The functional border zone in conscious dogs

KIM P. GALLAGHER, PH.D., RICHARD A. GERREN, PH.D., XUE-HAN NING, M.D., SHAUN P. MCMANIMON, B.S., MACK C. STIRLING, M.D., MARSHAL SHLAFER, PH.D., AND ANDREW J. BUDA, M.D.

ABSTRACT Studies focusing on the functional border zone have been performed largely with anesthetized, open-chest preparations. Therefore, we instrumented 14 dogs at sterile surgery with sonomicrometers arrayed to measure systolic wall thickening across the perfusion boundary produced by circumflex coronary occlusion. We fitted sigmoid curves to the data to model the distribution of wall thickening impairment as a function of distance from the perfusion boundary, which was delineated with myocardial blood flow (15 μm diameter microspheres) maps. Using this approach, we defined the functional border zone as the distance from the perfusion boundary to 97.5% of the sigmoid curve’s nonischemic asymptote. The lateral extent of the functional border zone, measured 10 min and 3 hr after occlusion, was 32 and 28 degrees of circumference, respectively. To evaluate the severity of nonischemic dysfunction, we measured average systolic wall thickening within the functional border zone. It was reduced from 3.69 ± 1.10 (mean ± SD) mm to 2.98 ± 1.07 mm (p<.01) and 2.74 ± 1.12 mm (p<.01) early and late after coronary occlusion. Thus, a narrow functional border zone was evident during circumflex coronary occlusion in conscious dogs. Its lateral extent was limited to approximately 30 degrees (similar to findings in open-chest, anesthetized dogs), severe dysfunction was restricted to the immediate vicinity of the perfusion boundary, and the average severity of nonischemic dysfunction within the functional border zone was mild.


NONISCHEMIC regional dysfunction at the lateral borders of damaged or ischemic myocardium has been demonstrated in several studies. To identify myocardium exhibiting nonischemic contractile dysfunction adjacent to the ischemic area, the term “functional border zone” was introduced recently. Describing this area in terms of contractile performance distinguishes it from the conventional concept of a border zone defined in terms of myocardial blood flow distribution, histology, or biochemical changes. The functional border zone begins at the sharply delineated interface between ischemic and nonischemic tissue and extends laterally into normally perfused myocardium. Most previous investigations were performed with anesthetized, open-chest preparations. For example, studies from our laboratory demonstrated that contractile dysfunction, measured with sonomicrometers or two-dimensional echocardiography, extended 25 to 30 degrees of circumference into nonischemic myocardium during occlusion of the circumflex artery. The functional border zone, however, has been evaluated in few studies with conscious animals. Because there are substantial differences between conscious and anesthetized dogs and because the lateral extent of nonischemic myocardial dysfunction may be important in the clinical evaluation of ischemic risk area and infarct size, we performed the following study. Our objectives were to measure the distribution of functional impairment across the lateral margins of the ischemic area and to determine the extent and severity of the functional border zone in conscious dogs.

Sonomicrometric measurements of systolic wall thickening were made in conscious dogs before and after occlusion of the left circumflex artery. Myocardial blood flow was measured with tracer-labeled microspheres and circumferential perfusion maps were constructed to delineate the position of the perfusion boundary and identify the location of the functional measurements. We evaluated the distribution of func-

From the Thoracic Surgery Research Laboratory, Departments of Surgery (Thoracic Section) and Physiology, and the Departments of Internal Medicine (Cardiology Division) and Pharmacology, The University of Michigan Medical School, Ann Arbor.

Supported in part by NIH grants ROI HL32043 and ROI HL29716. Dr. Gallagher is recipient of NIH Research Career Development Award K04 HL04120. Dr. Ning is recipient of NIH Fogarty International Fellowship F05 TW03542.

Address for correspondence: Kim P. Gallagher, Ph.D., Thoracic Surgery Research Laboratory, 3484 Kresge I, Box 0548, The University of Michigan, Ann Arbor, MI 48109.

Received Dec. 29, 1986; revision accepted July 2, 1987.

Presented in part at the American Heart Association Scientific Sessions, November 1985, Washington, D.C.

Vol. 76, No. 4, October 1987
tional impairment across the perfusion boundary by fitting sigmoid curves to the wall thickening data during coronary occlusion.

Methods

Animal preparation. The study was performed in conditioned mongrel dogs weighing between 21 and 29 kg. After induction with sodium thiopental, anesthesia was maintained with halothane and a sterile thoracotomy was performed through the left fifth intercostal space. To measure left ventricular pressure (figure 1), a Konigsberg (P7) high-fidelity micromanometer was introduced through the apex of the left ventricle. Tygon (2.2 mm inside diameter) catheters were placed in the left atrium (for injection of microspheres) and left ventricle (via the apex for calibration of the micromanometer in mm Hg). The proximal circumflex artery was dissected free to allow placement of a hydraulic occluder that was used to produce coronary occlusion. In eight of the dogs, a pulsed Doppler flow probe was placed around the circumflex artery proximal to the occluder to monitor coronary blood flow velocity. The signals from the flow probe were processed with a flowmeter constructed at the Dalton Research Center, University of Missouri (Dean Franklin, Mort Caldwell; Columbia, MO).

