Intermittent Coronary Sinus Occlusion in Dogs: Reduction of Infarct Size 10 Days After Reperfusion

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Intermittent balloon occlusion of the coronary sinus was applied to 11 open chest dogs subjected to 3 hours of ligation of the left anterior descending coronary artery followed by 8 to 12 days of reperfusion. Anticoagulants were not given during the reperfusion period. Risk region was assessed by planimetry of autoradiographs made from ventricular slices. Infarct size was equivalent when assessed by planimetry of ventricular slices before and after staining with triphenyltetrazolium chloride. In the seven survivors, 30 ± 8% of the risk region was infarcted. Seven of 11 control dogs survived (p = NS); 75 ± 4% of the risk region was infarcted in the control animals ($p < 0.01$ versus treated survivors). Light microscopic inspection of specimens stained with hematoxylin-eosin confirmed the border between necrotic and preserved myocardium. Thrombus was observed in the coronary sinus in all survivors in the treatment group.

These findings confirm earlier short-term studies that demonstrated a potent anti-ischemic effect of intermittent coronary sinus occlusion. At the same time, coronary sinus thrombosis warrants caution in the application of this technique to myocardial ischemia in humans.

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Intermittent balloon occlusion of the coronary sinus has been shown to reduce infarct size by nearly 50% in dogs subjected to 6 hours of ligation of the left anterior descending coronary artery (1) and by approximately 70% in dogs subjected to 3 hours of coronary ligation (2,3). This latter result has been observed with or without 3 hours of reperfusion after the ischemic period (2).

Despite these results, persistent doubt surrounds the anti-ischemic efficacy of intermittent occlusion of the coronary sinus. Part of this skepticism is due to the assessment of infarct size by triphenyltetrazolium chloride staining. Although such staining is an established means of delineating viable and necrotic myocardium several days after infarction (4), its use in short-term studies is controversial (5). A second factor contributing to this skepticism is that the anti-ischemic mechanism of intermittent coronary sinus occlusion has not been clearly established. Finally, although most observers (1–3) have found a potent anti-ischemic effect with this technique, one recent report (6) failed to identify any benefit associated with its use.

The purpose of this study was to evaluate the anti-ischemic effect of intermittent coronary sinus occlusion in dogs subjected to 3 hours of ligation of the left anterior descending coronary artery followed by 8 to 12 days of reperfusion. Eight to 12 day old infarcted myocardium is grossly visible. Infarct size in these dogs was assessed by triphenyltetrazolium chloride staining and by gross examination of unstained tissue. The demarcation between areas of viable and necrotic myocardium was subsequently confirmed by light microscopy.

Methods

Experimental preparation. Twenty-two large mongrel dogs (20 to 27 kg) were anesthetized with pentobarbital, intubated and ventilated with a Harvard volume cycled ventilator. The chest was opened in the left fifth intercostal space and a snare was placed around the left anterior descending coronary artery after the first diagonal branch. A fluid-filled catheter was inserted into the left atrium and the carotid artery. A balloon-tipped double lumen catheter (Da-

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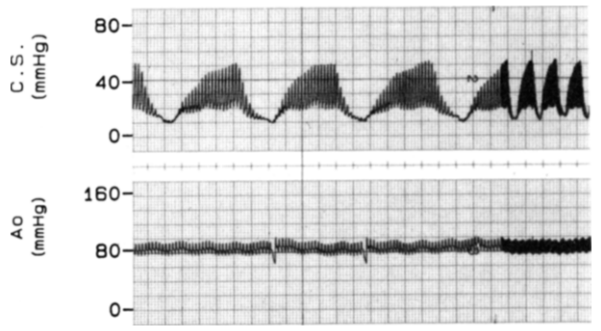


Figure 1. Representative tracing of coronary sinus (CS) and aortic (Ao) pressures during intermittent coronary sinus occlusion. During the 10 second period of coronary sinus balloon inflation, coronary sinus pressure increases to approximately 50/24 mm Hg. During the 5 seconds of balloon deflation, coronary sinus pressure decreases to approximately 10 mm Hg. Aortic pressure is unaffected.

tascope, Inc.) was inserted into the jugular vein and advanced to the coronary sinus. When inflated, the 5 cm long, 1.3 cm diameter balloon completely occluded the coronary sinus (Fig. 1), and the tip of the catheter was 3 to 5 cm inside the ostium of the coronary sinus. Pressures were measured with Statham P23 dB transducers and recorded on a Brush 240 recorder.

