Repeated Stunning Precedes Myocardial Hibernation in Progressive Multiple Coronary Artery Obstruction

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OBJECTIVE
The aim of this study was to characterize a regional myocardial flow-function relationship in collateral dependent myocardium produced by multiple coronary artery obstruction.

METHODS
Ameroid constrictors were placed around the proximal right (RC) and circumflex (CX) coronary arteries and a silicon tubing cuff around the proximal LAD (left anterior descending artery) (luminal stenosis ≥77%) in 18 dogs. Weekly two-dimensional echocardiography was performed for regional function (anterior [A], inferoposterior [IP], wall thickening [WT]), and fractional shortening (FS). Colored microspheres injected at baseline and before sacrifice, before and after dipyridamole (0.5 mg/kg) injection, determined resting flow (RF) and coronary reserve (CR), respectively.

RESULTS
Coronary angiography performed at four weeks after surgery confirmed occlusion of RC and CX with collateralization and a tight stenosis of LAD. Initially, an episodic reduction in A and IP WT was observed which became persistent later (AWT: 16 ± 3%; IPWT: 16 ± 4%; FS: 20 ± 4%; p < 0.005 vs. baseline [BS]). With dobutamine a biphasic response (improvement in A and IP WT between 5–15 and dysfunction between 20–30 μg/kg/min) was observed. Seven dogs were sacrificed at eight weeks and showed normal RF but reduced transmural CR (A: 75 ± 18%; IP: 46 ± 22% of control). Seven dogs underwent PTCA of the LAD at eight weeks and showed gradual improvement in AWT with normalization at 12 weeks (AWT: 30 ± 5%, p < 0.001 vs. eight weeks). At sacrifice RF and CR in the A wall were normal but there was reduced subendocardial RF in the IP region (64% of BS). Further, biopsy samples showed normal histological findings and high energy phosphate content in all dogs. Radioligand binding assays using 125I-iodocyanopindolol showed downregulation of beta-adrenergic receptor density in the dysfunctional regions compared with control.

CONCLUSIONS
In this canine model of viable, collateral dependent and reversibly dysfunctional myocardium, there was early episodic dysfunction followed by persistent dysfunction which was initially associated with normal RF and later with subendocardial hyperperfusion. (J Am Coll Cardiol 1999;34:2126–36) © 1999 by the American College of Cardiology

The dynamic nature of coronary artery disease can give rise to various ischemic syndromes, and in the past two decades there has been increased awareness of the contractile abnormalities that accompany ischemia and reperfusion. Myocardial stunning is a well-defined phenomenon of postischemic myocardial dysfunction which is a result of an acute ischemic insult. There are many excellent animal models of myocardial stunning (1–3), and evidence for the occurrence of myocardial stunning in man includes: stunning occurring after lytic therapy, coronary angioplasty, unstable angina and cardiac surgery (4). On the other hand, myocardial hibernation—a chronic phenomenon—is defined as persistently impaired myocardial function at rest due to decreased coronary flow that can be partially or completely restored to normal if the myocardial oxygen supply and demand relationship is favorably altered either by improving blood supply or by reducing demand (5). There is some evidence that both myocardial stunning and hibernation can coexist (6), and some authors even favor the concept that myocardial hibernation is caused by repeated episodes of stunning (7,8). The difficulty in developing chronic experimental models of coronary artery stenosis has hampered our detailed understanding of the multiple mechanisms responsible for regional and global dysfunction in patients with coronary artery disease. Earlier studies have shown that it is...
possible to produce multiple chronic coronary occlusions without infarction in dogs (9–11). The regional resting flow remains normal but the maximum dilatory capacity is compromised (12,13). Later studies showed that the resting function in such a collateral dependent region was also normal, and ischemia only ensued upon increase in metabolic demand (14,15). Chronic dysfunction of a collateral dependent zone with normal resting flow has also been reported (7,16). The mechanisms underlying chronic dysfunction in a collateral dependent bed, however, remain largely unexplored.

The aim of this study was primarily to characterize the relationship between regional myocardial blood flow and mechanical function in a canine model of collateral dependent myocardium produced by multiple vessel coronary artery stenosis, and second, to study the effect of revascularization on the regional flow and function, and third, to study the prevalent energetic, structural and adrenergic receptor status.