Regional myocardial function was measured with sonomicrometers arrayed to measure transmural wall thickness. The sonomicrometers were implanted in four locations, as schematically depicted in figure 1. One pair was placed in the central ischemic area perfused by the left circumflex artery and a second pair was placed in the central nonischemic (or control) area, perfused by the left anterior descending artery. The remaining pairs were placed on either side of the estimated position of the perfusion boundary (inset, figure 1) produced by occluding the circumflex artery. As a guide to estimate the position of the perfusion boundary we used the epicardial vascular anatomy. Previous experience indicated that the boundary produced by circumflex occlusion was approximately midway between the epicardial branches of the circumflex and left anterior descending arteries. As shown in the inset of figure 1, one crystal of each wall thickness pair was in the subendocardium and the other crystal was attached to the epicardium, over the position of the inner crystal. The inner crystal was inserted tangentially through the myocardium to the endocardium. The epicardial crystal, attached to a Dacron patch, was sewn to the epicardium with shallow sutures after locating the position of least distance between the two crystals while monitoring the signals with an oscilloscope. The signals from the ultrasonic dimension gauges were processed with a Triton (Model 120) sonomicrometer. The criterion for acceptance of functional data from the dimension gauges was the position of the wall thickness measurements in myocardial blood flow maps, which were used to define the position of the perfusion boundary. Data were not used if crystals were improperly aligned. Location of the inner crystals within the subendocardial third of the myocardial wall and correct alignment of the crystals across the wall was confirmed at the time of autopsy during the careful sectioning required for tissue sample preparation to determine myocardial blood flows.

The thoracotomy was closed after bringing the wires and catheters subcutaneously to the back of the neck. The dogs were

![FIGURE 1](image-url) Schematic depiction of the instrumentation used in the dogs of this study. Sonomicrometers were implanted in four locations, on both sides of the perfusion boundary produced by circumflex coronary occlusion (dashed line on the heart). The lower right inset indicates the manner in which ultrasonic crystals were arrayed to measure wall thickness. One crystal of each pair was positioned near the endocardium to act as a receiver (REC) of ultrasound from the transmitter (TRANS) crystal sutured to the epicardial surface. LV = left ventricular; LA = left atrial; RV = right ventricle; LAD = left anterior descending artery; LCX = left circumflex artery; WT = wall thickness; IS = ischemic; IS BZ = ischemic border zone; NIS BZ = nonischemic border zone; NIS = nonischemic (control).
treated with antibiotics and given morphine sulfate (0.13 to 0.25 mg/kg) for postoperative analgesia. Studies were performed 14 to 21 days after surgery, when the dogs exhibited normal activity and were afebrile.

**Myocardial blood flow measurements.** Regional myocardial blood flow was measured with tracer-labeled microspheres (15 μm diameter, New England Nuclear) by the reference withdrawal method. Three injections were made in every experiment with the use of one of six available isotopes (14C, 125I, 51Cr, 103Ru, 99mTc, 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 2 million microspheres were injected into the left atrium for the measurement of blood flows. The reference arterial sample was obtained from the femoral artery at a constant rate with a Harvard withdrawal pump; withdrawals were initiated before the injection of microspheres and completed 2 min later. Each bottle of microspheres was placed in an ultrasonic bath and was 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 KCl after the induction of deep anesthesia with sodium pentobarbital.

Each heart was removed and placed in formalin to facilitate sectioning. Dimension gauges were left in the heart to allow careful evaluation of their position in the wall at the time of heart sectioning. 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. The location of each piece of tissue and the position of the ultrasonic crystals were recorded, and the tissue samples were weighed and placed in counting vials for assay of radioactivity in a Tracor (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:

\[ Q_m = \frac{C_m \times Q_r}{C_r} \]

where \( Q_m \) = myocardial blood flow (ml/min); \( C_m \) = counts/min in tissue samples; \( Q_r \) = withdrawal rate of the reference arterial sample (ml/min); \( C_r \) = counts/min 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 plus microcomputer.

These procedures enabled construction of myocardial blood flow maps around the circumference of the left ventricle in which the position of the dimensional measurements could be located. Tissue samples in myocardium straddling the perfusion boundary were approximately 3 mm wide at the endocardium and weighed 200 to 500 mg, similar to the experimental preparations described by Murdock et al. and Hearse et al. Given the small size of the tissue samples, the number of microspheres in the ischemic samples was undoubtedly very low. We have demonstrated previously, however, that accurate delineation of the boundary between ischemic and nonischemic territories is possible by this approach. Although the estimates of blood flow in the ischemic samples may have a large statistical error, associated with them, the ischemic zone flows were so low that even a large error would influence the absolute flow values minimally.

The location of the “perfusion boundary” was determined by the position of the flow gradient in the circumferential blood flow map, as shown in figure 2. This figure shows blood flow maps from one of the experiments included in the present study. The locations of the sonomicrometers are indicated with the cross-hatched bars, the heights of which convey the relative change in wall thickening after coronary occlusion.

To provide a reference point for localizing the dimension measurements, we used the position of the perfusion boundary, which we defined as the junction between the high and low flow samples (figure 2). Because the true boundary is irregular in its course from endocardium to epicardium, tissue samples containing the irregular boundary are characterized by intermediate levels of blood flow, depending on the relative proportions of ischemic and nonischemic tissue. The point we called the “perfusion boundary” lies within a transitional area. The width of the transitional area may be narrow (as in figure 2) or wide depending on the irregularity of the true perfusion boundary and how well we cut the tissue samples to follow the true boundary. When tissue samples with intermediate blood flow values were encountered, the position of the perfusion boundary (used as the reference location for the sonomicrometers) was defined as passing through the center of the tissue sample if the level of intermediate blood flow was between 40% and 60% of the nonischemic area average. If blood flow was less than 40% or greater than 60% of the nonischemic average, the perfusion boundary was assigned to the left or right margins, respectively, of the intermediate flow sample. In so doing, we established a reference point that represents an estimate of the true perfusion boundary’s location. Because the true boundary is variably

![Figure 2](http://circ.ahajournals.org/)

**FIGURE 2.** Examples of circumferential maps of myocardial blood flow (MBF) from one of the experiments in this study. Blood flow in the subendocardium (ENDO, closed circles) and subepicardium (EPI, open circles) is shown. Sharp demarcations between ischemic and nonischemic areas are evident early (TCO1, top) and late (TCO2, bottom) after circumflex coronary occlusion. Although the position of the perfusion boundary was not changed, a trend for an increase in ischemic area blood flow is apparent, especially in the subepicardium, approximately 3 hr after occlusion. Three of the wall thickness measurements were made in this ventricular cross section and their locations are indicated with cross-hatched bars. The height of the bars (referenced to the right y axis) represents the relative change in thickening (dWT) compared with control conditions.