Experimental protocol. All dogs were given heparin (100 U/kg body weight) and lidocaine (3 mg/kg) before ligation of the left anterior descending artery. This artery was then ligated for 3 hours in both the treatment and the control groups. As in our previous studies, the dogs randomized to the treatment group received 2½ hours of intermittent coronary sinus occlusion beginning 30 minutes after coronary artery ligation. Intermittent occlusion consisted of 10 seconds of balloon inflation followed by 5 seconds of balloon deflation. To delineate the region at risk, 5×10^6 cerium-141–labeled 15 μm microspheres (New England Nuclear) were injected into the left atrium 20 minutes after coronary ligation (7).

At the end of the 3 hour ischemic period the left anterior descending artery snare and all catheters were removed. The chest was closed in layers and evacuated of air. All dogs were given an intramuscular injection containing 2 million U of procaine penicillin and 1 g of streptomycin and returned to their cages.

Pathologic studies. Eight to 12 days after the period of ischemia, the dogs were given a lethal injection of pentobarbital and potassium chloride. The heart and epicardial fat were removed and both atria and the right ventricle were trimmed away from the left ventricle. Gross inspection of the ligated coronary artery detected no evidence of coronary thrombus. The left ventricle was cut into five or six slices, each of which was weighed. For the first eight studies, tracings of left ventricular slices were made on clear acetate sheets and photographs were taken before and after triphenyltetrazolium chloride staining. For subsequent studies, tracings and photographs were made only after staining. Tracings were made of the basal side of the apical slice and the apical side of all other slices by an observer unaware of the treatment group.

Triphenyltetrazolium chloride staining was accomplished by incubating the slices at 37°C for 30 minutes in a mixture consisting of 5 mg triphenyltetrazolium chloride/ml stock solution, 1 *M* Sorenson's phosphate buffer and water in a 1:1:8 ratio.

Calculation of infarct size and risk region. Slices were immersed in 10% formalin for 48 hours. They were then placed over Kodak X-OMAT-AR film for 10 days. The developed films were placed under the acetate tracing of each slice and risk region was marked (7). Infarct size and

Table 1. Hemodynamic Variables Before and During Intermittent Coronary Sinus Occlusion

		Ischemic Period (minutes)					
	Baseline	25		35		180	
Aortic pressure (mm Hg)							
Control	118 ± 13/98 ± 10	106 ± 8/87 ± 8		95 ± 5/78 ± 5		102 ± 7/80 ± 3	
ICSO	108 ± 7/93 ± 6	100 ± 5/86 ± 4		90 ± 5/80 ± 5		94 ± 5/77 ± 5	
Heart rate (beats/min)							
Control	138 ± 6	138 ± 5		137 ± 4		144 ± 4	
ICSO	137 ± 6	134 ± 6		136 ± 5		137 ± 6	
Coronary sinus pressure (mm Hg)							
Control	8 ± 2	8 ± 3		9 ± 2		8 ± 2	
ICSO	9 ± 4	10 ± 5	9 ± 4	45 ± 5*/22 ± 3*	10 ± 5	43 ± 5*/21 ± 3*	

*$p < 0.01$ versus controls; no other significant differences exist between control and treated dogs. All values are expressed as mean ± SEM. ICSO = intermittent coronary sinus occlusion.

