Dissociation between epicardial and transmural function during acute myocardial ischemia

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ABSTRACT  The relationship between epicardial and transmural function (measured with sonomicrometers) was examined in 13 anesthetized open-chest dogs. Systolic wall thickening was used as a standard of integrated transmural function to compare with epicardial function measured as segment shortening parallel to surface fibers. Three levels of coronary inflow restriction were produced by using decrements in systolic wall thickening as an index of changes in the transmural distribution of myocardial blood flow (microspheres) in myocardium perfused by the left anterior descending artery (antero-apical group, n = 7) or circumflex artery (postero-basal group, n = 6). Levels 1 and 2 were characterized by reductions in systolic wall thickening of 35% and 80%, respectively, and marked decreases in deep myocardial blood flow. In the subepicardium, myocardial blood flow was minimally affected at levels 1 and 2 and there was no change in posterior-basal epicardial segment shortening, but anterior segment shortening decreased significantly (by 21% and 37%, respectively). At level 3 myocardial blood flow was reduced transmurally, producing systolic wall thinning and marked epicardial dysfunction in both groups. Parallel epicardial segment shortening underestimated the extent of transmural dysfunction in both groups at levels 1 and 2 but the degree of underestimation was greatest in the posterior-basal group. Anterior-apical segment shortening was impaired at levels 1 and 2, whereas posterior-basal segment shortening was unaffected, suggesting that significant regional variability exists in the epicardial response to nontransmural ischemia.


EPICARDIAL contractile function has been used extensively to assess regional myocardial performance during experimental myocardial ischemia. Important findings demonstrating the dependence of regional myocardial function on graded reductions in total coronary blood flow were obtained with mercury-in-silicone rubber length gauges or Walton-Brodie strain gauges sutured to the epicardium.1-5 More recent studies have focused on the functional consequences of changes in myocardial blood flow distribution produced by coronary stenosis. With tracer-labeled microspheres or hydrogen desaturation curves to document blood flow distribution within the myocardial wall and sonomicrometers to measure segment length shortening or wall thickening during systole, close coupling between myocardial perfusion and contractile performance in the same area has been well established.6-14

Because coronary stenosis produces a nonuniform pattern of flow distribution, characterized by more severe subendocardial than subepicardial flow restriction, investigators have begun addressing whether or not corresponding differences also develop in epicardial and subendocardial contractile function.15-21 Of particular interest is epicardial shortening when subepicardial perfusion is normal (or near normal) and only the deeper myocardial layers are ischemic. The findings and conclusions on this issue are contradictory. Weintraub et al.16 and Hattori et al.18 suggested that epicardial shortening is constrained or tethered in these circumstances, despite apparently adequate outer myocardial perfusion. However, we presented data showing that epicardial shortening parallel to local fiber orientation is not altered as long as subepicardial perfusion remains normal.17 This suggested that parallel epi-
cardial shortening is sensitive primarily to changes in outer myocardial blood flow alone, providing a poor index of changes in regional function across the entire myocardial wall during conditions characterized by nontransmural ischemia.

The objective of the present study was to determine the relationship between epicardial and transmural function during varying degrees of flow-limiting coronary stenosis. We used systolic wall thickening, a measurement across all myocardial "layers," as a standard of integrated regional function to compare with epicardial function, which was measured as segment shortening parallel to surface fiber orientation. In addition, because the functional response to ischemia may differ by location in the left ventricle, we compared functional responses to acute ischemia in the areas of myocardium supplied by the left anterior descending and circumflex arteries. Alignment of the ultrasonic dimension gauges relative to epicardial fiber orientation was carefully examined, since this is a critical factor in measurements of epicardial shortening during ischemia and because surface fiber orientation differs markedly in different myocardial locations.

Methods

Experimental preparation. The study was performed in 13 open-chest dogs anesthetized with halothane (end-tidal concentration 1% or less) and artificially ventilated. Arterial blood gases and pH were measured periodically to ensure they were in the normal range and that PaO2 exceeded 100 mm Hg. Dextran was infused intravenously to sustain normal arterial pressures, and hematocrits were determined at intervals to make certain hemodilution (hematocrit less than 30%) was not produced. The dogs were instrumented as shown in figure 1. A Millar high-fidelity micromanometer was passed into the left ventricle via the carotid artery and aorta for measurement of left ventricular pressure. Tygon catheters were placed in the left ventricle (via the apex to verify calibration of the Millar micromanometer in mm Hg), left atrium (for injection of microspheres), and femoral and carotid arteries (for obtaining two simultaneous reference withdrawal samples of arterial blood for calculation of myocardial blood flow).

The animals were divided into two groups. In six animals the left circumflex artery was exposed and a screw clamp was placed around it to produce coronary stenosis or occlusion. In seven animals the left anterior descending artery was exposed and equipped with a screw clamp occluder. Because the circumflex artery supplies primarily the posterior wall of the left ventricle, regional functional measurements were made in this area and this set of experiments is designated the posterior-basal group. The set of experiments in which the left anterior descending artery was used is designated the anterior-apical group.