**METHODS**

**Animal preparation and surgical procedure.** The study protocol was approved by the ethical committee of our institution. Eighteen mongrel dogs of either gender (23 ± 2.6 kg) were premedicated intramuscularly (i.m.) with piritramide (3 mg/kg, Dipidolor [Janssen-Cilag, Belgium]), and anesthesia was induced and maintained by intravenous (IV) administration of sodium pentobarbital (Nembutal, [Sanofi, Belgium] 15–20 mg/kg and 3 mg/kg/h, respectively). The animals were intubated and artificially ventilated (30% oxygen and 70% room air, [Mark 7A Bird Respirator, Palm Springs, California]) with the ventilation being adjusted to maintain normal blood gas values. The tip of a femoral artery cannula was placed in the descending aorta for monitoring the arterial blood pressure, withdrawal of samples for blood gas analysis and withdrawal of reference blood for microsphere analysis. The electrocardiogram (ECG) and arterial pressure were monitored throughout the entire procedure. In all dogs a left thoracotomy was performed through the fourth intercostal space under sterile conditions. After placing a left atrial cannula, the coronary vessels were dissected free in the proximal portion and amiodar occluders (Dimed Medical Engineering, Antwerp, Belgium) were placed around the left circumflex and right coronary artery. A silicon tubing (outside diameter 4 mm, inside diameter 2 mm) cuff was placed around the proximal portion of the left anterior descending artery (LAD) (always distal to the major septal perforating branch and before or after the first diagonal branch) and secured with two fine sutures so as to produce a tight stenosis. The chest incision was closed in layers and the pneumothorax evacuated. Antibiotics were administered preoperatively, and daily for three days postsurgery (Albipen LA 16 mg/kg i.m., ampicillinum anhydricum 100 mg/ml). There was full recovery within one to two days, and all dogs were fed ad libitum on a standard diet. Three sham operated dogs were treated in a similar fashion and were used to study regional histology and beta-adrenergic receptor density.

**Experimental protocol (Fig. 1).** Coronary angiography, angioplasty and quantitative coronary analysis. Between four to five weeks after surgery, all animals were reanesthetized and artificially ventilated (see above). The left carotid artery was isolated and cannulated (6F Sheath, USCI Bard, Belgium), heparin (200 IU/kg IV) and Xylocaine 2% (1 mg/kg IV) were administered before manipulation of the coronaries, and a selective left and right contrast coronary angiography was performed using a 5F coronary catheter. Fluoroscopic images were filmed for later quantitative coronary analysis (QCA). The left carotid artery was ligated after the procedure, the incision closed and a single dose of antibiotics administered i.m. In seven dogs during the eighth week postsurgery, the right carotid artery was cannulated (9F sheath), and percutaneous transluminal coronary angioplasty (PTCA) of the LAD was performed using an 8F multipurpose guiding catheter (USCI). Conventional 2.5 to 3 mm coronary angioplasty balloon catheters were inserted and positioned at the site of the LAD stenosis and inflated for 90 s at 8 to 10 atm. Repeated inflations were performed (max. 4 inflations) until a satisfactory dilatation (∆≤50% residual stenosis) of the LAD was achieved. Contrast angiography was performed after administration of nitroglycerine (200 μg) and the fluoroscopic images filmed for later analysis. The procedures were of short duration and
tolerated well by the animals. After PTCA the dogs were given an IV shot of aspirin (lysine acetylsalicylic 0.900 g, S.A. Synthélabo, Belgium) and received a daily oral dose of aspirin 300 mg. The patency of dilated vessels was confirmed angiographically just before sacrifice via a femoral or carotid approach. The degree of coronary stenosis was determined by performing QCA using a commercially available semiautomated system (Angiographic workstation, AWOS, Siemens AG, Erlangen, Germany). All measurements were performed on selected end-diastolic frames. Briefly, after selecting a digitized stillframe as displayed on the video monitor, the beginning and end point of the segment to be analyzed was defined by placing a point inside the vessel contours proximal and distal to the stenosis. The algorithm automatically drew a "path line" inside the vessel connecting these two points. An interpolated reference diameter was used for calculation of percent diameter stenosis and geometric area stenosis. The absolute values for minimal luminal diameter and reference diameter as well as percent stenosis were immediately displayed.

