Effects of a calcium-entry blocker (diltiazem) on regional myocardial flow and function during exercise in conscious dogs

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ABSTRACT We examined the effects of diltiazem, a calcium-entry blocker, on exercise-induced myocardial ischemia in nine conscious dogs with chronic coronary stenoses. An ameroid constrictor, Doppler flow probe, and hydraulic occluder were placed around the left circumflex coronary artery, and left ventricular pressure was measured (Konigsberg micromanometer). Pairs of ultrasonic crystals were implanted for measuring left ventricular systolic wall thickening (%ΔWTh) in control (left ventricular anterior wall) and ischemic (left ventricular posterior wall) regions, and regional myocardial blood flow was measured with the microsphere method. Eighteen days (average) after surgery mean coronary blood flow velocity had decreased and reactive hyperemic flow velocity after 10 sec of coronary occlusion was markedly reduced, but %ΔWTh at rest remained normal, indicating collateral development. Control treadmill exercise was performed for 3.7 min (average), and 2 hr later administration of 0.3 mg/kg diltiazem was followed by an identical exercise bout. Control exercise increased %ΔWTh in the normal region, while in the ischemic region %ΔWTh decreased markedly and ischemia was evident (subendocardial flow, 0.29 ± 0.12[SD] ml/min/g). After diltiazem hemodynamic and %ΔWTh values at rest were not changed; during exercise the heart rate was significantly lower (204 ± 24 vs 227 ± 33 beats/min, p < .01), but values for other hemodynamic measures were similar to those during the control run. %ΔWTh in the control region was not changed during exercise after diltiazem, but compared with control exercise in the ischemic zone there was less dysfunction and subendocardial flow was greater. Recovery from exercise-induced dysfunction in the ischemic region occurred within 5 min, compared with over 30 min after control exercise. Thus, in a preparation of chronic coronary stenosis, the calcium-entry blocker improved the relationship between regional myocardial flow and function during exercise and led to more rapid recovery of regional myocardial dysfunction.


THE CALCIUM-ENTRY BLOCKER diltiazem was developed as a drug that inhibits the influx of calcium ions during cell depolarization in vascular smooth muscle, thereby producing coronary vasodilation.1-4 Several studies have documented the beneficial effect of diltiazem in controlling symptoms of coronary artery spasm5-7 and effort angina pectoris.8-12 Diltiazem is also effective in increasing the total duration of exercise and the time to the first onset of angina, and the pressure-rate product is significantly reduced at submaximal exercise after administration of the drug.8,10 Experimental studies have suggested that after coronary occlusion, diltiazem increases regional myocardial blood flow in moderately ischemic zones in anesthetized dogs13 and augments collateral blood flow in conscious dogs.14 There is little information concerning how calcium-entry blockers such as diltiazem may produce antian- ginal effects in the presence of chronic coronary stenosis, and relationships between regional myocardial blood flow and function in normal and ischemic regions during exercise-induced regional ischemia before and after drug administration have not been studied. Recently we reported the development of a canine preparation that allows repeated, reproducible induction of regional dysfunction in a collateral-dependent zone supplied by a chronically stenosed coronary ar-
tery with an amiodar constrictor during free-running exercise. This setting resembles that in patients with effort angina pectoris due to fixed coronary lesions. In this study, we have validated the use of this animal preparation with treadmill exercise to allow microsphere injections and applied it to investigate the effects of diltiazem on hemodynamic variables, regional myocardial function, and simultaneously measured regional myocardial blood flow distribution.

Methods

Before surgery all dogs were familiarized with the laboratory and trained to run on a motorized treadmill.

Surgical instrumentation. Nine adult mongrel dogs weighing 22 to 35 kg (mean, 26.9 kg) were premedicated with acepromazine maleate (0.5 mg/kg) 30 min before induction of anesthesia with pentobarbital sodium (30 mg/kg). The trachea of each was intubated, and the dog was ventilated with a Harvard respiration pump. Through a sterile thoracotomy, a high-fidelity micromanometer (Konigsberg P-7) was introduced through the apex of the left ventricle and a Tygon tube (1.27 mm, inside diameter) was inserted through the apex for direct measurement of left ventricular pressure as previously described. The same types of Tygon catheters were introduced into the left atrial appendage and the descending aorta for injection of radioactive microspheres and for withdrawing reference arterial blood, respectively.

An amiodar constrictor was positioned distal to a Doppler flow probe on the left circumflex coronary artery and a hydraulic cuff was then placed around the vessel beyond the constrictor. The amiodar constrictor was encased in a slotted stainless steel ring; the constrictor was 5 mm long, the external dimension 8 mm, and the lumen 2.5 to 3 mm. The constrictor was designed to produce gradual constriction of the coronary artery, thereby promoting the development of collateral vessels.

For the measurement of regional wall thickening dynamics, two pairs of miniature ultrasonic crystals (5 MHz) were implanted in the left ventricular wall, as described elsewhere. One pair was positioned in the left ventricular posterior free wall supplied by the left circumflex coronary artery to measure "ischemic wall" thickness, and the other was placed in the left ventricular anterior wall to measure "control wall" thickness. The crystals were placed so that the ultrasonic transit time between the crystals was shortest on an oscilloscope.

The position of the crystals was carefully examined at the time of necropsy to verify correct alignment. Data from nine dogs are reported in this study. Of the 18 available pairs of crystals measuring ischemic and control wall thickness, data from 17 were acceptable. One pair of crystals implanted to measure control wall thickness could not be used for data collection because of improper alignment. At necropsy 13 inner crystals (seven in the ischemic wall and six in the control wall) were perfectly positioned just inside the subendocardial surface spanning 100% of the wall, and the remaining four inner crystals (two in the ischemic wall and two in the control wall) were found within 2 mm of the endocardial surface, spanning about 80% of the full wall thickness. There was no gross infarction between crystals, and the inner wall crystals were surrounded by a fibrous rim (approximately 1 mm) of connective tissue, as previously described. This process involves the subendocardial crystal only and it does not seem likely that it could have caused functional impairment, since normal wall thickening values have been obtained at rest under these conditions.