Two pairs of ultrasonic dimension gauges (6 MHz) were placed in the myocardium supplied by the circumflex artery or left anterior descending artery to measure regional dimensions. As shown in the insets of figure 1, one pair of crystals was arrayed to measure wall thickness.22, 23 One crystal of this pair (2 mm diameter) was inserted tangentially through the myocardium to the endocardium; we sewed the other crystal (4 mm diameter), attached to a Dacron patch, to the epicardium with shallow sutures after locating the position of least distance between the crystals while monitoring the signals with an oscilloscope.23 We used the thickness mode signal to achieve maximum spatial resolution. The position of the crystals was examined carefully at the time of necropsy to verify correct alignment. The other pair of crystals (2 mm diameter) was positioned in the epicardium, approximately parallel with the superficial fiber orientation. This orientation was confirmed at postmortem examination by directly measuring with a protractor the angle between surface fiber orientation and the axis through the center of the pair of epicardial crystals after gently stripping away the epicardial sheet of connective tissue, as described previously.22 The dimension gauges were connected to a Triton (Model 120) sonomicrometer for processing of signals.

Experimental protocol. The same protocol was followed in the posterior-basal and anterior-apical groups. After allowing 30 min for the animal to stabilize after completing instrumentation, we made a control injection of microspheres. Then we produced partial coronary stenosis while monitoring the wall thickness signal for reductions in systolic wall thickening. When wall thickening was reduced by less than 50% from control (level 1 of regional dysfunction), we waited 3 min to ensure stability and then injected another set of microspheres. Without releasing the occluder, a second, more severe, degree of partial coronary stenosis was produced, which was characterized by further reduction in systolic wall thickening greater than 50% from control but not complete elimination of thickening.
(level 2 of regional dysfunction), and another set of microspheres was injected. Finally, total or near total coronary occlusion was produced, associated with replacement of wall thickening by net systolic thinning or paradoxical motion (level 3 of regional dysfunction). Complete occlusion could not be verified precisely because we did not use an electromagnetic flowmeter to measure coronary blood flow. In the remainder of the text, levels 1, 2, and 3 will be used to refer to the three levels of regional dysfunction that were defined operationally by the changes produced in systolic wall thickening. Alterations in wall thickening were used as a guide to produce predictable changes in the distribution of myocardial blood flow across the wall, as well as to provide the standard of integrated transmural systolic function for comparison with epicardial systolic shortening (used as the parameter of epicardial function). The time required for performance of this protocol was approximately 1 hr. Total ischemia time from the first indication of regional dysfunction varied from 32 to 45 min.

**Myocardial blood flow.** Regional myocardial blood flow was measured with tracer-labeled microspheres (15 μm diameter, New England Nuclear) by the reference withdrawal method. Four injections were made in every experiment, with one of six available isotopes (¹⁴¹Ce, ¹¹⁷Sn, ⁵¹Cr, ⁱ⁰⁹Ru, ⁶⁹Nb, ⁴⁶Sc) for each flow determination. The choice of isotopes was determined by which isotopes were available at the time, and the order of their injection was randomized. Approximately 1 to 2 million microspheres were injected into the left atrium for measuring blood flow. Reference arterial samples were obtained simultaneously from both the femoral and carotid arteries at a constant rate (7.0 ml/min) with a Harvard withdrawal pump and were begun before the injection of microspheres and completed 2 min later. If the counts in the two reference samples varied by greater than 10%, indicating poor mixing, the data were discarded. The reference sample counts were averaged for the calculation of myocardial flows. Each batch of microspheres was placed in an ultrasonic bath and thoroughly mixed by vortex agitation before injection, and droplets of the microsphere suspension were periodically examined under a microscope to ensure that adequate dispersal had been achieved.

At the end of the experiments the dogs were killed with an injection of KCl. The heart was removed and placed in formalin to facilitate sectioning. Wall thickness dimension gauges were left in the heart to allow careful evaluation of their alignment across the wall, at the time of heart sectioning. Epicardial dimension gauges, implanted superficially, were removed, but small holes (1 to 2 mm depth) remained in the heart, making it possible to measure gauge alignment relative to surface fiber orientation.

To make certain that dimension measurements had been made within the ischemic area and that blood flows in tissue containing wall thickness gauges were comparable to tissue containing epicardial gauges, multiple full-thickness sections were obtained around complete rings of the left ventricle. Each block of tissue was divided into three pieces of approximately equal thickness from the endocardial to epicardial surfaces. After the location of each piece of tissue and the position of the ultrasonic crystals had been recorded, the tissue samples were weighed and placed in counting vials for assay of radioactivity in a Tracer (Model 1185) gamma scintillation counter. After correcting the counts in each tissue sample for background and overlapping counts with simultaneous equations, blood flow was calculated with the equation

\[ Q_m = \frac{(C_m \times Q_r)/Cr}{\text{for}}\]

where \( Q_m \) = myocardial blood flow (ml/min), \( C_m \) = counts per minute in tissue samples, \( Q_r \) = withdrawal rate of the reference arterial sample (ml/min), and \( Cr \) = counts per minute in the reference arterial sample. Flow per gram of tissue was calculated by dividing flow by the weight of the appropriate sample. Background and overlap corrections and blood flow calculations were performed on an Apple II+ microcomputer.

As shown in figure 2, we constructed myocardial blood flow "maps" (for subendocardial, midmyocardial, and subepicardial layers across the wall) around the circumference of the left ventricle in which the position of the dimensional measurements could be located. Approximate delineation of ischemic boundaries was achieved, allowing verification that the gauges were well contained in ischemic tissue. Blood flow distributions in the samples containing wall thickness and segment length dimension gauges were compared and no significant differences were detected; therefore, the blood flow data were pooled for additional statistical comparisons.