Vol. 76, No. 4, October 1987
irregular rather than a straight line, the specific locations we
designated for the perfusion boundaries are approximations
associated with a potential error of up to ±2 mm at the endo-
cardium (or ±5 to 7 degrees of circumference).

**Experimental protocol.** Systolic wall thickening and myo-
cardial blood flow measurements were made in 14 dogs. Ex-
periments were carried out with animals lying on their right sides
on a table in the laboratory. Morphine sulfate (0.5 mg/kg) was
given for analgesia 60 min before coronary occlusion. Addi-
tional morphine sulfate (0.13 mg/kg) was administered after
occlusion if the dogs exhibited signs of discomfort or pain. A
femoral artery cutdown was performed under local anesthesia to
insert an arterial catheter for measurement of arterial blood
pressure and for withdrawing a reference sample for myocardial
blood flow calculations. After completing control recordings and
injecting the first set of microspheres, the circumflex artery was
abruptly occluded with the hydraulic occluder. Occlusion was
verified by cessation of coronary blood flow velocity and/or
prompt elimination of systolic wall thickening. Ten minutes
after occlusion, when hemodynamics and regional dimensions
were stable, a second injection of microspheres was made. A
third injection of microspheres was made while simultaneously
recording hemodynamic and dimension variables approximately
3 hr after occlusion.

**Data analysis.** Recordings were made during each experi-
ment on an eight-channel Hewlett-Packard pressurized-ink
recorder and on magnetic tape for subsequent analysis. Variables
analyzed were 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 20 msec before peak negative
dP/dt), extent of wall thickening, mean ejection phase velocity
of thickening, left ventricular systolic and end-diastolic pressure,
heart rate, peak positive and negative dP/dt, and regional
myocardial blood flows. The extent of wall thickening was
calculated as the difference in millimeters between end-diastolic
and end-systolic dimensions and was also expressed as a per-
centage change from end-diastolic wall thickness. Dimensional
and hemodynamic data were analyzed by digitizing the recorded
data from analog tape with a DEC Micro PDP-11 computer
system. Ten to 20 cardiac cycles were averaged at each condi-
tion, using the beats occurring during the microsphere injec-
tions. The blood flow values reported from the ischemic and
nonischemic border zones represent blood flow from the tissue
samples spanned by the individual dimension measurements.
Central ischemic and nonischemic blood flows are averages from
several tissue samples remote from the perfusion boundary.

Hemodynamic, dimension, and myocardial blood flow data
were analyzed at three time periods with analysis of variance:
under control conditions, 10 min after total coronary occlusion,
and 3 hr after coronary occlusion. Paired t tests, adjusted for
multiple comparisons with the Bonferroni inequality, 19 were
used to discriminate which conditions differed significantly
when analysis of variance demonstrated a significant overall
change. Unpaired t tests were used to compare wall thickness
variables and blood flow data at four locations: (1) the central
ischemic area, (2) the ischemic border zone (defined as ischemic
myocardium 10 mm or less from the perfusion boundary), (3)
the nonischemic border zone (nonischemic myocardium less
than 10 mm from the perfusion boundary), and (4) the central
nonischemic or control area. Wall thickness data from myo-
cardial samples with intermediate blood flow values at the per-
fusion boundary were not included in the categorical analysis.
Because multiple comparisons were performed, the acceptable
alpha level was adjusted to 0.0083 (0.05 divided by six, the
number of possible comparisons) with the Bonferroni in-
equality. 19 When p<.05 or p<.01 is indicated in the tables or
text, it represents the corrected value.

In addition to the categorical analysis, we also evaluated wall
thickening data as a continuous function of distance from the
perfusion boundary. Sigmoid curves were fit to the wall thick-
ening data from individual experiments and composite data sets,
as described previously. 8 Because the sonomicrometers provide
measurements of regional function in a limited number of loca-
tions, we wanted a means of extrapolating the discrete data in
continuous terms. By fitting sigmoid curves to the wall thick-
ening data, the disadvantage of making relatively few measure-
ments in the vicinity of the perfusion interface could be mini-
mized. To mathematically model the distribution of wall
thickening change across the perfusion boundary we assumed
that the nonischemic tissue had a wall thickening asymptote, N,
and that ischemic tissue had a wall thinning asymptote, I. The
function we used that has these asymptotes and also changes
monotonically between them is the following:

\[ y = 1 + \frac{N-1}{(2\pi)^2} \int \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) dx \]

The value \( \mu \) corresponds to the position on the x axis of the
midpoint of the change between the asymptotes and \( \sigma \) is a value
that describes how rapidly the change is made. The modeling
function was fit to the data from the individual experiments in
which at least four dimension measurements spanning the per-
fusion boundary were available. The modeling function was also
fit to the pooled data from all of the dogs. To fit the sigmoid
curves to the wall thickening data, we minimized the variance
and used computerized nonlinear minimization techniques, as
previously described. 8 We used the sigmoid curve fits to define
the lateral extent of the functional border zone as the distance
(in degrees) from the perfusion boundary to 97.5% of the non-
ischemic asymptote, equal to \( \mu + 2 \sigma \). 8

**Results**

**Hemodynamics and blood flow.** Hemodynamic data are
summarized in table 1. Heart rate increased from 82 ±
23 (mean ± SD) to 123 ± 26 beats/min (p<.01) 10
min after circumflex coronary occlusion and remained
elevated significantly 3 hr after occlusion. Left ven-
tricular systolic pressure was not significantly changed
but end-diastolic pressure increased from 13.4 ± 4.8
to 18.6 ± 6.2 (p<.01) and 18.1 ± 6.8 mm Hg (p<.01)
10 min and 3 hr after occlusion, respectively. Peak
positive dP/dt decreased an average of 11% (p<.05)
from the control value of 3501 ± 576 mm Hg/sec early
after occlusion (table 1), indicating that there was a
significant global effect of circumflex occlusion. No
significant changes, however, were detected in peak
negative dP/dt.