Regional myocardial blood flow. Regional myocardial blood flow measurements were made with 15 μm diameter radioactive microspheres with Tween-80 added and labeled with 51Cr, 114In, 113Sn, 103Ru, 99mTc, or 46Sc (New England Nuclear) as previously described. For each blood flow determination, approximately 3 x 10^6 microspheres, followed by a 5 ml warm saline flush, were injected into the left atrium. Only data that indicated no hemodynamic changes or arrhythmias during and after the microsphere injections were used. Immediately after each experiment the dog was killed with an intravenous KCl injection after deep barbiturate anesthesia. The heart was fixed and cut as previously described.

The transverse slice of the left ventricle, which contained the posterior wall crystals, was divided into eight full-thickness samples weighing 3.5 to 4.5 g each, and a full-thickness sample of similar size containing the anterior wall crystals was also removed. Subendocardial, midwall, and subepicardial pieces were cut from all samples, those from the blocks containing crystals being taken to include the tissue between crystal pairs. For later histologic studies, pieces of tissue were also cut from the blocks containing the posterior (ischemic) and anterior (control) crystals, weighed, and placed in counting vials with 10% formalin added.

The myocardial and reference blood samples were counted in a Packard Autogamma Spectrometer (model 5912). The corrections for use of multiple isotopes were done with a matrix inversion technique for solving simultaneous equations, and corrected counts were used to calculate blood flow. Although not generally reported in studies on regional myocardial ischemia, we calculated the number of microspheres in all tissue samples. In the control area, the numbers of spheres in subendocardial samples, in counts per gram, were 918 ± 289 (SD) during control rest, 979 ± 357 during control running, and 905 ± 248 during running after diltiazem. Despite this relatively large number of microspheres injected (about 3 million), it was not possible to obtain sizeable sphere numbers during exercise in intensely ischemic zones supplied by the stenosed circumflex artery. In the subendocardial samples from the ischemic zone, the numbers of spheres were 763 ± 278 at rest, 151 ± 96 during control running, and 182 ± 119 during running after diltiazem. The fact that relatively low numbers of spheres reach regions of very reduced blood flow is well recognized in studies of this nature.

Data are reported from only the full-thickness samples containing the crystal pairs, but regional blood flows in the entire circumference of the ventricular slice (eight pieces) were determined. The piece containing the ischemic crystal pair was always within the zone of maximal ischemia and not in a border zone of intermediate blood flow.

Study protocol. Seven days after surgery all nine dogs studied were evaluated, with the conscious dogs resting quietly on their sides on the experimental table. After control recordings of the left ventricular pressure, coronary blood flow velocity and dimensions, the left circumflex coronary artery of each was completely and abruptly occluded by inflating the hydraulic occluder with a syringe. At 10 sec of occlusion the occluder was completely released and the subsequent reactive hyperemia was recorded. This procedure was then carried out in all dogs every 2 days. At 7 days after surgery mean blood flow velocity of the left circumflex coronary artery was 35.2 ± 8.2 cm/sec (mean ± SD), and mean blood flow velocity at peak reactive hyperemia was 86.0 ± 11.7 cm/sec (250 ± 32% of control rest). As time passed, dysfunction of the ischemic wall during total coronary occlusion gradually diminished, and reactive hyperemia also decreased progressively, as previously described.

At an average of 18 days after surgery (range 12 to 30), when reactive hyperemia was markedly decreased, the exercise stud-
ies were performed; on the study day in eight of nine dogs coronary blood flow velocity at rest was decreased, averaging 21.0 ± 8.7 cm/sec (p < .002 compared with 7 days after surgery). Peak reactive hyperemic flow velocity was also decreased significantly to 26.3 ± 11.1 cm/sec (129 ± 18% of rest, p < .001 compared with 7 days). In the remaining dog, blood flow through the circumflex coronary artery was absent because of complete obstruction by the amiodar constrictor. During total coronary occlusion with the hydraulic occluder, moderate dysfunction (about 40% reduction of systolic wall thickening in the ischemic area) was observed in three of the eight dogs, and in the remaining five dogs mild dysfunction (less than 20% reduction of systolic wall thickening) or no dysfunction was apparent during total coronary occlusion.

In present practice, when mean coronary flow velocity at the phase of peak reactive hyperemia is reduced to approximately 130% or less of that during resting conditions, treadmill exercise evaluation is performed, and if ischemic dysfunction is produced in the ischemic area during this brief evaluation run the experiment is carried out the same day. In this study, if exercise-induced dysfunction was not severe (systolic wall thickening not reduced to 50% or less of control) the data were not used.

Systolic wall thickening in both ischemic and control areas at 7 days after surgery (32.4 ± 8.8% and 31.2 ± 6.6%, respectively) was not significantly different from that on the study day. All nine dogs exhibited normal activity, were afebrile, had normal hematocrits, and were capable of repeated runs on the treadmill. In two of eight dogs in which circumflex coronary arteries were stenotic but still open, brief cyclic changes in coronary blood flow velocity without associated changes in regional function were observed at rest. Therefore, in these two dogs total occlusion of the left circumflex coronary artery was introduced with the hydraulic occluder throughout the study to avoid such cyclic changes in antegrade coronary blood flow.