**Data analysis.** Recordings were made during each experiment on an eight-channel Hewlett-Packard pressurized-ink recorder and on magnetic tape for subsequent analysis. Wall thickness, epicardial segment length, and left ventricular pressure were recorded simultaneously. The variables analyzed were segment length and wall thickness at end-diastole (identified as the point corresponding to the onset of the positive dP/dt signal) and end-systole (defined as the point approximately 20 msec before peak negative dP/dt). Extent of wall thickening or segment shortening, mean ejection phase velocity of thickening or shortening, left ventricular systolic and end-diastolic pressures, heart rate, and regional myocardial blood flow were computed from the analog tracings.}

**FIGURE 2.** Example of circumferential blood flow map to demonstrate how we verified that the ultrasonic dimension gauges were located within the ischemic zones. Fourteen samples were obtained from the heart slice containing the gauges. The samples are numbered on the x axis and begin (No. 1) with the posterior septal region and extend around the ring to No. 14, which adjoins the first sample. Subendocardial blood flow (ENDO BF) and subepicardial blood flow (EPI BF) during control conditions (Cont) and at dysfunction level 2 (Lev 2) are shown. The tissue samples containing dimension gauges are indicated with a large asterisk for the wall thickness (WTh) gauge and small asterisk for the epicardial segment length (EPI SL) gauge. Subendocardial blood flow was substantially reduced at level 2, but little change is evident in subepicardial blood flow. Examples of analog tracings from these gauges during the two conditions depicted are shown in the insets. These data are from the same experiment as shown in figure 3.
flows. The extent of segment shortening or wall thickening was calculated as the difference (in mm) between end-diastolic and end-systolic dimensions and was also expressed as a percentage change from the end-diastolic length or wall thickness. The same criteria for defining the end-diastolic and end-systolic time points were applied to the dimension data from the posterior-basal and anterior-apical groups. Mean ejection phase velocity was determined by dividing ejection phase excursion (difference between thickness or segment length at peak positive dP/dt and the end-systolic dimension) by the ejection time.11 Epicardial segment lengths during control conditions were normalized to a distance of 10 mm and changes in dimensions were referenced to this value.26 The reason for this normalization was the large degree of variability in the distance between epicardial segment length crystals (range 8 to 18 mm), in large part due to the limitations in available implantation sites posed by epicardial vasculature and the location of the wall thickness dimension gauge. Wall thickness dimensions were not normalized. For wall thickness and the epicardial segment length, we used three main parameters of systolic function: extent of systolic excursion (mm), percentage shortening or thickening, and mean ejection phase velocity (mm/sec).

Data were obtained from high-speed recordings (100 mm/sec) by hand calculation. A minimum of 10 cardiac cycles were averaged at each condition, with use of the beats occurring just after completion of the microsphere injections. Hemodynamic, dimensional, and myocardial blood flow data were analyzed in both groups at four times with repeated measures analysis of variances: (1) control conditions, (2) level 1 (of regional dysfunction), (3) level 2, and (4) level 3. When overall significance was detected, paired t tests were used to discriminate the significance of differences between conditions. Because multiple comparisons were performed, a Bonferroni correction of the acceptable p level was used.27 Routinely six comparisons were made, so the conventional p value (.05) was divided by six to give p < .0083 as the minimum acceptable alpha level. When "p < .05" is indicated in the tables and text, this will refer to the "corrected" value, actually corresponding to p < .0083.

Comparisons between parameters in the anterior and posterior groups were made with unpaired t tests (corrected for multiple comparisons) and with a profile analysis,27 a multivariate statistical technique used in situations where repeated measures are taken on more than one group and a group comparison across the repeated measurements is required. The profile analysis provided us with a trend analysis to compare the pattern of changes in the anterior and posterior groups. Parallel response curves (parallelism tested with Hotelling's T^2 statistic for two populations) indicated no interaction between group (anterior vs posterior) and time (control conditions and the three levels of dysfunction) effects and allowed testing of whether or not there existed differences between the groups and across interventions. If nonparallelism was demonstrated, testing for group or time effects was not warranted, which means the group responses were different for different times.

A major objective of this study was to compare the relationship between epicardial function and transmural function across the two groups to determine whether or not changes in parallel epicardial segment shortening reflect changes in contractile function across the wall. Consequently, an additional statistical approach was used to describe the relationship between change in epicardial segmental function (used as the dependent variable) and change in wall thickening (used as the independent variable). Separate regression functions were calculated for each experiment.28 After fitting various logarithmic, power, and exponential functions (to linearize the form of equations describing the relationships and stabilize variances), the linear model resulting in the best fit for the majority of subjects was imposed on the data for all subjects. The best fit was determined as that which yielded the highest coefficient of determination (R^2 value) and attained significance level.29 The means of the slope and intercept parameters were calculated for the anterior and posterior groups and a t test was applied to compare them. A difference in slope between the two groups indicated that their response patterns differed; a difference in intercepts, but not slope, indicated that the relative degree of change was not the same (a significant group effect) although the patterns were similar. The regression analysis provided an alternate statistical approach that was used to back up the profile analysis. Data are reported in the text, tables, and figures as mean ± SD.