Table 2. Ischemic Region and Infarct Size in Each Treatment Group

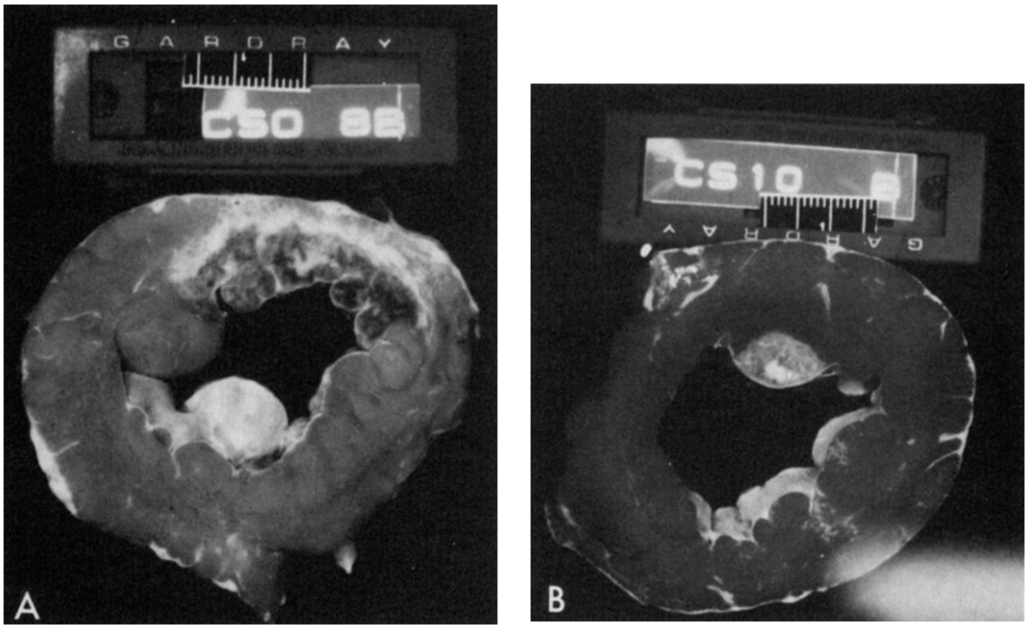
Study	Ischemic Region (g)	Ischemic Region (% of left ventricular mass)	Infarct Size (% of ischemic region)
	Control Dogs		
1	12.2	13.6	68.1
2	14.1	15.5	100.0
3	20.0	21.1	71.0
4	28.8	20.2	76.0
5	20.3	17.8	74.0
6	37.8	27.6	69.7
7	33.9	28.2	67.2
Mean ± SEM	23.9 ± 3.7	20.6 ± 2.1	75.1 ± 4.3
	Dogs With Intermittent Coronary Sinus Occlusion		
1	39.1	33.5	30.5
2	26.2	17.2	15.4
3	18.4	14.2	24.7
4	44.4	34.0	27.9
5	26.6	19.0	0.0
6	22.5	23.3	66.0
7	33.0	15.6	42.2
Mean ± SEM	30.0 ± 3.5	22.4 ± 3.1	29.6 ± 7.9*

*$p < 0.01$ versus control group.

risk region were calculated by planimetry using a Hewlett-Packard 9468A digitizer and 9810A calculator and multiplying the respective percentages by the weight of each slice. Confirmation of the margin between necrotic and viable tissue was made by light microscopic examination of hematoxylin-eosin–stained samples removed from the basal half of left ventricular slices before triphenyltetrazolium chloride staining. Samples were cut so that the grossly visible border between necrotic and viable myocardium was located in the middle of the tissue.

Statistics. Hemodynamic data from control and treated dogs were compared by repeated measures analysis of variance. Mortality, risk region and infarct size were compared by a *t* test. All data are presented as mean ± SEM.

Figure 2. Photographs of triphenyltetrazolium chloride-stained midleft ventricular slices from control (**A**) and intermittent coronary sinus occlusion-treated (**B**) dogs. Large areas of confluent infarction (**white areas**) are present in the control dogs. Treated dogs typically had patchy subendocardial necrosis.



The studies were performed in accordance with the animal welfare regulations of the Johns Hopkins Medical Institutions and the National Institutes of Health and the guiding principles of the American Physiologic Society.

Results

Hemodynamics and mortality. No significant differences were observed between the two groups in heart rate, blood pressure or baseline coronary sinus pressure (Table 1). Coronary sinus pressure did increase significantly during balloon inflation in the treated dogs. At the beginning of the 2½-hour treatment period, coronary sinus pressure rose from a baseline level of 9 ± 4 to 45 ± 5/22 ± 3 mm Hg at the completion of the 10 seconds of balloon inflation ($p < 0.01$). At the end of the study period, balloon inflation increased coronary sinus pressure from 10 ± 5 to 43 ± 5/21 ± 4 mm Hg ($p < 0.01$). Coronary sinus pressure decreased to baseline immediately after balloon inflation over the 2½ hour period of intermittent occlusion, indicating that the coronary sinus had not thrombosed while the catheters were in place.