Control recordings and the first microsphere injections were done while the dogs stood quietly on the treadmill. Each dog was then run on the treadmill at a speed and grade sufficient to substantially decrease systolic wall thickening in the ischemic area (average, 10.3 ± 1.2 km/hr, 5% grade). All treadmill runs were performed for 3.5 to 4.5 min (average of control run 3.7 ± 0.4 min), with microspheres injected approximately 2.5 min into the run, resulting in a steady-state condition. After confirming full recovery from the control exercise (2 to 3 hr later), diltiazem (0.3 mg/kg) was administered via the left atrial catheter over 5 min. Ten minutes later, control data for the second (diltiazem) exercise period were recorded with the dog standing quietly on the treadmill, and then the treadmill run was repeated at the same speed and grade and for the same running time.

Two of the experimental dogs and four additional dogs were exercised on a separate day with no intervention between runs to examine the reproducibility of two periods of running. The dogs ran at the same speed for the same running time in two exercise periods separated by 3 hr. Hemodynamic responses, myocardial dimensions, and regional myocardial blood flow were measured at rest and during exercise and the changes during the two exercise periods were compared.

Gross and histologic studies. Before cutting the fixed slices of the left ventricle, tracings were made on a Plexiglas overlay of the outlines of the endocardial and epicardial surfaces of the ventricle and the locations of the ultrasonic crystals. After the counting of radioactivity, three slices were made from each piece of tissue (subendocardial, midwall, and subepicardial) containing both the ischemic and control crystal pairs; therefore, nine slides stained with trichrome were available for each region containing crystals. The extent of necrosis was determined with a point-counting technique. The rim of scar tissue around the crystals was identified and excluded, and the amount of necrosis that was due to ischemic damage was determined.

Data analysis. Recordings for each experiment were made on a Brush forced-ink recorder and on magnetic tape for subsequent playback and computation. The parameters analyzed were heart rate, left ventricular systolic pressure, left ventricular end-diastolic pressure, peak positive and negative left ventricular dP/dt, end-diastolic wall thickness (at the time dP/dt crossed zero line), end-systolic wall thickness (defined as the maximum systolic excursion occurring within 20 msec before peak negative dP/dt), systolic excursion of wall thickening (in mm and as percent of end-diastolic wall thickness), and mean coronary blood flow velocity. Percent systolic wall thickening was determined and values for wall thickness were normalized to a 10 mm initial end-diastolic thickness. The absolute values for end-diastolic wall thickness in the ischemic and control areas during control standing rest before the control run ranged from 8.17 to 11.42 mm (average 10.12 ± 1.41 mm) and from 9.01 to 12.53 mm (average 10.82 ± 1.26 mm), respectively.

Hemodynamic, dimension, and coronary blood flow velocity data were digitized from magnetic tape with a computer system (PDP/11/03). Data from 20 consecutive cardiac cycles were collected and averaged at control, at points during the run, and during recovery. Statistical analysis of the difference between control exercise and exercise after diltiazem and comparison of the mean values during exercise with those during rest were conducted by analysis of variance with repeated measures. The level of statistical significance was p < .05, and all data are presented as the mean ± SD.

Results

Reproducibility studies. Hemodynamic responses were not significantly different between the first and the second bouts of exercise without drug intervention. Heart rates were 232.8 ± 13.5 vs 233.8 ± 22.1 beats/min (at the time of microsphere injection), left ventricular end-diastolic pressures 32.7 ± 7.2 vs 32.0 ± 5.5 mm Hg, left ventricular peak systolic pressures 170.0 ± 11.7 vs 171.8 ± 4.8 mm Hg, and left ventricular peak (+ dP/dt) 6249 ± 990 vs 6314 ± 1224 mm Hg/sec. Exercise-induced regional myocardial dysfunction in the ischemic area and the control wall responses were not different (figure 1, left), and changes in myocardial blood flow distribution were also not significantly different between the two exercise periods (figure 1, right). The differences between variables for the two exercise bouts (subtracting the smaller value from the larger value) were averaged and are shown in tables 1A and 1B. A high degree of reproducibility for results from sequential runs on the same day was observed in this animal preparation.

Hemodynamic data and the velocity of coronary blood flow at control and during diltiazem exercise. A tracing showing hemodynamic variables and dimensions at slow paper speeds during an entire running episode is reproduced in figure 2 and rapid-speed tracings are shown in figure 3. Within 10 sec after the beginning of exercise, heart rate, left ventricular systolic pressure,
and peak (+)dP/dt increased abruptly, and these values were maintained at high levels throughout the exercise period (table 2; figures 2 through 4).

Left ventricular end-diastolic pressure was slightly decreased at the early phase of exercise, but it was significantly elevated by 1 min. By the middle of the control exercise period (approximately 2.5 min into the run), average heart rate had increased (from control standing) to 227 beats/min, and left ventricular end-diastolic pressure and left ventricular peak (+)dP/dt were significantly increased (table 2, figure 4). During the recovery phase (by 10 min after the end of control exercise) heart rate remained significantly higher, while left ventricular systolic pressure, left ventricular end-diastolic pressure, and peak (+)dP/dt returned to near control values at 5 min after the end of the run.

Ten minutes after administration of diltiazem the heart rate at standing rest was slightly increased but other hemodynamic variables were not significantly different from resting values before the control run. During exercise after diltiazem heart rate increased to 204 beats/min (at the time of microsphere injection), the change being significant 10 sec after exercise was begun (p < .01). Increases in left ventricular systolic pressure, left ventricular end-diastolic pressure, and dP/dt were the same during control exercise, but left ventricular end-diastolic pressure was significantly reduced compared with that in the control exercise run.