Results

The average crystal axis deviation from surface fiber orientation in the posterior-basal group was 10 ± 4 degrees (range 6 to 14) and 11 ± 6 degrees (range 5 to 20) in the anterior-apical group. The posterior-basal epicardial segments roughly paralleled the long axis of the heart, described as a line from the point of left coronary artery bifurcation to the apical dimple (figure 1). In the anterior-apical region, the epicardial fiber orientation and consequently the alignment of the epicardial dimension gauges differed markedly from those of the posterior-basal group, relative to the long axis. The deviation of the epicardial fiber orientation from the long axis was 46 ± 7 degrees in the anterior-apical wall and 0 ± 15 degrees in the posterior-basal wall. Had the anterior group epicardial dimensions been measured parallel to either the long or short axis, the gauges would have been aligned improperly relative to the actual fiber orientation in the anterior wall near the apex. The average difference between anterior and posterior wall epicardial fiber orientation was 46 ± 10 degrees, consistent with observations reported by previous investigators.29, 30

Hemodynamic data are summarized in table 1. No significant changes in heart rate were observed in either the posterior-basal or anterior-apical groups during graded ischemia, compared with control conditions. There was a significant trend for peak systolic left ventricular pressure to decrease during coronary stenosis (by profile analysis), but only in the posterior group (at dysfunction level 3) was a significant reduction established by (corrected) t test. No significant change was observed in the anterior-apical group, probably reflecting the smaller portion of the left ventricle usually supplied by the left anterior descending artery compared with the circumflex artery.31 Left ventricular end-diastolic pressure increased significantly in both groups at dysfunction level 3 (table 1) and no difference was observed between the two groups in this parameter by profile analysis.

Examples of recordings from one experiment in the
TABLE 1

<table>
<thead>
<tr>
<th>Hemodynamic data (mean ± SD)</th>
<th>Control</th>
<th>Dysfunction level 1</th>
<th>p value A</th>
<th>Dysfunction level 2</th>
<th>p value A</th>
<th>Dysfunction level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>119 ± 15</td>
<td>122 ± 11</td>
<td>NS</td>
<td>121 ± 10</td>
<td>NS</td>
<td>121 ± 10</td>
</tr>
<tr>
<td>A</td>
<td>107 ± 12</td>
<td>110 ± 13</td>
<td>NS</td>
<td>110 ± 17</td>
<td>NS</td>
<td>114 ± 15</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>107 ± 9</td>
<td>105 ± 8</td>
<td>NS</td>
<td>99 ± 4</td>
<td>NS</td>
<td>93 ± 6*</td>
</tr>
<tr>
<td>A</td>
<td>112 ± 8</td>
<td>117 ± 7</td>
<td>NS</td>
<td>112 ± 10</td>
<td>NS</td>
<td>108 ± 12</td>
</tr>
<tr>
<td>LV end-diastolic pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>6.9 ± 1.7</td>
<td>7.2 ± 1.9</td>
<td>NS</td>
<td>8.0 ± 2.3</td>
<td>&lt;.05</td>
<td>10.8 ± 2.8*</td>
</tr>
<tr>
<td>A</td>
<td>9.2 ± 1.8</td>
<td>9.3 ± 1.4</td>
<td>NS</td>
<td>9.9 ± 1.9</td>
<td>&lt;.01</td>
<td>12.9 ± 2.8*</td>
</tr>
</tbody>
</table>

LV = left ventricular; P = posterior; A = anterior.

*Probability of difference between dysfunction levels 1 and 2 and between levels 2 and 3 (Bonferroni corrected t test).

**P < .05 compared with control value (Bonferroni corrected t test).

posterior-basal group and one in the anterior-apical group are shown in figures 3 and 4, respectively. Control recordings are shown in the left panels of each figure and characteristic patterns of epicardial segment shortening and transmural wall thickening are evident. Percentage systolic wall thickening, however, greatly exceeds that of percentage epicardial segment shortening. The remaining panels demonstrate the effects of the three different degrees of severe coronary stenosis. Progressive decrements in systolic wall thickening

FIGURE 3. Analog tracings and myocardial blood flow (MBF) data from one of the experiments in the posterior group. Recordings of wall thickness (WTh) and a parallel epicardial segment length (EPI SL) are shown during control (C) conditions and at three different levels of dysfunction (1, 2, and 3) produced by coronary inflow restriction. Blood flow data are presented in three layers across the wall (1, subendocardial third; 2, midmyocardial third; 3, subepicardial third) in tissue containing the dimension gauges in the ischemic zone and in nonischemic or control zone tissue. End-diastole (ED) and end-systole (ES) are indicated with the solid vertical lines. At each level, wall thickening dysfunction is evident and parallels the reductions in deep myocardial perfusion. Subepicardial blood flow was normal at levels 1 and 2 (middle panels) and little change is apparent in parallel epicardial shortening. Only when blood flow was reduced transmurally (level 3, right panel) is marked dysfunction evident in the epicardium.
were produced, which culminated in systolic thinning at dysfunction level 3, and these changes paralleled each other closely in the anterior and posterior examples. Epicardial segment shortening, comparable in both groups during control conditions, showed a different pattern. In the posterior group (figure 3), epicardial shortening was sustained at dysfunction levels 1 and 2 and appeared reduced only at dysfunction level 3. In the anterior group (figure 4) very similar changes in wall thickening and myocardial blood flow distribution were produced but epicardial segment shortening decreased at level 1, deteriorated further at level 2, and was replaced by systolic lengthening at level 3.

Myocardial blood flow. Myocardial blood flow data (ml/min/g) are presented in tables 2 and 3. No statistical comparisons of posterior-basal vs anterior-apical group data are presented in these tables because no significant differences were detected between flows in the posterior and anterior groups during control conditions or at any level of regional dysfunction by profile analysis. Significant parallelism was demonstrated but there were no significant group differences. This means we had achieved our objective of producing similar effects on myocardial blood flow in both groups of experiments. No significant alterations were observed in control zone blood flow (table 3). Examples of representative blood flow data from experiments in the posterior and anterior groups are shown in figures 3 and 4, respectively.