Left ventricular function at rest and under pharmacological stress. Two-dimensional transthoracic echocardiography was performed at baseline and on a weekly basis postsurgery until sacrifice, using a Toshiba Sonolayer (model SSH-160A) with a 5 MHz transducer in all dogs. The studies were performed in the right lateral decubitus position and under light sedation (Imalgene [100 mg Ketamine/ml] 2 mg/kg, and Rompun [xylazine hydrochloride 2%] 0.5 mg/kg, IM). Standard parasternal long and short axis views (midpapillary level) were recorded on videotape for off-line wall thickening (WT) analysis. In short axis the anterior (A) and the posterior papillary muscles were used as anatomical reference points whereby regional WT (%) was measured for five regions: anterior wall (AW), anteroseptum (AS), inferior wall (IW), posterior wall (PW) and midseptum (MS) as: (end systolic thickness - end diastolic thickness)/(end diastolic thickness). The fractional shortening (FS) (%), a parameter for global function was measured in long axis as: (end diastolic cavity area - end systolic cavity area)/(end diastolic cavity area); here the end systolic and end diastolic endocardial (endo) borders of each frame were manually traced excluding the papillary muscle. The end diastolic frame of echocardiographic images was selected using the Q-wave onset of the ECG, and the frame with the smallest left ventricular cavity was defined as end systole. All premature heart beats and postextrasystolic heart beats were excluded. The endo and epicardial (epi) borders of each frame were manually defined. The AW and AS were considered as regions supplied by the LAD, with inferoposterior (IP) being the collateral dependent region. The recordings of six beats were averaged for each measurement. In the seventh postoperative week, a dobutamine stress echocardiography was performed in all dogs after baseline (BS) measurements, using incremental doses of dobutamine at 3-min intervals (5, 10, 15, 20, 25, 30, 35 and 40 μg/kg/min). The stress test was terminated when significant wall motion abnormality was noted by visual evaluation, heart rate of ≥140 beats/min, ST depression or elevation or ventricular arrhythmias developed. A semiquantitative assessment of the wall motion was done using a standard scoring system with a 5-point scale: 1 = normal, 2 = slightly hypokinetic, 3 = hypokinetic, 4 = akinetic, 5 = dyskinetic. All echocardiographic measurements were performed by two observers (B.S. and M.S.) who had no knowledge of each others’ results.

Regional myocardial blood flow. Regional myocardial perfusion was determined using colored microspheres (CM) (15 μm diameter suspended in saline and 0.1% Tween 80 and thimerosal, Dye-Trak [Triton Technology, North Carolina]) as described previously (17). Briefly, approximately nine million CM (2.5 ml) were sonicated and vortex agitated before injection into the left atrium. One of five different colors (white, red, violet, blue or yellow) was administered at random as a bolus. Immediately before injection an arterial reference sample withdrawal was started from the femoral cannula at a rate of 9.23 ml/min and continued for 90 s. Before manipulation of the coronaries, two shots of CM were given before and after injection of dipyrindamole (0.5 mg/kg), to determine the BS flow and coronary reserve (CR). Two more shots of microspheres were given in a similar fashion just before sacrifice at eight or 12 weeks after surgery. The tissue samples were digested.
in 4M KOH with 2% Tween 80 and processed according to previously published techniques (18). After sacrifice and excision of the heart, the atria, great vessels, right ventricle and connective tissue were removed, and the left ventricle (LV) sliced into five sections of equal thickness perpendicular to the longitudinal axis. The first (basal) and fifth (apical) sections were disregarded, and the remaining three slices were used for determination of regional myocardial flow for the LAD perfusion region and the collateral dependent region. For each region ±3 g of myocardial tissue was obtained, divided into epi and endo portions, weighed and the amount of microspheres counted spectrophotometrically as described earlier.

Myocardial histology. At the end of the eighth (n = 7) and twelfth (n = 7) postoperative week, the animals were anesthetized, intubated and ventilated and the heart exposed via a thoracotomy as described before. After placement of an atrial catheter and injections of microspheres, several (3–5) transmural myocardial biopsy specimens (Tru-cut biopsy needle, Travenol Laboratories) were obtained from the A and IP wall of the LV, immersed in a fixative (containing needle, Travenol Laboratories) were obtained from the A and the IP wall of the LV were described earlier (19). Transmural needle myocardial biopsies obtained from the A and IP wall of the LV, immersed in a fixative (containing 3% gluteraldehyde buffered with 90 mmol/L KH2PO4 and adjusted to pH 7.4 with 0.1N KOH) and processed for light and electron microscopy. Toluidine blue staining was used to determine myocardial viability and degree of transmural fibrosis in all biopsies by using morphometry. A large dose of saturated KCL was then administered before excision of the heart. The heart was sliced into five slices of equal thickness after confirming the presence of silicon tubing cuff on the LAD and inspection of the tissue for macroscopic signs of infarction or regional thinning of myocardial muscle.