### TABLE 1A

Differences in hemodynamic and dimension responses to two runs completed 3 hr apart with no intervention in the interim (n = 6, mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Running</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 sec</td>
<td>10 sec</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>5.3±3.6</td>
<td>9.8±9.8</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>2.7±2.6</td>
<td>9.2±6.8</td>
</tr>
<tr>
<td>%ΔWT in the ischemic region</td>
<td>1.4±1.2</td>
<td>1.8±1.9</td>
</tr>
<tr>
<td>%ΔWT in the control region</td>
<td>1.3±0.8</td>
<td>2.4±0.7</td>
</tr>
</tbody>
</table>

HR = heart rate; LVSP = left ventricular peak systolic pressure; %ΔWT = % systolic wall thickening; Mid = approximately 2.5 min into run, at the time of microsphere injections.
TABLE 1B
Differences in myocardial blood flow (MBF) between two runs completed 3 hr apart without invention in the interim (n = 6, mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>ENDO</th>
<th>MID</th>
<th>EPI</th>
<th>TMBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic region</td>
<td>0.09±0.09</td>
<td>0.09±0.09</td>
<td>0.08±0.06</td>
<td>0.06±0.05</td>
</tr>
<tr>
<td>Control region</td>
<td>0.18±0.14</td>
<td>0.17±0.13</td>
<td>0.23±0.19</td>
<td>0.22±0.22</td>
</tr>
<tr>
<td>Normalized (ischemic region)</td>
<td>0.04±0.03</td>
<td>0.04±0.04</td>
<td>0.11±0.11</td>
<td>0.04±0.03</td>
</tr>
</tbody>
</table>

ENDO = subendocardium; MID = midmyocardium; EPI = subepicardium; TMBF = mean transmural blood flow; NORMALIZED = normalized myocardial blood flow in the ischemic region/myocardial blood flow in the control region.

During recovery after the diltiazem run all of these parameters returned to the resting control levels within 5 min (table 2; figure 4).

In six of nine dogs the left circumflex coronary artery remained open throughout the studies. Changes in flow velocity during running were not significant, nor were those at rest or during exercise after diltiazem (table 2).

Regional myocardial function at control and during diltiazem exercise. Dimension data are shown in table 3. Figure 3 illustrates changes in these measurements during exercise before and after diltiazem in a single dog, and the data in all dogs are summarized in figure 5.

Ischemic wall thickness. During control exercise there

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**FIGURE 2.** Tracings obtained during a control run. Within 5 to 10 sec after the beginning of exercise, heart rate, left ventricular pressure, and peak positive dP/dt increased abruptly and the latter two variables then decreased but were maintained at high levels throughout the exercise period. Systolic wall-thickening in both the ischemic and control regions increased at the early phase of running; however, augmentation of wall performance in the ischemic region was transient. Ischemic wall thickening decreased progressively at 2 min into the run dysfunction reached steady state. At that time, isotope-labeled microspheres were injected (open arrow) to obtain the myocardial blood flow during exercise-induced ischemia.
was severe dysfunction and late systolic thickening of the ischemic wall in all dogs (table 3; figures 2 and 3). Systolic wall excursion and percent systolic wall thickening were initially increased slightly (NS) and then markedly decreased from 2.92 (control rest) to 0.53 mm, and from 29.2% to 5.2%, respectively, at the middle phase of the control run (the time of microsphere injection). The systolic wall excursion and percent systolic wall thickening were not significantly different at 1 min and at points midway and late into the run, indicating that a relatively stable state was achieved. Thirty minutes after the control run, percent systolic wall thickening in the ischemic area remained depressed significantly (80% of the control value), but it had returned to control by 2 hr after the control run (table 3; figure 5).

Ten minutes after diltiazem injection, wall thickness variables were not changed from the control resting values. During exercise after diltiazem, significant lessening of wall dysfunction was observed (table 3; figures 3 and 5) and these reductions in systolic wall performance were significantly less than those during the control run.

After exercise with diltiazem the recovery of systolic wall performance in the ischemic area was faster than after control exercise, when it remained depressed at 30 min. One minute after exercise, systolic wall excursion and percent systolic wall thickening had re-

FIGURE 3. Recordings at fast paper speed from same experiment shown in figure 2. End-diastole and end-systole are indicated with solid vertical lines. Top, Control exercise. After diltiazem (bottom) hemodynamics and wall performance at control standing were unchanged, but deterioration of function in the ischemic area during the run was less marked. One minute after diltiazem exercise, wall performance in the ischemic area returned to near control.
covered to 71% and 70% of control, respectively, and at 5 min and thereafter these values were not significantly reduced compared with rest and remained significantly higher than those after control exercise.

**Control wall thickness.** During control exercise, average end-diastolic wall thickness increased significantly (by 4%) over the control resting value. Systolic wall excursion and percent systolic wall thickening were increased significantly during the entire period of running (table 3; figures 2, 3, and 5). After the end of the run, all variables returned to near control within 1 min. Ten minutes after diltiazem infusion, wall thickness variables were unchanged compared with the values before control exercise. Compared with control exercise the increases in systolic wall excursion and percent systolic wall thickening were slightly but not significantly higher during the entire period of diltiazem exercise (figure 5).

**Myocardial blood flow data at control and during diltiazem exercise.** Averaged myocardial blood flow data are presented in table 4A and summarized graphically in figure 6. Normalized blood flow data in the ischemic area (expressing flow as a fractional decimal of flow in the control area) are presented in table 4B. At rest, blood flows were not different in the control and the ischemic areas between each crystal pair (table 4A; figure 6). During control exercise, subendocardial blood flow in the ischemic area decreased compared with control at rest, but midmyocardial blood flow was not significantly different from that at rest. Subepicardial blood flow during exercise significantly exceeded the resting level of blood flow (+90%). The ratio of subendocardial to subepicardial blood flow significantly decreased during control exercise (table 4A).

During control exercise relative subendocardial blood flow decreased in the ischemic region to 13%, midmyocardial blood flow decreased to 33%, subepicardial blood flow decreased to 70% (p < .01), and mean transmural blood flow decreased to 39% of control area flow (table 4B).

During exercise with diltiazem, subendocardial blood flow was significantly higher than that during control exercise before diltiazem (p < .01; table 4A; figure 6). Midmyocardial blood flow was also significantly higher than during control exercise (p < .05). Subepicardial blood flow was not significantly different from the control exercise value, and the subendocardial to subepicardial flow ratio increased by 50%.