At dysfunction level 1 subendocardial blood flow was consistently reduced, an average of 45% in the posterior-basal group and 28% in the anterior-apical group. A significant reduction (−23%) was also detected in midmyocardial blood flow in the posterior group. No significant change was observed in subepicardial blood flow (table 2). The endocardial-to-epicardial flow ratio decreased in anterior-apical and posterior-basal groups from average values not significantly different from unity to $0.62 \pm 0.15$ (posterior) and $0.71 \pm 0.14$ (anterior).

Dysfunction level 2 was characterized by more substantial decrements in regional blood flow, which were significantly different from those at level 1 in the subendocardium and midmyocardium (table 2). Subendo-
cardiac blood flow decreased by 70% (posterior) and 59% (anterior) from control; midmyocardial flow decreased by 53% (posterior) and 42% (anterior). There was no significant difference in the relative change, as well as absolute data, between the anterior-apical and posterior-basal groups. In the posterior group a significant reduction in subepicardial blood flow (average change -23%) was observed compared with control. This change, however, was not significantly different from level 1 and no significant decrease from control was detected in the anterior group.

**TABLE 2**
Ischemic zone myocardial blood flow (ml/min/g, mean ± SD)

<table>
<thead>
<tr>
<th>ENDO</th>
<th>Control</th>
<th>Dysfunction level 1</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dysfunction level 2</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dysfunction level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>1.01±0.25</td>
<td>0.55±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.05</td>
<td>0.30±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
<td>0.10±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>0.90±0.15</td>
<td>0.65±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.05</td>
<td>0.37±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
<td>0.10±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MID</td>
<td>P</td>
<td>0.95±0.28</td>
<td>0.74±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;.05</td>
<td>0.45±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>A</td>
<td>0.93±0.14</td>
<td>0.81±0.19</td>
<td>&lt;.05</td>
<td>0.54±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
<td>0.16±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>EPI</td>
<td>P</td>
<td>0.96±0.39</td>
<td>0.89±0.41</td>
<td>NS</td>
<td>0.74±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>A</td>
<td>0.92±0.15</td>
<td>0.91±0.20</td>
<td>NS</td>
<td>0.78±0.17</td>
<td>&lt;.05</td>
<td>0.37±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean transmural flow</td>
<td>P</td>
<td>0.98±0.30</td>
<td>0.73±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;.05</td>
<td>0.49±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>A</td>
<td>0.92±0.14</td>
<td>0.79±0.19</td>
<td>&lt;.05</td>
<td>0.56±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
<td>0.21±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ENDO/EPI ratio</td>
<td>P</td>
<td>1.05±0.23</td>
<td>0.62±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
<td>0.41±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>A</td>
<td>0.98±0.05</td>
<td>0.71±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
<td>0.47±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;.01</td>
<td>0.27±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Probability of difference between levels 1 and 2 and between levels 2 and 3 (Bonferroni corrected t test).
<sup>b</sup>p < .05 compared with control values.
<sup>c</sup>p < .01 compared with control values (by Bonferroni corrected t test).

Significant reductions in blood flow to all three myocardial layers were produced at level 3. Subendocardial and midmyocardial blood flows were affected most profoundly (table 2). Subepicardial blood flow decreased 58% (posterior) and 60% (anterior).

**Wall thickness.** Average data on wall thickness are presented in table 4. There was a significant difference in end-diastolic and end-systolic wall thickness between the groups during control conditions, reflecting the thinning of the wall that occurs closer to the apex (anterior-apical group). The extent of control thicken-

**TABLE 3**
Control zone myocardial blood flow (ml/min/g, mean ± SD)

<table>
<thead>
<tr>
<th>ENDO</th>
<th>Control</th>
<th>Dysfunction level 1</th>
<th>p value</th>
<th>Dysfunction level 2</th>
<th>p value</th>
<th>Dysfunction level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.94±0.25</td>
<td>0.98±0.33</td>
<td>NS</td>
<td>0.91±0.27</td>
<td>NS</td>
<td>1.00±0.38</td>
</tr>
<tr>
<td>A</td>
<td>0.99±0.16</td>
<td>1.09±0.19</td>
<td>NS</td>
<td>1.04±0.20</td>
<td>NS</td>
<td>1.06±0.18</td>
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<tr>
<td>MID</td>
<td>P</td>
<td>0.89±0.28</td>
<td>0.95±0.35</td>
<td>NS</td>
<td>0.90±0.34</td>
<td>NS</td>
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<tr>
<td>A</td>
<td>0.96±0.19</td>
<td>1.08±0.20</td>
<td>NS</td>
<td>1.04±0.27</td>
<td>NS</td>
<td>1.05±0.24</td>
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<td>0.88±0.40</td>
<td>NS</td>
<td>0.82±0.36</td>
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<td>NS</td>
<td>0.96±0.23</td>
<td>NS</td>
<td>0.92±0.21</td>
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<tr>
<td>Mean transmural flow</td>
<td>P</td>
<td>0.89±0.30</td>
<td>0.94±0.36</td>
<td>NS</td>
<td>0.87±0.32</td>
<td>NS</td>
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<tr>
<td>A</td>
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<td>1.04±0.20</td>
<td>NS</td>
<td>1.01±0.23</td>
<td>NS</td>
<td>1.01±0.20</td>
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<tr>
<td>ENDO/EPI ratio</td>
<td>P</td>
<td>1.09±0.21</td>
<td>1.11±0.20</td>
<td>NS</td>
<td>1.11±0.19</td>
<td>NS</td>
</tr>
<tr>
<td>A</td>
<td>1.16±0.16</td>
<td>1.14±0.19</td>
<td>NS</td>
<td>1.08±0.11</td>
<td>NS</td>
<td>1.15±0.19</td>
</tr>
</tbody>
</table>

Abbreviations and statistical comparisons as in table 2.
ing, percentage thickening, and mean ejection phase velocity of thickening, however, were not significantly different.