Eight dogs, four in each group, died. Two in each group sustained ventricular fibrillation during the 3 hour study period. The other four were found dead in their cages 1 day after the study. One of the four, treated with intermittent coronary sinus occlusion, had a massive hemothorax, but no bleeding site was identified at autopsy. No obvious cause of death was found in the other three dogs at postmortem examination. All three dogs had awakened before being returned to their cages and are presumed to have died from arrhythmias. The size of the ischemic and infarcted regions was not analyzed in these three animals.

Infarct size. The percent of left ventricular myocardium at risk of infarction, determined by autoradiography, was 21 ± 2% (24 ± 4 g) in the control group and 22 ± 3% (30 ± 4 g) in the group treated with intermittent coronary sinus occlusion (p = NS for both absolute and percent values). Despite the similarity of region at risk in both groups, infarct size varied markedly: 75 ± 4% of the risk region was infarcted in the control dogs, compared with only 30 ± 8% in the group treated with intermittent coronary sinus occlusion ($p < 0.01$) (Table 2, Fig. 2). Infarct size was also analyzed on the basis of location within the ventricle (that is, apical slice, midventricular slice, and so on) (Table 3). Intermittent coronary sinus occlusion was associated with myocardial salvage in all but the most apical slice.

Pathologic findings. Planimetry of left ventricular slices demonstrated equivalence of infarct size as determined by gross inspection before or after triphenyltetrazolium chloride staining. The border between infarcted and living myocardium was also the same when determined by gross examination and by light microscopic inspection of tissue stained with hematoxylin-eosin (Fig. 3). Light microscopic examination of randomly selected examples of seemingly preserved myocardium confirmed the visual impression in all cases.

All dogs treated with intermittent coronary sinus occlusion had extensive thrombosis of the coronary sinus. It seems

Table 3. Infarct Size According to Ventricular Slice (apex to base)

Slice	Left Ventricular Tissue at Risk (g)	Slice at Risk (%)	Risk Region Infarcted (%)
1 (Apex)			
Control	2.9 ± 0.8	40 ± 9	61 ± 8
Treated	3.4 ± 0.6	35 ± 5	42 ± 11
2			
Control	6.1 ± 0.9	45 ± 5	69 ± 8
Treated	10.6 ± 1.3*	54 ± 4	35 ± 10*
3			
Control	7.0 ± 1.3	35 ± 5	79 ± 3
Treated	9.9 ± 1.0	35 ± 2	27 ± 8†
4			
Control	6.1 ± 1.5	26 ± 6	72 ± 7
Treated	3.4 ± 1.4	12 ± 5	21 ± 8†
5 (Basal)			
Control	3.0 ± 3.3	4 ± 5	67 ± 4
Treated	1.3 ± 2.4	5 ± 12	11 ± 5†

*$p < 0.05$ versus control; †$p < 0.01$ versus control. The left ventricle was cut into five or (in most cases) six slices. Slice 1 is the apex of the left ventricle, slices 3 and 4 are midventricular. Slice 5 is the most basal of the slices to contain an ischemic area. Because of the site of the coronary ligature (after the first diagonal branch of the anterior descending artery), slices 4 and 5 contain relatively little ischemic tissue.

likely that thrombosis occurred several hours after withdrawal of the coronary sinus catheter, as the heparin effect wore off. In most cases, thrombus appeared to totally occlude the coronary sinus. At the same time, the gross appearance of the epicardial veins and myocardium of the two groups was indistinguishable with the exception of the aforementioned differences in infarct size.

Discussion

Comparison with previous studies. Of four controlled studies of the anti-ischemic effect of intermittent coronary sinus occlusion, three have reported a reduction of infarct size. Using a cycle of 15 seconds of balloon inflation and 4 seconds of balloon deflation, and initiating intermittent coronary sinus occlusion 15 minutes after ligation of the left anterior descending coronary artery, Mohl et al. (1) observed a 44% reduction in infarct size after 6 hours of ischemia ($p < 0.001$). Using a cycle of 10 seconds of balloon inflation followed by 5 seconds of balloon deflation, we found (2) that 2½ hours of intermittent coronary sinus occlusion initiated 30 minutes after occlusion of the left anterior descending artery reduced infarct size from 57 ± 8% to 15 ± 6% of risk region ($p < 0.001$). Similar results were obtained when the same experimental protocol was followed by 3 hours of reperfusion. In control dogs, 49 ± 9% of the risk region was infarcted, compared with only 19 ± 7% in treated dogs ($p < 0.02$) (2). Similar results were obtained by Jacobs et al. (3) in a study in which treated animals received 2½ hours of intermittent coronary sinus occlusion beginning 30 minutes after coronary ligation. Intermittent coronary sinus occlusion was continued during 3 hours of reperfusion. Infarct size was reduced by 45%; 31 ± 4% of the risk region was infarcted in control animals compared with only 17 ± 4% in treated animals ($p < 0.03$).