During exercise after diltiazem, normalized subendocardial, midmyocardial, and subepicardial blood flows were decreased less compared with flows in the control area, but only the change in the subendocardium was significant (13% vs 25% of control area flow before and after diltiazem; table 4B).

During exercise after diltiazem, myocardial blood flow in the control area (anterior wall) decreased slightly compared with that observed during control exercise, but the differences were not significant (table 4A; figure 6).

**Regional blood flow-systolic function relationships during exercise.** Relationships between myocardial blood flow and systolic wall thickening are presented graphically in figure 7. Data are normalized by the expression of blood flow values as decimal fractions of blood flow in the control area (anterior wall) and by the expression of wall thickening as a decimal fraction of systolic thickening in the control resting state.

During exercise after diltiazem subendocardial blood flow significantly increased compared with that during control exercise (approximately twofold) and this was accompanied by lessening of dysfunction. Changes in subendocardial blood flow were relatively proportional to changes in systolic wall thickening, but changes in subepicardial blood flow were relatively small. The relationship between myocardial function and mean transmural blood flow, expressed graphically (dashed line, figure 7), lay between the subendocardial and subepicardial relationship.

**Gross and histologic findings.** At autopsy one of nine dogs had a small hemorrhage in the subendocardial layer of the ischemic area, but in the left ventricular free walls of the remaining eight dogs there was no gross damage. Microscopically, histologic changes due to ischemia constituted 1.9 ± 3.3% (range 0 to 9.8%) of the inner third layer between the crystals in the ischemic wall and 1.6 ± 2.5% (range 0 to 5.8%) of the inner third layer in the midmyocardium. There was no ischemic damage in the outer third of the myocardium in the ischemic area, and there was no histologic evidence of ischemic myocardial damage in the control area in any of the nine hearts. Scar formation due to subendocardial crystal implantation was 9.9 ± 5.0% of the inner third of the myocardium in the ischemic area, 0.6 ± 1.8% of the midwall region (around the wires), and 1.7 ± 3.3% of the epicardial third.

**Discussion**

We have previously developed a conscious canine preparation in which severe regional myocardial dysfunction occurs during exercise in zones that, at rest, have normal regional function; this preparation allows reproducible runs to be carried out sequentially on the same day.15–17 Our approach was modified in the present experiments to permit microsphere injections dur-
doubtedly different.

The treatment of the run.

from normal coronary blood flow velocity. CONT = control run; DILT = diltiazem run. Microspheres were injected at middle of run (about 2.5 min into run).

*p < .05, **p < .01 vs standing rest; p value column is for control vs diltiazem run.

In eight of nine dogs, the left circumflex coronary artery was still open on the study day (average 18 days after surgery), with significantly decreased velocity of coronary flow, but regional function in the ischemic area remained normal, indicating collateral development. From the observed degree of reduction in velocity of coronary flow it is likely that there was approximately 90% stenosis of the vessel, and that during running, left circumflex coronary flow velocity did not increase significantly. These conditions are similar to those in experimental studies of critical coronary stenosis produced acutely by a cuff occluder, although the degree of collateral circulation was undoubtedly different.

In many patients, severe coronary arterial stenosis is associated with normal ventricular function at rest, but exercise stress produces transient myocardial ischemia. Thus, in these patients, and in our animal preparation, areas of the left ventricles are supplied by limited antegrade and collateral flow sufficient only to meet resting metabolic demands. The findings of our study imply that exercise-induced regional and global dysfunction in such patients may be associated with subendocardial ischemia.

Experimental regional myocardial ischemia has previously been shown to impair the normal augmentation of overall hemodynamic function of the left ventricle during exercise, with a lower systolic peak pressure and dp/dt and higher end-diastolic pressure during acute coronary stenosis than during control exercise without coronary stenosis. In the present study, with the initiation of running (5 to 10 sec into the run), end-diastolic pressure decreased (NS) and this was associated with significantly increased end-diastolic wall thickness, indicating that left ventricular size initially became smaller. At this phase, systolic wall thickening in the ischemic area increased transiently above the resting control value, accompanied by an abrupt in-

| TABLE 2 |
|------------------|------------------|------------------|------------------|------------------|
| Hemodynamic changes during control and diltiazem runs (mean ± SD, n = 9) | Standing | 10 sec | 1 min | Middle | End |
| | HR (beats/min) | | | | | |
| CONT | 92.0 ± 11.7 | 234.7 ± 24.0a | 226.1 ± 26.6b | 226.7 ± 32.9 | 227.6 ± 31.6 |
| DILT | 108.6 ± 7.0 | 221.8 ± 25.3a | 203.4 ± 24.1b | 204.0 ± 24.2b | 206.1 ± 27.1b |
| p value | .05 | NS | <.01 | <.01 | <.01 |
| LVEDP (mm Hg) | | | | | |
| CONT | 13.3 ± 3.8 | 10.0 ± 3.2 | 27.2 ± 4.6b | 28.0 ± 4.9b | 28.1 ± 5.4b |
| DILT | 12.7 ± 3.9 | 11.2 ± 3.3 | 25.7 ± 4.1b | 25.1 ± 4.5b | 25.7 ± 4.1b |
| p value | NS | NS | NS | <.01 | <.01 |
| LVPSP (mm Hg) | | | | | |
| CONT | 135.9 ± 12.9 | 177.0 ± 24.8b | 170.1 ± 20.5b | 170.8 ± 22.4b | 168.9 ± 21.7b |
| DILT | 140.1 ± 15.1 | 174.3 ± 21.2b | 166.9 ± 19.8b | 166.1 ± 23.8b | 163.8 ± 20.5b |
| p value | NS | NS | NS | NS | NS |
| LV peak (+)dp/dt (mm Hg/sec) | | | | | |
| CONT | 3596 ± 527 | 6877 ± 833b | 6174 ± 833b | 5982 ± 896b | 5958 ± 926b |
| DILT | 3839 ± 518 | 7031 ± 1082b | 6132 ± 974b | 6062 ± 976b | 5968 ± 916b |
| p value | NS | NS | NS | NS | NS |
| LV peak (−)dp/dt (mm Hg/sec) | | | | | |
| CONT | 2970 ± 483 | 4204 ± 866a | 4045 ± 774a | 4043 ± 991b | 3834 ± 986a |
| DILT | 3158 ± 602 | 4031 ± 861a | 3865 ± 824a | 3929 ± 1016b | 3969 ± 1034a |
| p value | NS | NS | NS | NS | NS |
| Mean CBFV (cm/sec) (n = 6) | | | | | |
| CONT | 25.3 ± 11.7 | 31.7 ± 17.5 | 31.2 ± 16.1 | 32.7 ± 16.0 | 32.2 ± 16.0 |
| DILT | 23.5 ± 9.9 | 28.1 ± 12.2 | 30.0 ± 15.1 | 30.6 ± 16.6 | 29.9 ± 16.1 |
| p value | NS | NS | NS | NS | NS |