During ischemia, changes produced in wall thickness dimensions paralleled each other closely in both groups. Profile analysis indicated that progressive reductions in end-diastolic wall thickness occurred during coronary stenosis, which were statistically significant by corrected t test at levels 2 and 3 of the anterior group (table 4). Each level of dysfunction was characterized by substantial decrements in parameters of systolic wall function. The degree of change at every level, evaluated by comparison of raw data or by the extent of relative change, was the same for both groups (confirmed by the profile analysis), demonstrating that we had achieved our objective of producing comparable transmural dysfunction in the posterior and anterior groups. The relative reductions in systolic excursion were −38%, −81%, and −117% (greater than −100% indicating dyskinesia or net systolic thinning) at levels 1, 2, and 3, respectively, in the posterior-basal group. The corresponding reductions in the anterior-apical group were −35%, −78%, and −117%. Percentage wall thickening decreased −37%, −80%, and −118% (at levels 1, 2, and 3, respectively) in the posterior-basal group; the changes in the anterior-apical group averaged −34%, −76%, and −120% (table 4). The relative reductions in mean ejection phase velocity of thickening were smaller in both groups than the changes in systolic excursion or percentage wall thickening, in part related to the development of significant isovolumic thinning during coronary stenosis that appeared to increase as a function of ischemic severity. However, the reductions in ejection phase velocity were significant (table 4), and the pattern of relative change (by profile analysis) was the same for the posterior-basal (−29%, −63%, and −93% at levels 1, 2, and 3, respectively) and anterior-apical (−24%, −58%, and −97%) groups.

The relationship between changes in transmural systolic wall thickening and changes in regional myocardial blood flow are depicted in figure 5. Strong dependence of transmural thickening on reductions in subendocardial blood flow (upper graph) is evident. The distribution of data points for changes in wall thickening vs normalized subepicardial blood flow was similar in both groups also, as shown in figure 5 (lower graph), reflecting a pattern in line with the results of previous investigations that indicated relatively poor correspondence between outer perfusion and transmural thickening. 8, 10, 13, 14

**Epicardial segment length.** Data on epicardial segment shortening are presented in table 5. There were no significant differences during control conditions between the anterior-apical and posterior-basal groups in the three parameters of epicardial systolic function: systolic excursion, percentage shortening, or mean ejection phase velocity of shortening. Profile analysis
Demonstrated that systolic excursion and percentage shortening data were not parallel, indicating that the patterns of change in these parameters were significantly different. In the posterior-basal group, no significant change in systolic function was observed at levels 1 and 2 compared with control (table 5). Only at level 3 was significant reduction in epicardial systolic excursion (−73%), percentage shortening (−74%), and shortening velocity (−64%) detected. It is noteworthy that some element of systolic shortening was sustained in five out of six experiments at level 3, despite systolic wall thinning in all six studies.

In the anterior-apical group the response of epicardial segment length to ischemia differed significantly from that in the posterior-basal group. In contrast to that in the posterior group, epicardial systolic function was impaired at levels 1 and 2 (table 5). Systolic excursion and percentage shortening were reduced significantly (by 21% from control) at level 1. A further decrease (−37%) was evident at level 2 and the velocity of shortening was also significantly diminished (−18%). In five of the seven experiments at level 3, epicardial shortening was replaced by lengthening (dyskinesia), but no significant difference in epicardial data between the anterior-apical and posterior-basal groups was demonstrable at this level.

The relative change in epicardial systolic excursion is plotted against relative change in transmural systolic excursion in figure 6. The shape of the relationship (regression equation in the form $y^2 = ax + b$) required to fit the data most appropriately was curvilinear. Statistical comparison of the slope and intercept values derived from the regression analysis of normalized epicardial systolic excursion (y axis) on normalized wall thickening (x axis) demonstrated that the slopes were not significantly different (indicating similarity of the relationship pattern). The intercepts were significantly different, however ($p < .01$), indicating that the anterior-apical epicardial response to ischemia was characterized by greater reductions in shortening than the posterior-basal group (figure 6) at each decrement in wall thickening.

Although a significant difference existed in terms of epicardial function between the anterior-apical and posterior-basal groups, both underestimated the extent of the regional contractile defect (measured as systolic wall thickening). Nearly all of the data points lie above the line of identity (dotted line in figure 6), indicating that epicardial functional changes (measured as parallel segment shortening) underestimate the extent of transmural dysfunction. The same observations were made for function measured as percentage shortening and percentage thickening. Mean ejection phase velocity of shortening was also strongly dissociated from the mean ejection phase velocity of thickening (tables 4 and 5). However, a significant difference was not detected between changes in anterior-apical and posterior-basal shortening velocities (by profile analysis).