Myocardial high energy phosphate content. The adenine nucleotides (ATP, ADP, AMP and IMP) and their metabolites (adenosine, inosine, hypoxanthine) were determined by high performance liquid chromatography (HPLC) as described earlier (19). Transmural needle myocardial biopsy specimens obtained from the A and the IP wall of the LV were immediately immersed and snap frozen in liquid nitrogen and stored at −80°C for later analysis with HPLC. Tissue contents are calculated as micromoles per gram dry weight.

Beta-adrenergic receptor binding studies. All radioligand binding studies were carried out in triplicate using 125I-Cyanopindolol (ICYP, 2200 Ci/mmol; 10 to 1200 pM). In brief, frozen myocardial tissue stored at −80°C (weight 500 to 1,000 mg) was powdered, homogenized and filtered through Nitex cloth (149 μm). Homogenates were diluted with an equal volume of 1 M KCl, incubated at 4°C for 10 min, and then centrifuged (48,000 g for 20 min). Bradford protein microassay was used, with bovine serum albumin as a standard for determination of protein concentration. The assays were performed by incubating 40 μl (100 μg) protein in 50 mM Tris-HCl containing 4 mM MgCl2 and 100 μM guanosine triphosphate at pH 7.5 in a total volume of 150 μl at various concentrations of ICYP (1 pM → 300 pM) for 90 min at 37°C in an oscillating water bath. Isoproterenol (10−4 M) was used to determine nonspecific binding. Assays were terminated by addition of 4 ml of ice cold buffer with a 50 mM concentration of Tris HCl at pH 7.5. Bound and free ligand were separated by filtration over glass fiber filters followed by an additional wash of 2 × 4 ml. Radioactivity retained on the filters was quantified by gamma counting. Scatchard analysis of binding data was performed to obtain the dissociation constant (Kd) for ICYP binding, and the maximum number of binding sites (Bmax).

Statistics. All data are expressed as mean values ± SD. The data were analyzed using one-way analysis of variance (ANOVA) for repeated measures. When significant differences were detected, paired post-hoc comparisons were performed using Student t test with modified Bonferroni correction. A value of p < 0.05 was considered to be statistically significant. Inter- and intraobserver variability was assessed for measurements of regional WT% and FS% using data from 10 animals. The reproducibility of the measurements was determined with the intraclass-correlation coefficient, which is the ratio of the variabilities within the subjects over the total variability. The reproducibility between the two observers was calculated from the average values of the two measurements of each observer separately. A linear mixed model incorporating fixed effects (i.e., main effects of interest) and random effects (i.e., variation between subjects) was used to analyze the variation in individual weekly WT data of all dogs. The statistical calculations were performed using statview 4.12+ and statistical analysis system (SAS).

RESULTS

Survival. Fourteen of the 18 dogs completed the study period of either eight weeks or 12 weeks. Four dogs died suddenly between the third to fifth week after surgery. Autopsy examination revealed occluded circumflex and right coronary artery, with a tight stenosis of LAD in all four dogs.

Hemodynamic data (Table 1). Heart rate. The BS heart rate was 77 ± 10 and 73 ± 12 beats/min in the non-PTCA and PTCA dogs, respectively with a significant increase in heart rate after injection of dipyridamole (ranging between 15 to 26 beats, 20 ± 5 beats, p < 0.05). This returned to BS levels shortly.

Mean arterial blood pressure. The BS mean arterial blood pressure was 98 ± 14 mm Hg and 99 ± 10 mm Hg for non-PTCA and PTCA dogs, respectively, and remained essentially stable after dipyridamole (96 ± 12 mm Hg and 98 ± 16 mm Hg).

Cardiac catheterization data. The selective coronary angiography performed during the fourth to fifth week after surgery showed occlusion of the circumflex and right coronary artery (with visible collateral formation between the circumflex, right coronary artery and the LAD) in all dogs, and an important stenosis of the LAD at 77 ± 7% (range
67% to 93%). Seven dogs undergoing PTCA of the LAD showed a reduction of stenosis to 38 ± 4% (p < 0.05). Patency of the dilated vessel was confirmed just before sacrifice in four of these animals (vessel stenosis 36 ± 8%, p = ns vs. immediately after PTCA).