HR = heart rate; LVEDP = left ventricular end-diastolic pressure; LVPSP = left ventricular peak systolic pressure; mean CBFV = mean coronary blood flow velocity; CONT = control run; DILT = diltiazem run. Microspheres were injected at middle of run (about 2.5 min into run).
wall performance. Redistribution of coronary flow away from the subendocardium to the subepicardium can be induced by exercise during partial coronary stenosis, an effect that may relate primarily to limitation of the diastolic time per minute available for subendocardial perfusion and to a vasodilator response in the subepicardium. After diltiazem, initial hemodynamic responses and regional contraction during exercise were similar to those during control exercise; however, the severe ischemia that developed in the ischemic area during control exercise was significantly attenuated, especially in the inner half of the myocardium, in which it was associated with significantly improved regional myocardial wall thickening.

As has been reported previously, in experimental and clinical studies, diltiazem evokes negligible changes in hemodynamic and regional function at rest, a finding confirmed in our study. Other calcium-entry blockers, such as verapamil and nifedipine, appear to have a more pronounced direct negative inotropic effect than diltiazem. Also, although diltiazem and verapamil have a negative chronotropic effect, nifedipine increases in heart rate, left ventricular pressure, and peak (+)dP/dt (figure 2). Subsequently, however, systolic wall thickening, peak (+)dP/dt, and left ventricular pressure became progressively and markedly reduced as running continued and as a steady state (minimum systolic wall thickening) was approached after 60 sec of running (figure 2), as shown in previous reports. Thus, the effects of exercise-induced ischemia with limited antegrade and collateral flow appear to be time dependent. During the initial phase of running, we speculate that myocardial oxygen demand increased abruptly due to augmentation of sympathetic activity, with enhanced contractile function per beat and per minute, and during this phase coronary flow became inadequate to meet increased regional O₂ demands, leading to a condition of "relative ischemia." However, this condition appeared to be transient, with insufficient myocardial blood flow to the affected region resulting in a progressive decrease in regional myocardial performance. This, in turn, probably led to reduced O₂ demand in the involved region, so that the steady state of myocardial dysfunction during exercise with coronary stenosis may be characterized by absolute (rather than relative) ischemia, especially in the subendocardium, which primarily determines overall
TABLE 3
Regional myocardial performance during control and diltiazem runs (mean ± SD, n = 9)

<table>
<thead>
<tr>
<th></th>
<th>Standing</th>
<th>10 sec</th>
<th>1 min</th>
<th>Middle</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic wall thickness (n = 9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDWTh (mm)</td>
<td>10.00</td>
<td>10.07 ± 0.19</td>
<td>10.06 ± 0.27</td>
<td>10.11 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>∆WTh (mm)</td>
<td>2.92 ± 0.69</td>
<td>3.38 ± 0.87</td>
<td>3.38 ± 0.87</td>
<td>0.83 ± 0.38B</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>%ΔWTh</td>
<td>29.2 ± 6.9</td>
<td>32.5 ± 7.7</td>
<td>32.5 ± 7.7</td>
<td>9.8 ± 3.7B</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Normalized %ΔWTh (fraction of control %ΔWTh at standing × 100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONT</td>
<td>100</td>
<td>105.7 ± 6.4</td>
<td>21.7 ± 13.4B</td>
<td>19.0 ± 13.9B</td>
<td>15.1 ± 9.3B</td>
</tr>
<tr>
<td>DILT</td>
<td>99.9 ± 5.2</td>
<td>112.1 ± 12.4</td>
<td>36.3 ± 14.4B</td>
<td>34.8 ± 13.2B</td>
<td>33.3 ± 14.8B</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Control wall thickness (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDWTh (mm)</td>
<td>10.10 ± 0.30</td>
<td>10.36 ± 0.47</td>
<td>10.31 ± 0.39</td>
<td>10.30 ± 0.46</td>
<td>10.27 ± 0.44</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>∆WTh (mm)</td>
<td>2.93 ± 0.76</td>
<td>3.73 ± 0.92B</td>
<td>3.56 ± 0.94B</td>
<td>3.61 ± 0.81B</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>%ΔWTh</td>
<td>30.0 ± 7.6</td>
<td>35.9 ± 8.5B</td>
<td>34.0 ± 8.8B</td>
<td>34.9 ± 7.7B</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

EDWTh = end-diastolic wall thickness; ∆WTh = excursion of thickening in systole; %ΔWTh = extension of thickening; ischemic wall = left ventricular posterior wall supplied by left circumflex coronary artery; control wall = anterior wall supplied by left anterior descending coronary artery.