**Discussion**

The main findings of this study were: (1) epicardial shortening parallel to local fiber orientation underestimates the extent of transmural dysfunction during acute ischemia limited to deep myocardial layers and (2) this underestimation is more pronounced in the posterior-basal ventricle, perfused by the circumflex artery, than in the anterior-apical ventricle supplied by the left anterior descending artery.
TABLE 5
Epicardial segment length (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dysfunction level 1</th>
<th>p value</th>
<th>Dysfunction level 2</th>
<th>p value</th>
<th>Dysfunction level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDL (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>10.00</td>
<td>10.02 ± 0.13</td>
<td>NS</td>
<td>10.09 ± 0.18</td>
<td>&lt;.05</td>
<td>10.27 ± 0.19</td>
</tr>
<tr>
<td>A</td>
<td>10.00</td>
<td>10.07 ± 0.16</td>
<td>NS</td>
<td>10.14 ± 0.23</td>
<td>&lt;.01</td>
<td>10.48 ± 0.30b</td>
</tr>
<tr>
<td>ESL (mm)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>8.78 ± 0.23</td>
<td>8.79 ± 0.22</td>
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<td>8.94 ± 0.23b</td>
<td>&lt;.01</td>
<td>9.94 ± 0.43c</td>
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<tr>
<td>A</td>
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<td>9.11 ± 0.32</td>
<td>&lt;.01</td>
<td>9.37 ± 0.31b</td>
<td>&lt;.01</td>
<td>10.53 ± 0.67c</td>
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<td>dL (mm)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>1.23 ± 0.14</td>
<td>1.23 ± 0.21</td>
<td>NS</td>
<td>1.15 ± 0.25</td>
<td>&lt;.01</td>
<td>0.33 ± 0.39c</td>
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<tr>
<td>A</td>
<td>1.21 ± 0.14</td>
<td>0.96 ± 0.19</td>
<td>&lt;.05</td>
<td>0.77 ± 0.13c</td>
<td>&lt;.01</td>
<td>−0.06 ± 0.39c</td>
</tr>
<tr>
<td>%dL (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>12.3 ± 2.4</td>
<td>12.3 ± 2.0</td>
<td>NS</td>
<td>11.4 ± 2.4</td>
<td>&lt;.01</td>
<td>3.2 ± 3.7B</td>
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<tr>
<td>A</td>
<td>12.1 ± 1.5</td>
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<td>&lt;.05</td>
<td>7.6 ± 1.4C</td>
<td>&lt;.01</td>
<td>−0.4 ± 3.8C</td>
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<tr>
<td>MEP dL/dt (mm/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P</td>
<td>10.3 ± 1.7</td>
<td>10.4 ± 1.3</td>
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<td>9.6 ± 1.3</td>
<td>&lt;.01</td>
<td>3.7 ± 2.2C</td>
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<tr>
<td>A</td>
<td>8.9 ± 2.2</td>
<td>8.3 ± 2.7</td>
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<td>7.3 ± 2.8C</td>
<td>&lt;.01</td>
<td>2.7 ± 2.5C</td>
</tr>
</tbody>
</table>

EDL = end-diastolic length; ESL = end-systolic length; dL = EDL − ESL; %dL = (dL/EDL) × 100; MEP dL/dt = mean ejection phase velocity of thickening; P = posterior; A = anterior.

*p < .05 compared with control values.

*p < .01 compared with control values (by Bonferroni corrected t test).

Epicardial underestimation of transmural dysfunction.

In an earlier study, epicardial segment lengths (parallel to surface fiber orientation) and subendocardial segment lengths were measured with sonomicrometers in the circumflex-supplied myocardium of open-chest dogs.17 Partial coronary stenosis reduced subendocardial and midmyocardial blood flow but subepicardial perfusion remained at control levels. Segment shortening decreased in the ischemic subendocardium but persisted in the nonischemic subepicardium, consistent with our data in the posterior-basal group at levels 1 and 2 of the present study. We speculated that sustained parallel epicardial shortening may have limited importance in terms of contractile performance across the entire wall.17 This was confirmed in the present study with measurements of transmural systolic wall thickening rather than subendocardial segment length shortening. It is noteworthy that epicardial shortening was observed in the posterior-basal group even when systolic wall thinning had replaced thickening (level 3), emphasizing the degree of dissociation that can exist between epicardial shortening (measured in this manner) and total wall function, measured as systolic thickening.

Only one other study, to our knowledge, has compared epicardial shortening with transmural wall thickening. Heikkila et al.12 implanted radiopaque markers on the endocardium and epicardium of anesthetized pigs. They tracked the motion of the markers with a radiographic technique that enabled them to measure epicardial segment shortening (average 15.7% of end-diastolic length) and wall thickening (average 31.2% of end-diastolic thickness). To evaluate the importance of epicardial shortening, a metal bar was immersed in liquid nitrogen and applied to the epicardial surface of the heart to freeze the tissue, creating an acute subepicardial infarct. Epicardial shortening was eliminated by this maneuver, but there was no significant change detected in the extent of transmural wall thickening in the area of the subepicardial “infarct.”32 Unfortunately, no data on the depth of the frozen and damaged tissue through the wall were reported, but their functional data support the concept that epicardial shortening (or the lack of it) may be relatively unimportant in terms of integrated contractile function across the entire wall. A limited role for epicardial shortening is also consistent with experimental observations demonstrating that systolic thickening of the outer wall contributes a relatively small fraction to total wall thickening during systole.33-35

Using Walton-Brodie strain gauges to measure deep and superficial force development in the anterior wall, other investigators have also documented subendocardial dysfunction without substantial changes in epicardial mechanical performance during nontransmural ischemia.1,3 These findings and ours appear to contrast with those of Weintraub et al.,16 in whose study epicardial and subendocardial segment shortening were mea-
assured in the anterior wall supplied by the left anterior descending artery. Their epicardial segments exhibited dysfunction (during progressive inflow restriction) that closely paralleled reductions in subendocardial blood flow and segment shortening. Because epicardial shortening was markedly impaired even when subepicardial perfusion was normal, they concluded that a substantial tethering effect constrained motion in the outer muscle layers during these conditions.