Myocardial blood flow data are summarized in table
2 and examples of blood flow maps from one of the
experiments are shown in figure 2. Complete blood
flow data were available from 11 of the 14 dogs; com-
plete data from three of the dogs could not be used
because of technical problems with the withdrawal
samples. Perfusion boundaries, however, could be
delineated in all 14 experiments. There were no sig-
nificant differences in myocardial blood flow between
Table 1
Hemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>TCO1</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TCO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>82 ± 23</td>
<td>123 ± 26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NS</td>
<td>113 ± 37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>132 ± 16</td>
<td>125 ± 12</td>
<td>NS</td>
<td>132 ± 18</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>13.4 ± 4.8</td>
<td>18.6 ± 6.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NS</td>
<td>18.1 ± 6.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(+ dP/dt) (mm Hg/sec)</td>
<td>3501 ± 576</td>
<td>3130 ± 434&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
<td>3021 ± 606</td>
</tr>
<tr>
<td>(- dP/dt) (mm Hg/sec)</td>
<td>2497 ± 624</td>
<td>2293 ± 360</td>
<td>NS</td>
<td>2495 ± 674</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

C = control; TCO1 = early coronary occlusion (10 min); TCO2 = late coronary occlusion (2.5 to 3.0 hr); HR = heart rate (n = 14); LVSP = left ventricular systolic pressure (n = 14); LVEDP = left ventricular end-diastolic pressure (n = 14); (+ dP/dt) = peak positive first derivative of left ventricular pressure (n = 13); (- dP/dt) = peak negative first derivative of left ventricular pressure (n = 13).

<sup>a</sup>Probability of difference between TCO1 and TCO2 groups.
<sup>b</sup>p < .05.
<sup>c</sup>p < .01, compared with control values.

Experiments are shown in figure 3 to illustrate the rapid onset of dimensional alterations after coronary occlusion. Beat-averaged waveforms from the same experiment are presented in figure 4 to demonstrate in closer detail the pattern of changes we observed in regional function. The corresponding myocardial blood flow map is presented in figure 2. Under baseline conditions, wall thickness data were similar in all four locations. There were no significant differences among the locations in the extent of wall thickening, percentage wall thickening, or mean ejection phase velocity of thickening (table 3). All of the control condition variables of regional systolic performance were within the normal range reported for conscious animals.

When the circumflex artery was occluded, striking changes occurred in the central ischemic area and ischemic border zone (figure 3). End-diastolic wall thickness decreased by 5% in the ischemic region but was not significantly changed in the nonischemic border zone or central nonischemic area (table 3). The average distances from the perfusion boundary of the wall thickening measurements were 71 ± 19 degrees or 21 ± 7 mm (of endocardial circumference) in the central ischemic area, 11 ± 9 degrees or 3 ± 2 mm in the ischemic border zone, 18 ± 9 degrees or 5 ± 2 mm in the nonischemic border zone, and 51 ± 11 degrees or 15 ± 4 mm in the central nonischemic area.

Relative changes in wall thickening after coronary occlusion are depicted graphically in figure 5 to summarize the results of the categorical analysis. In the central ischemic area and ischemic border zone, systolic wall thickening was eliminated, on the average, 10 min after occlusion. There were no significant differences between the two locations in terms of the extent of systolic thickening, percentage thickening, or mean ejection phase velocity of thickening (table 3). Small, statistically significant improvement in thickening was detected 3 hr after coronary occlusion, but both the central ischemic area and ischemic border zone remained characterized by severe dysfunction (figure 5).

In the nonischemic border zone (10 mm or less from the perfusion boundary) systolic wall thickening was reduced significantly from 3.69 ± 1.10 to 2.98 ± 1.07 mm (−19%) 10 min after coronary occlusion. The marked disparity between wall thickening on each side of the perfusion boundary is illustrated in figures 3 and 4. The ischemic and nonischemic border zone dimension gauges, separated by 9 mm in this example, were characterized by dyskinetic and moderately reduced wall thickening, respectively. After 3 hr of coronary occlusion, nonischemic border zone wall thickening.

The size of the ischemic area averaged 44 ± 4% (157 ± 14 degrees of circumference) of the left ventricular cross sections containing sonomicrometers. There were no significant differences between blood flow in the central ischemic area and that in the ischemic border zone at 10 min postocclusion. At 3 hr after occlusion, small but significant increases in blood flow to the circumflex bed occurred that were most pronounced in the ischemic border zone, the periphery of the ischemic area (table 2). There were no significant differences, however, detected between perfusion in the central ischemic area and ischemic border zone at 3 hr after occlusion.

Ten minutes after circumflex occlusion, perfusion in the nonischemic area was augmented by approximately 30%, consistent with the elevated heart rate observed at that time. Blood flow in tissue samples containing nonischemic border zone sonomicrometers was not significantly different from that in the central nonischemic area at 10 min or 3 hr after occlusion (table 2). This means we were successful in implanting the nonischemic border zone sonomicrometers in normally perfused tissue.

Wall thickness data. These data are summarized in table 3. Examples of analog tracings from one of the the dogs with flow probes and those without flow probes.