*p < .05, †p < .01 vs standing rest; p value column is for control vs diltiazem run.

does not, and the peripheral vasodilatory action of diltiazem is smaller than that of either verapamil or nifedipine.42

There are several possible mechanisms by which diltiazem may have improved exercise-induced regional ischemia in the area supplied by limited antegrade and collateral blood flow. First, the small but significant reduction in exercise heart rate (approximately 10% less than control exercise) after diltiazem could have improved regional blood supply to the ischemic area, especially to the deeper layers of the myocardium, by prolongation of the diastolic period.41,42 In the present study, the diastolic period per minute was increased slightly, but significantly, after diltiazem compared with at control exercise (41.6 sec/min average during control standing, 30.4 sec/min during control exercise, and 32.4 sec/min during exercise with diltiazem, p < .05). Second, the vasodilatory action of diltiazem may have led to improved perfusion in the ischemic region through increased blood flow in the coronary collateral vessels.6,14,44 We did not measure peripheral coronary pressure and therefore cannot calculate coronary collateral resistance in these experiments, although the direct or indirect effects of diltiazem to increase coronary flow must have been mediated through collaterals in the three dogs in which the coronary artery was occluded during exercise. We have reported values for velocity of antegrade blood flow only (not volume of flow) obtained with the Doppler flowmeter. However, it is of interest that results with this method, as well as with the microsphere technique, revealed no change during exercise before or after diltiazem; this might suggest that the average collateral blood flow changes were not large since,
were that the case, a larger change might be detected by the microsphere method, which measures both antegrade and collateral flow. Nakamura et al.\textsuperscript{15} found that diltiazem did not significantly increase collateral flow into the severely ischemic zone after acute occlusion of the left anterior descending coronary artery in anesthetized, open-chest dogs, although myocardial blood flow to the border areas surrounding the severely ischemic area did increase significantly. However, the border area includes both the collateral-dependent and normally perfused myocardium.\textsuperscript{45} Franklin et al.\textsuperscript{14} reported that in conscious dogs diltiazem significantly increased blood flow to collateral-dependent myocardium when pacing-induced tachycardia caused ischemia-related myocardial hypofunction, but that significantly increased blood flow was not associated with improvement of regional myocardial function. They used myocardial segmental shortening to evaluate regional function, and the blood flow reduction in the collateral-dependent area was much smaller during pacing before diltiazem than in the present study. When myocardial ischemia is not severe (nontransmural), considerable nonuniformity of transmural contraction occurs,\textsuperscript{21} and the use of intramural crystals to measure the segmental shortening can sometimes lead to variable results under the above conditions. We measured transmural wall thickening to evaluate regional myocardial function, which correlates well with the degree of myocardial ischemia, even when ischemia is nontransmural.\textsuperscript{20, 35}

A third mechanism may relate to production by diltiazem of a favorable redistribution of transmural blood flow.\textsuperscript{46} The calcium-entry blocker nifedipine has been shown by Bache and Tockman\textsuperscript{46} to limit reactive

\begin{table}[h]
\centering
\caption{Recovery}
\begin{tabular}{lcccc}
\hline
 & 1 min & 5 min & 10 min & 30 min \\
\hline
10.18±0.17 & 10.15±0.19 & 10.04±0.24 & 10.03±0.17 & \\
10.20±0.25 & 10.15±0.37 & 10.02±0.32 & 10.02±0.34 & \\
NS & NS & NS & NS & \\
1.50±0.88\textsuperscript{a} & 2.17±0.86\textsuperscript{a} & 2.19±0.59\textsuperscript{a} & 2.36±0.61 & \\
2.10±0.74\textsuperscript{a} & 2.56±0.86 & 2.52±0.79 & 2.72±0.83 & \\
<.01 & <.01 & <.01 & <.01 & \\
14.8±8.6\textsuperscript{b} & 21.3±8.2\textsuperscript{b} & 21.8±5.9\textsuperscript{b} & 23.5±6.0\textsuperscript{b} & \\
20.5±7.2\textsuperscript{b} & 25.2±8.3 & 25.1±7.8 & 27.1±7.7 & \\
<.01 & <.01 & <.01 & <.01 & \\
48.7±19.8\textsuperscript{b} & 71.1±12.8\textsuperscript{b} & 74.5±7.0\textsuperscript{b} & 80.8±8.5\textsuperscript{b} & \\
69.4±11.7 & 84.7±9.7\textsuperscript{a} & 84.7±8.4\textsuperscript{a} & 92.1±7.0 & \\
<.01 & <.01 & <.01 & <.01 & \\
10.15±0.26 & 10.08±0.22 & 10.09±0.25 & 10.06±0.26 & \\
10.11±0.42 & 10.23±0.33 & 10.17±0.36 & 10.05±0.23 & \\
NS & NS & NS & NS & \\
2.82±0.70 & 2.89±0.75 & 2.95±0.74 & 3.02±0.82 & \\
2.87±0.73 & 2.94±0.67 & 3.01±0.69 & 3.05±0.69 & \\
NS & NS & NS & NS & \\
27.8±6.9 & 28.6±7.4 & 29.2±6.9 & 30.0±8.3 & \\
28.3±7.1 & 28.7±6.1 & 29.5±6.3 & 30.3±6.7 & \\
NS & NS & NS & NS & \\
\hline
\end{tabular}
\end{table}

\begin{figure}[h]
\centering
\includegraphics{image}
\caption{Changes in systolic wall thickening in ischemic (top) and control regions (bottom) during exercise before and after diltiazem. During diltiazem exercise, systolic wall thickening in the ischemic area significantly improved compared with that of control exercise, and recovery of wall performance from the exercise-induced dysfunction was faster than control after the end of run. \textsuperscript{*p < .05, **p < .01} compared with control standing. \textsuperscript{tp < .01 for comparison of control and diltiazem values.}
\end{figure}
TABLE 4A
Myocardial blood flow during control and diltiazem exercise (mean ± SD, n = 9)