Our results suggest that Weintraub et al. overestimated the degree of epicardial dysfunction. We think the reason for this discrepancy is related to the alignment of the epicardial dimension gauges. Weintraub et al. reported that they implanted the epicardial gauges parallel to the long axis. As discussed earlier (Results), the surface fiber orientation of myocardium supplied by the left anterior descending artery is not parallel with the long axis. Therefore we suggest that the gauge alignment in the study of Weintraub et al. may have deviated from the actual fiber orientation (and the alignment of our epicardial gauges) by 40 to 50 degrees. We speculate that the observations of Weintraub et al. of marked epicardial dysfunction are the results expected for gauges that are out of alignment with local surface fibers, consistent with the findings we presented in an earlier article addressing the fiber orientation issue relative to epicardial shortening. Thus the difference between our data and those of Weintraub et al. may be primarily methodologic. Their findings, however, raise a potentially important possibility. Epicardial shortening measurements, appropriately out of alignment with local fiber orientation, may provide a means of accurately assessing ischemic changes in transmural function while minimizing myocardial trauma caused by implantation of crystals. A recent study, in which different methods were used, supports the possibility that epicardial shortening characteristics (not parallel to local fibers) provide valid information on contractile performance of deeper myocardial layers.

**Difference between anterior and posterior locations.** The difference in the epicardial response to deep myocardial ischemia (at levels 1 and 2) between the anterior-apical and posterior-basal groups was a consistent observation (figure 6), qualitatively similar to that recently reported by Hattori et al. In this respect, our results are also in agreement qualitatively with those of Weintraub et al. in that significant epicardial dysfunction was observed in the anterior wall without significant restriction of subepicardial flow. We think that the functional disparity is related primarily to differences between the basal and apical regions of the left ventricle. The anterior-apical group data were obtained in the lower third of the left ventricle, while the posterior-basal group data were obtained in the upper half of the ventricle. One contributing factor to this difference may be that the myocardial wall is thinner near the apex compared with the base. Given a wall that averaged 7.6 mm in thickness in the anterior group and 10 mm in the posterior group (table 4), the outer 2 mm of the myocardium containing the dimension gauges would seem to be more sensitive to deep and midlayer ischemia in the thinner wall (anterior-apical area) because 2 mm represents a larger percentage of the total thickness. If this is the case, the discrepancy between anterior and posterior epicardial function may be partly artifactual because of the size of the ultrasonic crystals relative to the thickness of the wall.

An additional factor that may play a more significant role is surface fiber orientation, which varies considerably between the two areas. We defined epicardial contractile function for the purposes of this study as segment shortening parallel to surface fiber orientation. This means that shortening was measured nearly parallel to the long axis in the posterior group but was...
measured at an angle approximately 45 degrees from the long axis in the anterior group. The majority of fibers in the left ventricle course in a circumferential orientation within the middle 50% to 60% of the wall.\(^{29}\) Therefore the posterior epicardial segments were measured nearly perpendicular to this mass of fibers, while those in the anterior group were deviated much less substantially from the circumferential direction.

When the inner and middle layers of the posterior basal wall are ischemic and dysfunctional, a constraining effect is apparent on epicardial shortening measured in a circumferential orientation (i.e., not parallel to surface fiber orientation), even when epicardial shortening parallel to the surface fibers remains normal.\(^{17}\) This suggests that the influence of middle layer (circumferential) fibers on epicardial motion during ischemia is related to how closely the axis of the epicardial measurement approaches the circumferential direction. We propose that the reduced shortening in the nonischemic anterior epicardial segments (levels 1 and 2) is the result of a greater influence of tethering in that area because the orientation of the surface fibers is closer to that of the underlying circumferentially arrayed fibers making up the bulk of the wall. Consequently, a more substantial influence of deep and midlayer ischemia and dysfunction is evident in the anterior-apical group than in the posterior-basal group, where surface fibers are arrayed at a steeper angle relative to the circumferential muscle mass. The relative thinness of the wall in the anterior-apical area could compound the difference. This explanation assumes that the largest fraction of the myocardial muscle is arranged circumferentially, in accordance with the data reported by Streeter,\(^{29}\) an assumption that has not been verified for all parts of the ventricle. In addition, the speculation that the degree of tethering between “layers” depends on the relative orientation of the layers to one another will require additional study.

We would like to express our appreciation to Russell A. Grinage and Thomas B. McClanahan for expert technical assistance and to Tarry Goble for word processing. We also thank Dr. Tony Schork (Department of Biostatistics, School of Public Health) for statistical advice and Dr. Gregory Freeman (Department of Medicine, University of Texas, San Antonio) for valuable discussions on issues relating to fiber orientation and contractile performance.

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_Circulation_. 1985;71:1279-1291
doi: 10.1161/01.CIR.71.6.1279

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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