Under baseline conditions, blood flow was similar in all four locations. After circumflex coronary occlusion, blood flow decreased drastically in the ischemic area and a steep perfusion boundary was evident (figure 2).
averaged 2.74 ± 1.12 mm (− 26% compared with baseline conditions), which did not constitute a significant change compared with the data obtained early after occlusion.

In contrast to the nonischemic border zone, wall thickening in the central nonischemic category (greater than 10 mm from the perfusion boundary) was not significantly changed compared with baseline (table 3). Evident in figure 5, however, is the tendency for wall thickening to increase in the nonischemic area. Under baseline conditions, there was no difference in wall thickening between the nonischemic border zone and central nonischemic area. After coronary occlusion, central nonischemic wall thickening significantly exceeded thickening closer to the perfusion boundary in the nonischemic border zone in absolute (table 3) or relative (figure 5) terms.

To express the discrete wall thickening information as a continuous function of distance from the perfusion boundary, sigmoid curves were fitted to the thickening data after coronary occlusion. In figures 6, 7, and 8 regional contractile performance is plotted on the y axis as a percentage of control condition wall thickening (equal to 100%). Each data point corresponds to one wall thickness measurement and its position relative to the x axis corresponds to the distance of the measurement from the perfusion boundary which is designated as zero. Positive numbers (right of the perfusion boundary) indicate nonischemic myocardium, and negative numbers indicate ischemic myocardium.

Four (or more) wall thickness measurements were available for eight of the 14 dogs, which was the minimum number we required to apply sigmoid fits to the data from individual experiments. Plots from each of the eight experiments are presented in figures 6 and 7 to illustrate that the quality of the fits was excellent and to demonstrate the range of variability we encountered across experiments. The size of the functional border zone (defined as the distance from the perfusion boundary to 97.5% of the nonischemic asymptote, equal to μ + 2σ) averaged 32 ± 17 degrees at 10 min after circumflex coronary occlusion. The composite data set and sigmoid curve from all 14 experiments are shown in figure 8. The composite value for the size of the functional border zone, using all available data, was 32 degrees (29 degrees for the composite data set limit.
TABLE 3
Transmural wall thickness data in four myocardial zones under control conditions and 10 min and 3 hr after circumflex coronary occlusion

<table>
<thead>
<tr>
<th></th>
<th>Ischemic area (n = 8)</th>
<th>Ischemic border zone (n = 14)</th>
<th>Nonischemic border zone (n = 14)</th>
<th>Nonischemic area (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>p value</strong></td>
<td><strong>p value</strong></td>
<td><strong>p value</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDWT (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>9.07 ± 1.35</td>
<td>NS</td>
<td>10.38 ± 1.28</td>
<td>NS</td>
</tr>
<tr>
<td>TCO1</td>
<td>8.63 ± 1.29</td>
<td>NS</td>
<td>9.90 ± 1.30C</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>p valueA</td>
<td>NS</td>
<td>&lt;.05</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>TCO2</td>
<td>8.99 ± 1.40</td>
<td>NS</td>
<td>10.19 ± 1.35</td>
<td>NS</td>
</tr>
<tr>
<td>ESWT (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>12.63 ± 1.58</td>
<td>NS</td>
<td>13.87 ± 1.99</td>
<td>NS</td>
</tr>
<tr>
<td>TCO1</td>
<td>8.43 ± 1.39C</td>
<td>NS</td>
<td>9.91 ± 1.63C</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>p valueA</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>TCO2</td>
<td>9.05 ± 1.55C</td>
<td>NS</td>
<td>10.44 ± 1.59C</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>dWT (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.56 ± 0.66</td>
<td>NS</td>
<td>3.49 ± 0.92</td>
<td>NS</td>
</tr>
<tr>
<td>TCO1</td>
<td>-0.20 ± 0.36C</td>
<td>NS</td>
<td>0.01 ± 0.70C</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>p valueA</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>TCO2</td>
<td>0.06 ± 0.37C</td>
<td>NS</td>
<td>0.25 ± 0.68C</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>%dWT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>40.0 ± 9.6</td>
<td>NS</td>
<td>33.3 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>TCO1</td>
<td>-2.3 ± 4.05C</td>
<td>NS</td>
<td>-0.2 ± 7.2C</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>p valueA</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>TCO2</td>
<td>0.5 ± 3.9C</td>
<td>NS</td>
<td>1.9 ± 6.2C</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>MEP dW/dt (mm/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>18.3 ± 2.1</td>
<td>NS</td>
<td>18.2 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>TCO1</td>
<td>0.8 ± 1.3C</td>
<td>NS</td>
<td>2.1 ± 2.2C</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>p valueA</td>
<td>NS</td>
<td>&lt;.05</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>TCO2</td>
<td>1.8 ± 1.6C</td>
<td>NS</td>
<td>2.9 ± 2.7C</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

C = control; TCO1 = early coronary occlusion (10 min); TCO2 = late coronary occlusion (3 hr); EDWT = end-diastolic wall thickness; ESWT = end-systolic wall thickness; DWT = ESWT − EDWT; %dWT = (DWT/EDWT) × 100; MEP dW/dt = mean ejection phase velocity of thickening.

*Probability of differences between or across groups.

*p < .05.

*p < .01, compared with control values.

lated to the eight individual experiments shown in figures 6 and 7). Severe dysfunction (defined as wall thickening reduced to less than 50% of baseline values) extended 6 ± 5 degrees beyond the perfusion boundary. In the composite sigmoid curve, the value was also 6 degrees.