**Left ventricular function (Tables 1 and 2).** At rest. The BS regional WT% for the A, MS and IP regions was similar in the non-PTCA and PTCA dogs. Immediately after surgery the wall motion was normal in all regions. In the initial weeks after surgery, an episodic nature of dysfunction was observed in individual dogs (Fig. 2) with later more persistent hypokinesis in both the A and IP WT% in both groups. The MS WT, on the other hand, remained relatively stable (see Fig. 3, panels A and B). After PTCA an improvement in the AWT% was observed which became significant from the second week onward (p < 0.04). At sacrifice, (fourth to fifth week post-PTCA) the A WT% had almost normalized at 30 ± 5%, but there was persistent hypokinesis in the IP region. Similarly for the global function, there was a significant drop in the FS% by eight weeks (non-PTCA dogs: 23 ± 3%, p < 0.03 vs. BS; PTCA dogs: 20 ± 4%, p < 0.05 vs. BS). After PTCA there was an improvement in the FS% to 30 ± 6% (p < 0.02 vs. eight weeks).

During dobutamine stress. A biphasic response was observed in all dogs, with initially a significant improvement at low dose (p < 0.05) in the hypocontractile regions and a subsequent deterioration in function at higher doses (Table 2). Normal function was observed between 10 to 15 μg/kg/min, with subsequent hypo-, a- to dyskinesis developing between 20 and 30 μg/kg/min. The heart rate increased significantly around 25 μg/kg/min (82 ± 12 to 118 ± 12 beats/min, p < 0.05).

**Table 1.** Summary of Hemodynamic Parameters

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<th>HR</th>
<th>MAP</th>
<th>FS</th>
<th>AWT</th>
<th>IPWT</th>
<th>MSWT</th>
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<td></td>
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<tr>
<td>Non-PTCA</td>
<td>77 ± 10</td>
<td>98 ± 14</td>
<td>33 ± 6</td>
<td>35 ± 8</td>
<td>35 ± 7</td>
<td>35 ± 3</td>
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<tr>
<td>PTCA</td>
<td>73 ± 12</td>
<td>99 ± 10</td>
<td>33 ± 5</td>
<td>37 ± 7</td>
<td>39 ± 5</td>
<td>39 ± 6</td>
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<td>8 weeks</td>
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<tr>
<td>Non-PTCA</td>
<td>68 ± 15</td>
<td>94 ± 14</td>
<td>23 ± 3*</td>
<td>16 ± 4*</td>
<td>18 ± 5*</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>PTCA</td>
<td>72 ± 16</td>
<td>96 ± 10</td>
<td>20 ± 4*</td>
<td>16 ± 3*</td>
<td>16 ± 4*</td>
<td>33 ± 7</td>
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<tr>
<td>12 weeks</td>
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<tr>
<td>PTCA</td>
<td>85 ± 22</td>
<td>98 ± 12</td>
<td>30 ± 6</td>
<td>30 ± 5*</td>
<td>21 ± 5</td>
<td>32 ± 7</td>
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</table>

*p < 0.05

AW = anterior wall; BS = baseline; FS = fractional shortening; HR = heart rate (beats/min); IPWT = infroposterior wall thickening; MAP = mean arterial pressure (mm Hg); MSWT = midseptum wall thickening.

**Inter- and intraobserver variability.** The differences in measurements of WT% and FS% between the two observers and between the two measurements made by the same observer were nonsignificant (p > 0.1). The reproducibility of the measurements varied between 0.77 and 0.98 (IP WT%: 0.77, AWT%: 0.98, MSWT%: 0.94, FS%: 0.92) for the two observers and between 0.82 and 0.96 for the measurements made by the same observer.

**Linear model.** A random starting point followed by a drop and constant process was fitted. The variance of AWT (36.25) was not significantly higher than the variance of IPWT (35.69). The serial correlation for AWT was −0.23 (p = 0.018) and for IPWT −0.21 (p = 0.028). Negative serial correlation means that when a given measurement is high the next measurement will tend to be low, and vice versa. At timepoint 0, there was, on average, no significant difference (p = 0.445) between AWT (35.17, s.e. 1.57) and IPWT (36.83, s.e. 1.60). From timepoint 1 to 8 (week) there was no significant difference (p = 0.49) between AWT (19.16, s.e. 0.60) and IPWT (19.60, s.e. 0.60). This means that AWT and IPWT appear to change in a similar fashion, showing a weekly variation in function (Fig. 2).

