the adequacy of subendocardial perfusion. In pathologic conditions like severe aortic insufficiency, coronary diastolic pressure is low and ventricular diastolic pressure is high. An increased heart rate under these circumstances would reduce the time of regurgitation, raise mean aortic (coronary diastolic) pressure, and lower mean left ventricular diastolic pressure. If these pressures were changed to a greater extent than diastolic time was reduced, then DPTI per minute would increase and subendocardial perfusion might improve. The beneficial effect of tachycardia in patients with aortic insufficiency (44) may in part be explained on this basis.

Acknowledgment
We acknowledge the invaluable assistance of Miss Elizabeth Shapkin, Mr. Lesley A. Williams, and Mr. Bruce D. Payne.

References
7. GRIGGS, D.M., JR., AND NAKAMURA, Y.: Effects of coronary constriction on myocardial distribu-


during ventricular contraction and relaxation.
Experimental Subendocardial Ischemia in Dogs with Normal Coronary Arteries
GERALD D. BUCKBERG, DAVID E. FIXLER, JOSEPH P. ARCHIE and JULIEN I.E. HOFFMAN

Circ Res. 1972;30:67-81
doi: 10.1161/01.RES.30.1.67
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1972 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/30/1/67

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/