The functional data and sigmoid curve fits 3 hr after coronary occlusion from six of the individual experiments are included in figures 6 and 7. We were unable to apply sigmoid fits to data at 3 hr from two of the animals because of problems in maintaining technically satisfactory waveforms from all four dimension gauges. There was a consistent tendency for the asymptotes to contract, compared with the early occlusion curve fits. The size of the functional border zone, however, was not changed. It averaged 27 ± 10 degrees at 3 hr of occlusion, which was not significantly different from the average of 25 ± 9 degrees obtained in the same six experiments after 10 min of occlusion. The composite data from all of the experiments at 3 hr of occlusion (included in figure 8) yielded a comparable value for the extent of nonischemic dysfunction, 28 degrees. Severe dysfunction extended 5 degrees in the composite sigmoid curve (6 ± 5 degrees in the six individual experiments).

Discussion

The objective of this study was to characterize the functional border zone in conscious dogs. By using sonomicrometers we were able to make precise measurements of regional wall thickening, the locations of which could be identified accurately relative to the
position of the perfusion boundary. Sonomicrometers provide narrow measurements oriented in a radial direction. Consequently, they are sensitive to local systolic thinning during ischemia but are relatively insensitive to circumferential bulging, which may influence measurement techniques that “average” function over a wider area, such as segment length shortening or cross-sectional echocardiography. Unlike some applications of two-dimensional echocardiography, sonomicrometers do not require external references that may introduce error into the calculations of wall thickening.7

Relatively few sonomicrometers, however, can be implanted in each animal. Therefore, limited sampling is a potential drawback to the use of this approach for evaluation of functional changes across large areas of myocardium. To minimize the sampling problem, we fitted sigmoid curves to the wall thickening data to

---

FIGURE 3. Examples of analog recordings from the same experiment shown in figure 2. Left ventricular pressure (LVP), measured with a Konigsberg micromanometer, is shown in the top row; dP/dt, derived with a differentiating circuit, is shown in the bottom row. Wall thickness (WT) tracings, shown in the middle four rows, were obtained by implanting sonomicrometers in four locations: the central ischemic area (IS), ischemic border zone (IS BZ), nonischemic border zone (NIS BZ), and central nonischemic area (NIS). End-diastole (ED) and end-systole (ES) are indicated with solid vertical lines. Baseline recordings, shown at high paper speed on the left, illustrate that comparable levels of wall thickening were measured in all four locations before occlusion. In the center portion, slow paper speed recordings demonstrate the rapid onset of dimensional and hemodynamic changes after the onset of circumflex coronary occlusion. Electronic artifacts (“jumped cycles”) were evident soon after occlusion in the IS WT signal, but were eliminated by adjustment of the sonomicrometer unit. High paper speed recordings (right) show that substantial dysfunction developed on the ischemic side of perfusion boundary. Thickening close to the perfusion boundary on the nonischemic side (NIS BZ WT) was reduced compared with baseline, but further from the perfusion boundary (NIS WT), wall thickening was unchanged.
model the distribution of functional impairment across the perfusion boundary. The sigmoid curves complemented the categorical analysis of data (table 3), provided a formal means of describing the data distribution, and enabled quantitative estimation of the extent of nonischemic dysfunction.

The principle source of error in the position of the sigmoid curves is related to the accuracy with which we determined the position of the perfusion boundary. Although the boundary between ischemic and nonischemic myocardial cells is sharp, it is irregular,18 with peninsulas of ischemic and nonischemic tissue interdigitating with one another.23–28 Consequently, the position we designated for the perfusion boundary is approximate and may be associated with a potential error of up to 2 mm (or roughly 5 to 7 degrees of circumference). We attempted to convey this point in figures 6 to 8 by bracketing the position of the perfusion boundary to indicate that its designated location is contained within a transition zone representing an admixture of ischemic and nonischemic tissue. It is important to note, however, that an adjustment of ± 5 degrees in the positions of the sigmoid curves (figures 6 to 8) would result in minor modification of our results and no change in our conclusions.

Another potential source of error associated with use of curve fitting to model the distribution of wall thickening impairment is the type of curve that is used. The shape of the curve itself will influence the derived estimates of lateral nonischemic dysfunction. A sigmoid curve probably oversimplifies the relationship between location and contractile performance during ischemia, but the quality of the individual and composite fits (figures 6 to 8) suggests that a sigmoid curve represents a reasonable approximation.

In addition, the nonischemic asymptote of the sigmoid curves is dictated primarily by the wall thickening measurements located the farthest distance from the

**FIGURE 4.** Beat-averaged waveforms of wall thickness (WT) during control (CONT) conditions, 10 min after coronary occlusion (TCO1), and 3 hr after coronary occlusion (TCO2) from the same experiment shown in figures 2 and 3. The tracings represent average waveforms from 20 digitized cardiac cycles in the four location categories described in the text. End-diastole (ED) is indicated with the dashed vertical line and end-systole (ES) is indicated with the open diamond. Percentage wall thickening values under control (C) conditions and early (1) and late (2) during occlusion are superimposed on each panel. Wall thickening was replaced by wall thinning in the central ischemic area (IS) and ischemic border zone (IS BZ) 10 min after circumflex occlusion. At 3 hr after occlusion, improvement in both dimensions was evident, but each remained characterized by severe dysfunction. In the nonischemic border zone (3 mm or 12 degrees from the perfusion boundary) wall thickening was reduced by approximately 30% from control. In the central nonischemic (NIS) or control area (16 mm from the perfusion boundary), wall thickening was maintained at control levels after coronary occlusion.