The MSWT, on the other hand, did not show such a variation and remained relatively stable.

**Myocardial perfusion (Table 3).** The data are expressed as absolute flow values (ml/min/100 g) for the epi and endo of the A and IP region.

**Non-PTCA group.** The absolute flow and the CR were similar at BS in the two regions (p > 0.1). At eight weeks the resting flow in both the epi- and endocardium of A and IP regions were not significantly different compared with BS (p > 0.1, Table 3). The maximum vasodilatory flow, when compared with the BS maximum vasodilatory flow,
was substantially depressed in the collateral dependent IP zone (epi: 0.56 ± 0.30, p = 0.03; endo: 0.36 ± 0.22, p < 0.002), and moderately depressed in the LAD perfused region (epi: 0.77 ± 0.32, p = 0.14; endo: 0.71 ± 0.22, p = 0.048). See Figure 4 for simultaneous changes in perfusion and function.

PTCA group. The absolute flow and the CR were similar at BS in the two regions. At 12 weeks the resting flow and CR in both the epi- and endocardium of A wall were not significantly different from BS denoting that PTCA had brought about improvement. The IP region, on the other hand, showed a significant reduction in the CR and subendocardial resting flow compared with BS (66 ± 19 ml/100 g/min, p = 0.03).

Myocardial structural changes. Two dogs showed a small localized subendocardial infarction of the posterior papillary muscle. Light microscopy showed predominantly normal myocardial structure (Fig. 5, top panel) in all dogs, with a small percentage of myocytes (5% to 10%) showing mild to moderate degree of myolysis with glycogen accumulation on electron microscopy (Fig. 5, bottom panel). Further, there was an increase between 5% and 10% in interstitial connective tissue compared with sham operated dogs.

Myocardial high energy phosphate content (Table 4). There was no loss of high energy content in the groups with or without PTCA and the energy charge (ATP + 1/2 ADP)/(ATP + ADP + AMP), a measure of the energy supply in an ATP consuming system, appears to be in the range (0.89 to 0.94) which is described in normal animal and human hearts.

Beta-adrenergic receptor binding study (Table 5). The beta-adrenergic receptor (AR) density (Bmax [maximum bound ligand]) and the Kd value were significantly decreased for both the A and IP regions compared with the sham operated control dogs (n = 3) (p < 0.001 and p < 0.005, respectively). In the PTCA group, there was a small but significant increase in the Bmax of the A region after PTCA (69 ± 5 fmol/mg protein, p = 0.04 vs. A region Bmax of non-PTCA dogs) without normalization of Bmax.

DISCUSSION

We developed a canine model of multiple coronary artery obstruction giving rise to collateral dependent, noninfarcted and reversibly dysfunctional myocardium.

Perfusion-contraction relationship in the collateral dependent myocardium. Earlier studies employing the athero-oid constrictor to produce a gradual coronary occlusion found the collateral dependent region to be noninfarcted and with normal resting flow (10–13). Increase in heart rate, vasodilation and changes in perfusion pressure were shown to cause an underperfusion of the collateral dependent areas (20,21), and the dilatory capacity was found to be compromised (9). Later it was shown that basal function in these collateral dependent areas was normal (8,12–14) and became compromised in conditions of increased metabolic demands. Also hypokinesis could be induced in the collateral dependent noninfarcted dog heart by reducing the collateral forming potential (by ligation of epicardial anastomoses) (22).