<table>
<thead>
<tr>
<th>Ischemic area (between PT crystals, ml/min/g)</th>
<th>Standing</th>
<th>Control run</th>
<th>Diltiazem run</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENDO</td>
<td>0.92 ± 0.18</td>
<td>0.29 ± 0.12a</td>
<td>0.50 ± 0.15a</td>
</tr>
<tr>
<td>p value</td>
<td>.01</td>
<td>.05</td>
<td>NS</td>
</tr>
<tr>
<td>MID</td>
<td>0.85 ± 0.17</td>
<td>0.61 ± 0.28</td>
<td>0.88 ± 0.32</td>
</tr>
<tr>
<td>EPI</td>
<td>0.72 ± 0.13</td>
<td>1.37 ± 0.49b</td>
<td>1.52 ± 0.41b</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MEAN</td>
<td>0.83 ± 0.17</td>
<td>0.78 ± 0.23</td>
<td>0.96 ± 0.23</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ENDO/EPI</td>
<td>1.27 ± 0.13</td>
<td>0.24 ± 0.13b</td>
<td>0.36 ± 0.13b</td>
</tr>
<tr>
<td>p value</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Control area (between AT crystals, ml/min/g)</td>
<td>0.86 ± 0.16</td>
<td>2.27 ± 0.52b</td>
<td>2.07 ± 0.45b</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MID</td>
<td>0.80 ± 0.18</td>
<td>2.23 ± 0.59b</td>
<td>2.13 ± 0.46b</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EPI</td>
<td>0.68 ± 0.16</td>
<td>2.04 ± 0.60b</td>
<td>1.96 ± 0.51b</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MEAN</td>
<td>0.78 ± 0.15</td>
<td>2.18 ± 0.56b</td>
<td>2.06 ± 0.45b</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ENDO/EPI</td>
<td>1.26 ± 0.10</td>
<td>1.11 ± 0.10</td>
<td>1.07 ± 0.17</td>
</tr>
<tr>
<td>p value</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
</tr>
</tbody>
</table>

ENDO = subendocardium; MID = midmyocardium; EPI = subepicardium; MEAN = mean transmural blood flow; PT = posterior transmural; AT = anterior transmural.

*p < .05, **p < .01 standing vs both runs; p value column is for control run vs diltiazem run.

TABLE 4B
Normalized myocardial blood flow in the ischemic regions during control and diltiazem runs (fractions of the control area; mean ± SD, n = 9)

<table>
<thead>
<tr>
<th>ENDO</th>
<th>Standing</th>
<th>Control run</th>
<th>Diltiazem run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.06 ± 0.17</td>
<td>0.13 ± 0.07a</td>
<td>0.25 ± 0.08a</td>
</tr>
<tr>
<td>p value</td>
<td>.05</td>
<td>.05</td>
<td>NS</td>
</tr>
<tr>
<td>MID</td>
<td>1.06 ± 0.11</td>
<td>0.33 ± 0.18b</td>
<td>0.44 ± 0.21b</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EPI</td>
<td>1.08 ± 0.15</td>
<td>0.70 ± 0.29a</td>
<td>0.82 ± 0.31a</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MEAN</td>
<td>1.06 ± 0.10</td>
<td>0.39 ± 0.17a</td>
<td>0.49 ± 0.18a</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

ENDO = subendocardium; MID = midmyocardium; EPI = subepicardium; MEAN = mean transmural blood flow.

*p < .01 standing vs both runs; p value column is for control run vs diltiazem run.

hyperemia after coronary occlusion and also to inhibit the redistribution of blood flow away from the subendocardium to the subepicardium in the presence of a coronary stenosis that limits reactive hyperemia; this effect appeared to be independent of any change in myocardial O₂ consumption.46 Such an effect could have been present in this study during the active hyperemia of exercise since an increase in the systolic portion of coronary flow due to subepicardial vasodilation has been shown to occur during exercise in the presence of coronary stenosis.35 In addition, myocardial blood flow in the control area slightly decreased (although not significantly) during exercise with diltiazem compared with during the control run. This reduction in myocardial blood flow in the control area probably resulted from reduced myocardial O₂ requirements after diltiazem (significantly decreased heart rate and left ventricular diastolic pressure, and slightly but not significantly decreased left ventricular peak pressure). In clinical studies, heart rate at submaximal exercise after a high dose of diltiazem has been shown to be significantly lower than that during exercise with placebo. Despite a lack of effect on systolic blood pressure, diltiazem administration has been associated...
with a significant reduction in submaximal pressure-rate product and an increase in exercise tolerance in patients with effort angina and fixed chronic coronary artery disease.\textsuperscript{8-11} This reduction in heart rate could have reduced O\textsubscript{2} requirements per minute in the ischemic zone during the initial development of dysfunction, as well as in the overlying epicardial region, thereby favorably affecting transmural blood flow distribution. Finally, it is possible that the mild reduction in left ventricular end-diastolic pressure during exercise after diltiazem could have favored enhanced subendocardial blood flow in the ischemic zone.

Our conscious canine preparation was designed to simulate the physiologic effects of exercise-induced myocardial ischemia in patients with fixed coronary artery stenoses. It should be recognized, however, that the atherosclerotic lesion may not mimic the dynamics of an atherosclerotic lesion, and that in the clinical setting collateral channels may originate from coronary arteries in which there is significant atherosclerotic disease. We have demonstrated in our animal preparation that exercise-induced myocardial ischemia is reduced by diltiazem, and our findings may provide a basis for understanding its efficacy in the treatment of patients with exercise-induced angina.

We gratefully acknowledge the technical assistance of Margaret Hill and Barbara Ann Martin and our word-processing operator, Marilyn Conwell. We are also grateful to Elizabeth A. Gilpin for statistical analyses.

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