**FIGURE 5.** Relative changes in wall thickening (dWT) during coronary occlusion. Data are presented as percentages of control values in the four categories defined by location of the sonomicrometers relative to the perfusion boundary (PB): central ischemic area (IS), ischemic border zone (IS BZ), nonischemic border zone (NIS BZ), and central nonischemic area (NIS). Data (mean ± SD) 10 min after circumflex occlusion are shown as closed circles; data at 3 hr after occlusion are shown as open circles. The corresponding absolute data are presented in table 3. The salient features of the figure are: (1) the similarity of dysfunction in the center (IS) and periphery (IS BZ) of the ischemic area, (2) the substantial difference in wall thickening on either side of the PB, although the IS BZ and NIS BZ sonomicrometers were separated by an average of only 29 degrees (or 8 mm of endocardial circumference), (3) the small but significant reduction in NIS BZ wall thickening (in normally perfused tissue an average of 18 degrees or 5 mm from the PB), and (4) the disparity between nonischemic wall thickening near (NIS BZ) and far (NIS) from the PB.
perfusion boundary (nonischemic area category). The assumption was made that they were representative of remote nonischemic function. It is possible, however, that what we described as an asymptote would have a significant slope if wall thickening were augmented in myocardium that we were unable to sample with the sonomicrometers, such as in the septum. Accordingly, we must acknowledge that our estimates of lateral nonischemic dysfunction are limited by how well the nonischemic area sonomicrometers reflect function remote from the perfusion boundary. That they did reflect the relative lack of change in nonischemic wall thickening accurately is suggested by the preliminary report of Buda et al. They measured wall thickening with two-dimensional echocardiography in full circumference cross sections of the left ventricle in conscious dogs before and after circumflex coronary occlusion. In the nonischemic myocardium of the interventricular septum, directly opposite the central ischemic area, no significant change in percentage wall thickening was observed acutely after circumflex occlusion, similar to our results with sonomicrometers in the nonischemic area situated closer to the perfusion boundary (table 3).

The combined data and composite sigmoid curves in figure 8 illustrate our main findings. A narrow zone of intermediate function was evident between the dyskinetic, ischemic area and normally contracting, nonischemic myocardium. Although not as sharply de-

**FIGURE 6.** Individual examples of sigmoid curves fitted to wall thickening data during coronary occlusion. Wall thickening (dWT) is expressed on the y axis as a percentage of control condition values. Distance on the x axis is in degrees, with the position of the perfusion boundary (PB) designated as zero. The position of the PB is bracketed by dashed lines to emphasize that the true boundary is irregular and that the location we designated for the PB is associated with a potential error of approximately ±2 mm. Positive numbers are on the nonischemic side of the PB; negative numbers are on the ischemic side. Data obtained after 10 min of circumflex occlusion are shown with the solid circles and solid lines; data obtained after 3 hr of occlusion are shown with open circles and dashed lines. The numbers superimposed on the plots represent the lateral extents of the functional border zone, defined as the distance from the perfusion boundary to 97.5% of the nonischemic asymptote (equal to µ + 2σ). The data and sigmoid curves in panel 2 are from the same experiment shown in figures 2 to 4. Data at 3 hr after occlusion were not available from the experiment shown in panel 4.
marcated as the perfusion boundary, a gradient of mechanical dysfunction extended across the perfusion boundary, resulting in a functional border zone that was restricted in size and severity. Severe nonischemic dysfunction was apparent close to the perfusion boundary, but wall thickening recovered rapidly over approximately 30 degrees of circumference.

Wall thickening within the functional border zone (measured an average of 5 mm from the perfusion boundary) was reduced only 20% from control levels (figure 5, table 3), demonstrating that the relative severity of nonischemic dysfunction was mild. The size of the functional border zone and the distribution of functional impairment across the perfusion boundary were altered minimally between 10 min and 3 hr of occlusion. It was notable that severe dysfunction (defined as wall thickening less than 50% of control values) extended only 6 degrees on the average beyond the perfusion boundary. Given that our level of imprecision was approximately 5 degrees, our results suggest that the circumferential extent of severe dysfunction corresponds closely to that of the ischemic area. Consequently, evaluating the size of regions at risk may prove sound with clinical methods such as two-dimensional echocardiography or contrast ventriculography, despite the limited spatial and temporal resolution of some of these techniques, if severe dysfunction is used to define the margin of the ischemic area.

The present findings, obtained in conscious animals, are in remarkably close agreement with results of our previous study using similar means to evaluate the functional border zone in open-chest, anesthetized dogs during circumflex coronary occlusion. To demonstrate this point more clearly, the individual data points and composite sigmoid curves from the present study and from our earlier study are presented together in figure 9. The only obvious differences in the sigmoid curves are in the values of the ischemic and nonischemic areas.

FIGURE 7. Individual examples of sigmoid curves fitted to wall thickening data during coronary occlusion. Data at 3 hr after occlusion were not available from the experiment shown in panel 8. Abbreviations and notes are as in figure 6.
mic asymptotes. The extent of the functional border zone, defined as the distance from the perfusion boundary to 97.5% of the nonischemic asymptote, was equally narrow in both conscious and anesthetized dogs. The average severity of dysfunction within the functional border zone was quite similar also, if wall thickening in the nonischemic border zone is compared with that in the nonischemic (or control) area further removed from the perfusion boundary. In anesthetized\textsuperscript{8} and conscious dogs (figure 5), nonischemic border zone wall thickening was approximately 20% to 30% less than that in the control area at a greater distance from the perfusion boundary. Thus, the present results confirm our earlier conclusions and extend them by demonstrating the characteristics of the functional border zone after acute occlusion of the circumflex coronary artery in conscious dogs.

Most previous studies on nonischemic dysfunction were performed with anesthetized preparations.\textsuperscript{1, 2, 4, 5, 7–9} With the exception of the report by Lima et al.,\textsuperscript{5} studies in anesthetized animals that included data on the location of functional measurements relative to the perfusion boundary\textsuperscript{3, 4, 7, 9} agree well with our results and conclusion. For example, Buda et al.\textsuperscript{9} and Force et al.\textsuperscript{7} used two-dimensional echocardiography to evaluate the extent of nonischemic dysfunction after circumflex coronary occlusion. Buda et al.\textsuperscript{9} reported that dysfunction extended approximately 25 degrees beyond one lateral margin of the ischemic area. In similar fashion, Force et al.\textsuperscript{7} demonstrated that regional wall thickening recovered to normal levels within a distance of 8 to 9 mm from the perfusion boundary.