In our model of progressive coronary artery obstruction using atheroid occluders (which are known to exert a stenotic effect as early as one week, and to occlude the coronary between 17 and 28 days after implantation), extensive collateral formation was observed in the IP region. Immediately after surgery the function was normal; however, reduced contractility was observed both in the IP region and the A wall during the early follow-up. Analysis of the weekly WT data from individual dogs showed that both IP and A wall initially showed an almost weekly variation in function, an “episodic dysfunction” (Fig. 2), which was not observed in the MS region in the same dogs. Later, the function tended to remain low and did not return to BS value. At eight weeks there was persistent dysfunction; the resting flow was normal in both IP and A wall, but the maximum vasodilatory capacity was compromised, and
the myocardium was viable. It is conceivable that the most likely cause of the early episodic dysfunction is demand ischemia (i.e., conditions of increased metabolic demands or even situations of intermittent platelet plug formation), with repeated superimposed episodes of demand ischemia producing a state of perpetual stunning. At 12 weeks after surgery (and four weeks after PTCA of LAD) the flow, CR and function were normal in the LAD perfused region, whereas there was persistent hypocontractility in the collateral dependent IP region with a clear reduction of subendocardial flow (64% of BS flow), once again in the presence of predominantly viable myocardium, with some myocytes showing a mild to moderate degree of myolysis (Fig. 5, bottom panel). We speculate that the persistently depressed function with a local decrease in oxygen consumption acts as a trigger for an autoregulatory response of the regional flow to a lower level. This model is in fact the first animal model where a chronic physical stenosis was successfully dilated to show an improvement in the contractility and perfusion and suggests many parallels with actual clinical situations existing in patients (Fig. 6).

**Metabolic, structural and adrenergic aspects of collateral dependent myocardium.** The dobutamine echocardiography provided us with evidence of viability before PTCA was performed. Increasing doses of dobutamine infusion produced a biphasic response in both the A and IP regions. Recruitment of inotropic reserve with low dose dobutamine

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**Table 3.** Regional Perfusion Data; A: Non-PTCA Group, B: PTCA Group

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<tr>
<td></td>
<td>epi</td>
<td>endo</td>
<td>epi</td>
<td>endo</td>
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<tr>
<td>BS</td>
<td>85 ± 29</td>
<td>96 ± 34</td>
<td>BS</td>
<td>69 ± 21</td>
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<tr>
<td></td>
<td>309 ± 114</td>
<td>360 ± 155</td>
<td>85 ± 25</td>
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<td>BS + dip</td>
<td>83 ± 33</td>
<td>85 ± 33</td>
<td>65 ± 20</td>
<td>60 ± 17</td>
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<td>8 week</td>
<td>240 ± 100</td>
<td>254 ± 154*</td>
<td>12 wk + dip</td>
<td>232 ± 175</td>
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<tr>
<td>8 week + dip</td>
<td>188 ± 41*</td>
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<td></td>
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<td>12 week</td>
<td>140 ± 148*</td>
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*p < 0.05.

A = anterior; BS = baseline; dip = dipyridamole treatment; endo = subendocardium; epi = subepicardium; IP = inferoposterior. Absolute flow values are given in ml/min/100 g.
is not exclusively related to hibernation (23), as stunned myocardium can be also stimulated by any intervention which increases cytosolic calcium concentration; however, worsening of the function with an increasing dose of dobutamine (biphasic response) is typical of hibernating myocardium. The contractile dysfunction observed in our model cannot be explained by decreased high energy phosphate content (HEPC), as this was within the normal range, and mitochondrial function as estimated by the ATP/ADP ratio and energy charge also did not appear to be impaired. Further, myocardial structure in all our dogs was predominantly normal. Only two dogs showed a small localized subendocardial infarction of the posterior papillary muscle. The extensive morphological changes observed in patients with hibernating myocardium (24,25) were not to be found in this model. Only 5% to 10% of the myocytes showed moderate degrees of myolysis and glycogen accumulation. This could be due to species differences (dogs develop collaterals faster and more extensively than humans) or is simply a question of time. Finally, we found consistent changes in the beta AR properties of the dysfunctional myocardium, with a tendency towards reversibility as the function improved. Studies of beta AR systems in a setting of chronic (reversible) ischemia are almost nonexistent. One study (15) showed downregulation of beta AR in the collateral dependent zone in a chronic porcine model of exercise induced ischemia. The \(G_{s}\) (G-receptor stimulating protein) content was increased with maintained adenylyl cyclase activity and reduced \(G_{i}\) (G-receptor inhibitory protein) content, which could provide a means by which adrenergic activation is maintained in the setting of chronic episodic myocardial ischemia. These findings suggest the involvement of beta-adrenergic systems in chronic reversible forms of myocardial ischemia and implicate similar neurohormonal changes as seen in heart failure models with downregulation of beta AR. Another mechanism recently implicated in the contractile dysfunction in stunning involves partial troponin I degradation as a result of activation of \(Ca^{2+}\) dependent proteases during reperfusion (26).