The short-term studies described in this report confirm and extend the earlier long-term experiments outlined. When 3 hours of left anterior descending artery occlusion was followed by 8 to 12 days of reperfusion, 2½ hours of intermittent coronary sinus occlusion during the ischemic period reduced infarct size from 75 ± 4% of risk region to 30 ± 8% ($p < 0.01$). Two separate measures of infarct size, gross inspection before and after triphenyltetrazolium chloride staining, provided equivalent estimates of infarct size. The border between necrotic and living myocardium was confirmed by light microscopic examination of samples stained with hematoxylin-eosin.

Against the consistent pattern of these studies stands the report by Zalewski et al. (6), in which intermittent coronary sinus occlusion applied throughout 6 hours of left anterior descending artery occlusion failed to reduce infarct size. Their cycle of 15 seconds of coronary sinus occlusion and 4 seconds of deflation was the same as that used by Mohl et al. (1), as was the 6 hour duration of ischemia. One possible explanation for the discrepancy between these results is that myocardial oxygen demand was probably greater in the study by Zalewski et al. (6). Mean aortic pressure ranged from 90 ± 4 to 84 ± 4 mm Hg in the series of Mohl et al. In contrast, mean aortic pressure varied from 113 ± 8 to 124 ± 9 mm Hg in the experiments of Zalewski et al. These values are significantly different ($p < 0.05$ to $p < 0.002$). Although differences between heart rates in the two studies were not significantly different, there was also a trend toward higher heart rates in the experiments of Zalewski and coworkers. Thus, the rate-pressure product may have been higher in the latter experiments (6), and this could account for the discrepancy between their results and those of other investigators.

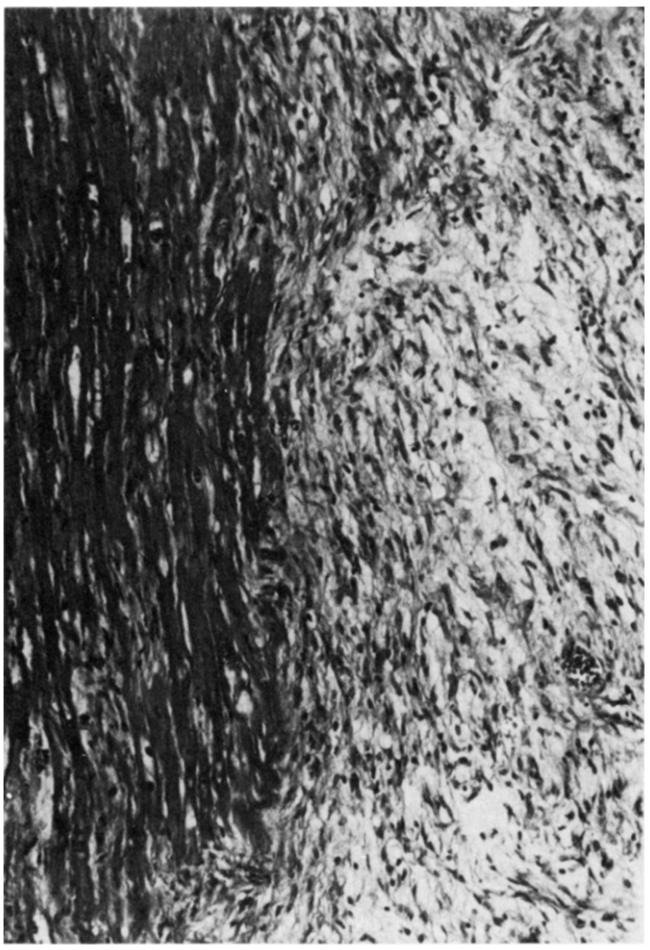


Figure 3. Photomicrograph of border between infarcted (**white**) and noninfarcted (**gray**) myocardium. The border corresponded to grossly identified margins of infarct, such as those seen in Figure 2.