Relatively few studies in conscious animals have been reported that bear on the issue of the functional border zone. Guth et al.\textsuperscript{11} measured wall thickening with sonomicrometers in conscious pigs during 2 min occlusions of the left circumflex artery. They observed reductions in wall thickening in normally perfused myocardium adjacent to the ischemic area, but precise information on the distance between the sonomicrometers and the perfusion boundary was not obtained. Thus, Guth et al.\textsuperscript{11} showed that nonischemic dysfunction occurs in conscious, closed-chest animals, but the lateral extent and distribution of functional impairment were not determined. The same limitation also applies to the study by Cox and Vatner.\textsuperscript{10} They demonstrated that subendocardial segments arrayed with one crystal in the ischemic area and one in the nonischemic area exhibited as much dysfunction as central ischemic seg-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{Composite data sets and composite sigmoid curve fits from all of the experiments. The x and y axis are arranged in the same manner as in figures 6 and 7. The perfusion boundary (PB) is shown with the dotted line bracketed by dashed lines to represent the approximate limits of the transition zone defined by the irregular course of the true perfusion boundary across the myocardial wall. Data obtained after 10 min of coronary occlusion are shown with solid circles and the solid line; data obtained 3 hr after coronary occlusion are shown with open squares and the dashed line. The numbers superimposed on the graph represent the composite fit values (in degrees) for the lateral extent of the functional border zone.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure9}
\caption{Composite data sets and composite sigmoid curve fits obtained early after coronary occlusion in conscious dogs (solid circles and line) and open-chest, anesthetized dogs (open squares and dashed line). Data from anesthetized dogs were from a previous study in which comparable means of evaluating the functional border zone were used.\textsuperscript{8} The main point of the figure is to illustrate the striking similarity in the extents of the functional border zone (values in degrees) measured in conscious and open-chest anesthetized dogs. PB = perfusion boundary.}
\end{figure}
ments entirely contained in the ischemic area during coronary occlusion in awake dogs. Although supporting the functional border zone concept, insufficient data were reported on the location of the functional measurements compared with the position of the perfusion boundary to reconstruct the lateral extent or severity of nonischemic dysfunction.

Two recent studies performed in conscious animals provide support for our findings. Gibbons et al. used two-dimensional echocardiography to study the time course of regional wall motion abnormalities for 6 weeks after permanent coronary occlusion. Myocardial blood flow and wall motion were measured in 36 radial segments of left ventricular short-axis cross sections. The circumferential extent of the wall motion abnormalities correlated closely with the extent of the ischemic area at 30 min after coronary occlusion. Although they did not focus attention on the topography of dysfunction across the ischemic-nonischemic interface, their data support our conclusion that the perfusion boundary and the lateral limit of severe dysfunction (wall thickening reduced more than 50%) are nearly identical. In similar fashion, Buda et al. reported in preliminary form the results of using two-dimensional echocardiography to evaluate circumferential flow-function relations in conscious dogs. A functional border zone averaging 27 degrees at one lateral margin of the ischemic area was evident early after circumflex coronary occlusion, in close agreement with our results based on sonomicrometric measurements.

The possible mechanisms of nonischemic dysfunction have been discussed in previous reports. We think the functional border zone is a mechanical phenomenon that is dictated by the geometry of the left ventricle and relative levels of stiffness in ischemic and nonischemic areas but is unrelated to flow restriction or relative ischemia. The similarity of the functional border zone in open-chest, anesthetized, and conscious dogs, despite substantial hemodynamic differences between the two types of preparations, supports this view. There is little evidence for a zone of intermediate blood flow surrounding the ischemic area. Perfusion may appear to be intermediate in tissue samples at the lateral margins of the ischemic area because they contain the interdigitating peninsulas of ischemic and normally perfused myocardium that characterize the ischemic-nonischemic interface. Likewise, the boundary of biochemical abnormalities is also abrupt, reinforcing the view that nonischemic dysfunction is not due to relative ischemia in the area corresponding to the functional border zone.

The mechanical alternatives proposed to explain nonischemic dysfunction include tethering and stress concentration at the ischemic margin. Lacking rigorous definition, the concept of tethering cannot be readily tested or distinguished from other mechanical explanations such as stress concentration. Because the distribution of stress concentration appears to correspond well to the distribution of wall thickening impairment (figure 8), our current view remains that elevated stress at the ischemic-nonischemic interface is the most likely possibility. Additional investigation, however, will be required to substantiate its role in the phenomenon of nonischemic dysfunction.

We conclude that the clinical importance of the functional border zone during acute occlusion is limited because it extends a relatively short distance into normally perfused myocardium and is characterized by mild dysfunction in conscious animals. Furthermore, its size and relative severity appear to be fixed in the first few hours after coronary occlusion. The effects of longer periods of ischemia, reperfusion, the replacement of infarcted myocardium with scar tissue, and ischemic zone locations other than that of the circumflex arterial bed remain to be determined.

We thank Thomas B. McClanahan, Russell A. Grinage, Diane Pace, and Laurel Wright for technical assistance. We also thank Tarry Goble for word processing of the manuscript.

References
25. Harken AH, Barlow CH, Harden WR III, Chance B: Two and three dimensional display of myocardial ischemic "border zone" in dogs. Am J Cardiol 42: 954, 1978
The functional border zone in conscious dogs.
K P Gallagher, R A Gerren, X H Ning, S P McManimon, M C Stirling, M Shlafer and A J Buda

_Circulation_. 1987;76:929-942
doi: 10.1161/01.CIR.76.4.929

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/76/4/929

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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_ is online at:
http://circ.ahajournals.org//subscriptions/