Collateral dependent myocardium—parallels with the clinical situation. In humans, the relationship between the severity of coronary stenosis and the degree of ischemia produced is not easy to predict, especially when irregular atherosclerotic plaques, variable collateral circulation and preexisting myocardial remodeling may alter the effects of any given degree of stenosis (27). It is therefore difficult to extrapolate an experimental situation in its entirety to a clinical situation. Another factor to bear in mind is species differences regarding preexistent collaterals and the poten-
tial for collateral formation. Pig, sheep and man have fewer collaterals compared with the dog. In some species, such as the guinea pig, the collateral flow is so extensive that even complete coronary occlusion by ligation produces no detectable ischemia, and, in others such as the rat, the flow is so low that very severe ischemia results. As Schaper (28) has stated so eloquently, “guinea pigs win the rat race.” In humans it is thought that gradual coronary occlusion provokes a variable growth of collaterals, and the result of a complete arterial occlusion varies from patient to patient (28). Never is the collateral flow high enough in humans to align them to the guinea pig. Data from human studies shows great variation with some showing low flow in the dysfunctional collateralized myocardium (29) and others showing near normal levels of resting flow in the collateralized regions (7,30) implicating repeated stunning in the persistent dysfunction observed. Further, recent studies (6,31) have shown that resting dysfunction can reflect a diversity of resting flow abnormalities which can actually coexist. Our canine model of progressive multivessel coronary artery obstruction producing reversible myocardial dysfunction parallels the clinical condition found in patients. The data suggest a transition from the initial episodic dysfunction to a state of perpetual stunning (due to repeated superimposed episodes of demand ischemia) with normal resting flow, and later towards an adapted state of myocardial hibernation where the flow autoregulates to a decreased local oxygen consumption.

**Study limitations.** There are several limitations to our model. Sequential flow measurements were not possible due to the limited number of colors available (at present only five colors are available). The instrumentation was kept to a minimum due to the relatively long study period (8 to 12 weeks), and, therefore, the function could not be monitored continuously or in awake animals. The experience from our laboratory with chronic heavily instrumented animals shows only a five to six week survival without problems of infections, dysfunction of equipment, etc. Chronic instrumentation could have allowed us to demonstrate better the episodic nature of dysfunction observed. A detailed study of the beta AR system (i.e., adenyl cyclase activity, Gi and Gs content) was not performed, nor were measurements of circulating or myocardial tissue norepinephrine levels made. This would have thrown more light on the possible molecular mechanisms.

**Conclusions.** We developed a canine model of chronic progressive multiple coronary artery obstruction whereby collateral dependent, structurally intact and reversibly dysfunctional myocardium was produced. The early phase characterized by episodic dysfunction was followed by per-
sistent dysfunction in the presence of normal resting flow, with a yet later stage being characterized by persistent dysfunction and subendocardial hypoperfusion. To conclude, therefore, in this model of collateral dependent viable myocardium, repeated stunning precedes myocardial hibernation. This reproducible model can be used to study various mechanistic aspects of myocardial hibernation.

Acknowledgments
The authors wish to thank Mr. Peter Lemmens for performing the HPLC analysis and Mr. Fred Thoné for performing the histology.

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Table 5. Summary of Data From the Beta-Adrenergic Receptor Study

<table>
<thead>
<tr>
<th>Bmax</th>
<th>Kd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ant</td>
</tr>
<tr>
<td>Non-PTCA</td>
<td>58 ± 11</td>
</tr>
<tr>
<td>PTCA</td>
<td>69 ± 5*</td>
</tr>
<tr>
<td>Control</td>
<td>149 ± 20</td>
</tr>
</tbody>
</table>

*p < 0.05 (PTCA vs. non-PTCA group for anterior wall). All Bmax and Kd values were significantly low (p < 0.005) compared with the sham operated controls.

ant = anterior; Bmax = maximum bound ligand (i.e. beta-adrenergic receptor density in fmol/mg protein); IP = inferoposterior; Kd = dissociation constant (pM); MS = midseptum; PTCA = percutaneous transluminal coronary angioplasty.

Figure 6. Top Panel: Coronary angiography performed at four weeks postsurgery confirmed the presence of tight stenosis on the LAD, and shows retrograde filling of the circumflex. Amoroid constrictors (placed around the circumflex and right coronary artery) appear as radio opaque rings. Bottom Panel: Percutaneous transluminal coronary angioplasty of the LAD was performed at eight weeks after surgery. LAD = left anterior descending artery.