Several lines of evidence indicate that intermittent coronary sinus occlusion retards but does not prevent necrosis. First, intermittent coronary sinus occlusion has no consistent

effect on such determinants of infarct size as heart rate, blood pressure or collateral flow. Moreover, the amount of the ischemic region infarcted in models of 3 hours of ischemia and intermittent coronary sinus occlusion (15 to 30%) is significantly less than infarct size after 6 hours of ischemia and intermittent occlusion (55%). Because canine infarction is ordinarily complete in 5 to 6 hours, increased myocardial oxygen demand may have accelerated the ischemic injury, abolishing any difference between infarct size in treated and control dogs at the end of the 6 hour study period in the experiment of Zalewski et al. (6).

Role of thrombotic occlusion of the coronary sinus. It is possible but unlikely that chronic occlusion of the coronary sinus played a role in the results of this study. The coronary sinus had not thrombosed while the catheters were in place, because coronary sinus pressure decreased to baseline immediately on balloon deflation. The dynamics of this process did not change over the 2½ hours of intermittent balloon occlusion, suggesting that there was not even a noncritical thrombosis present at the end of the ischemic period. In this and most other models, it is likely that the overwhelming majority, if not all, of the infarction occurred during the ischemic period and the first few minutes of reperfusion. Hearts were observed directly during this period of time and coronary sinus thrombosis was not observed. It seems more likely that coronary sinus thrombosis occurred several hours later, as the heparin wore off.

Mechanism of infarct size reduction. The mechanism by which intermittent coronary sinus occlusion reduces infarct size is not entirely clear. Several observations suggest that this technique results in perfusion of ischemic myocardium with venous blood from nonischemic regions. First, intermittent coronary sinus occlusion does not increase microsphere-determined arterial collateral flow (2). Second, intermittent coronary sinus occlusion raises coronary sinus pressure to levels above distal coronary pressure, thus establishing a gradient for venous blood flow from the coronary sinus to the ischemic region when the coronary sinus balloon is inflated. Finally, intermittent coronary sinus occlusion has been shown (2) to increase the washout of xenon-133 from the ischemic region. Together these results point toward perfusion from a venous source. Perfusion of the ischemic region with venous blood from nonischemic myocardium would result in the delivery of small amounts of oxygen and glucose and would enhance the removal of toxic metabolites. As such, venous retroperfusion of this magnitude would convert an ischemic insult into an injury consisting of profound hypoxia and partial ischemia. Each of these might be associated with an extended period of viability and tissue salvage at the end of a 3 hour period of arterial ligation. Several lines of evidence suggest that oxygen supply to an ischemic region affects the rate of infarction as well as ultimate infarct size (8–12). In addition, perfusion of isolated hearts with glucose-containing, oxygen-free solutions is associated with increased glycolytic flux and some preservation of mechanical function when compared with findings in globally ischemic hearts (13). Whether this metabolic and functional benefit translates into retardation of necrosis is unknown.

The possibility of venous retroperfusion is consistent with the recent report by Yoshida et al. (14), in which a substantial amount of microsphere-insensitive flow was demonstrated in ischemic tissue by inert gas washout. This microsphere-insensitive flow was unaffected by increasing coronary artery perfusion pressure, an observation that also suggests the possibility of perfusion from a venous source. On the other hand, when Zalewski et al. (6) perfused an ischemic segment for 6 hours with venous blood at approximately twice normal rates of flow, no reduction of infarct size was observed. It is not known whether venous perfusion of this type is associated with tissue preservation earlier in the course of infarction.

Clinical relevance. Thrombus found in the coronary sinus of treated dogs in our study raises questions about the clinical application of intermittent coronary sinus occlusion. Although thrombosis of the coronary sinus was not associated with any apparent ill effects in our study, its natural history is unknown in dogs and humans. Because the coronary sinus was not thrombosed while the catheters were in place, anticoagulation might have prevented the coronary sinus thrombosis, but this remains untested. Coronary sinus thrombosis notwithstanding, our study establishes that reduction of canine infarct size is achieved by intermittent coronary sinus occlusion beginning 30 minutes after the onset of a 3 hour period of myocardial ischemia.

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