Inability of Methylprednisolone Sodium Succinate to Decrease Infarct Size or Preserve Enzyme Activity Measured 24 Hours after Coronary Occlusion in the Dog

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SUMMARY Methylprednisolone sodium succinate (50 mg/kg) was given 30 minutes before or after the start of a 90 minute occlusion of the left circumflex coronary artery (LCX) in one group of dogs. In a second group, methylprednisolone sodium succinate was given 15 minutes after permanent occlusion of the left anterior descending artery (LAD). Infarct size was determined by dehydrogenase staining after 24 or 96 hours. Heart slices were incubated with nitro-blue tetrazolium and nonstaining infarcted tissue was dissected and weighed. Myocardial depletion of creatine phosphokinase activity (CPK) and lactate dehydrogenase activity (LDH) were determined 24 hours after temporary LCX occlusion. When measured after 24 hours, methylprednisolone sodium succinate treatment did not reduce infarct size or decrease enzyme loss. After temporary LCX occlusion infarct size was 30.4 ± 3.6% of left ventricular weight in control dogs and 30.0 ± 2.3% in treated dogs. No significant difference in infarct size was observed in hearts examined 24 or 96 hours after myocardial infarction. After permanent LAD occlusion, infarct size in control dogs was 39.2 ± 1.6% of left ventricular weight and 33.7 ± 3.5% in treated dogs. CPK activity in the LCX area decreased by 26.5 ± 7% in controls and by 28.1 ± 7% in treated dogs. LDH activity decreased by 26.4 ± 7% in controls and by 30.7 ± 7% in treated dogs. Treated dogs sustained a significantly greater fall in arterial blood pressure after LCX occlusion than did controls. During LCX occlusion and upon reperfusion, methylprednisolone sodium succinate treated dogs exhibited a significantly greater number of premature ventricular beats. Since infarct size and enzyme depletion were not reduced when measured after 24 hours, methylprednisolone sodium succinate treatment does not appear to have enhanced myocardial cell viability.

CORONARY OCCLUSION PRODUCES A ZONE of relatively ischemic cells around the borders of an expanding infarct. The ultimate fate of these cells may be influenced by a variety of interventions such as inotropic and pressor agents. Several studies have indicated that pharmacological doses of the glucocorticoids may salvage some ischemic tissue, thus decreasing infarct size. The first positive report of the efficacy of glucocorticoids was that of Johnson et al. who calculated infarct volume from visual inspection of the heart. No reduction in infarct size after cortisone treatment, however, could be detected by other investigators using similar methods. Interest in the glucocorticoids has been renewed by recent work using very large doses of these steroids. The corticosteroids have been reported to decrease the loss of such myocardial enzymes as creatine phosphokinase and β-glucuronidase from the ischemic or anoxic heart as well as decreasing electrocardiographic signs of ischemic injury. Some investigators have observed improved in hemodynamic and metabolic indices after glucocorticoid treatment, while others report no such improvement. Two attempts have been made to assess the effects of glucocorticoids on human infarct size using serial plasma creatine phosphokinase determinations to predict infarct size. These studies have produced contradictory results.

The parameters measured in these recent studies are indirect expressions of cell viability. While these measurements may indicate qualitative changes in the progression of ischemic injury, they do not permit a quantitative determination of the amount of myocardium salvaged by corticosteroid treatment. A more direct approach has been taken by Shatney et al. who used a histochemical staining technique to measure the actual area of injury produced by six hours of coronary occlusion. Steroid treatment resulted in a slight decrease in infarct size at that time. However, Cox et al. have shown that the area of injury delineated by this method continues to expand for at least 18 hours after coronary occlusion. Hence, measurements obtained in the first several hours after occlusion may not predict accurately ultimate infarct size. Therefore, in the present study, the same histochemical technique, as well as enzyme depletion measurements, were employed to determine the effect of glucocorticoid treatment on infarct size when measured 24 to 96 hours after coronary occlusion. In the present study no reduction in infarct size or preservation of enzyme activity was observed. This suggests that apparently beneficial steroid effects observed in the first several hours after coronary occlusion may only reflect a delay in myocardial enzyme depletion, as determined by dehydrogenase staining.

Materials and Methods

Male, mongrel dogs ranging in weight from 8.8 to 17.0 kg were used in this study. Beagles were excluded since there is some indication that this breed is particularly resistant to the consequences of coronary artery occlusion. Dogs were anesthetized with an intravenous dose of sodium pentobarbital (30 mg/kg), intubated with a cuffed endotracheal tube and respired with room air by a Harvard positive pressure pump. Catheters implanted in the left jugular vein and carotid artery were exteriorized at the back of the neck. Arterial blood pressure was monitored using a Statham P23D pressure transducer connected to the carotid catheter. The jugular catheter was used for subsequent administration of drugs and for obtaining venous blood samples in certain experiments.
Coronary Occlusion

Two models of myocardial infarction were employed in this study. In one set of experiments (group I), temporary circumflex coronary artery occlusion was followed by reperfusion, a model described by Jennings et al.21 As Bruno et al.22 and Jarmakani et al.23 have pointed out, such a model may reflect the kind of changes that take place in man, in whom the process of coronary artery narrowing is gradual and the ischemic insult at the time of infarction is seldom associated with a complete deprivation of blood flow to the area. Whether the delivery of blood is sufficient to maintain the myocardial cells in a viable state is dependent upon the types of demands placed upon the myocardium subsequent to the ischemic insult. Since many investigators' have used permanent occlusion of the left anterior descending coronary artery as a model of myocardial infarction, this approach was utilized in a second group of animals (group II) to facilitate comparisons with previous work.

In group I, the circumflex branch of the left coronary artery (LCX) was isolated near its origin, proximal to any major ventricular branches. The LCX was constricted with a silk ligature tied around both the artery and a 19 gauge hypodermic needle. The needle was then removed, leaving the artery partially constricted. Immediately thereafter the LCX was completely occluded with a length of silicone elastic retraction tape. After 90 minutes of occlusion the retraction tape was removed and flow was restored through the partially constricted vessel. Because it was not known whether the maximum drug effect would be seen if treatment was administered before or after the onset of occlusion, group I was subdivided into two treatment schedules. Animals received 50 mg/kg methylprednisolone sodium succinate i.v. either 30 min prior to (pretreated) or 30 min after (posttreated) the onset of occlusion. These animals were compared to dogs which received 0.9% NaCl solution as controls. Since time of drug administration proved to have no effect on infarct size, posttreatment alone was utilized in all subsequent experiments.

In group II the anterior descending branch of the left coronary artery (LAD) was isolated at the tip of the left atrial appendage and partially constricted with a ligature tied around the artery and a 20 gauge needle. The needle was removed and after 30 min of partial constriction the LAD was completely occluded with a second ligature.24 The LAD remained occluded until the dogs were sacrificed 48 hours later. Group II dogs received an i.v. dose of either 50 mg/kg methylprednisolone sodium succinate 15 min after occlusion or 0.9% NaCl solution as a control.

Group I consisted of 35 controls, 20 pretreated and 22 posttreated dogs. Group II consisted of 10 controls and nine posttreated dogs. When ventricular fibrillation occurred no attempt was made to defibrillate since it was not known how this would affect measurements of infarct size. These dogs were eliminated from the study.

Hemodynamic Measurements

In both groups I and II arterial blood pressure and standard lead II of the electrocardiogram were monitored continuously for four hours after occlusion. In group II, hourly blood samples were drawn from the right atrium (via the jugular catheter) and also from the femoral artery. Oxygen content was measured using a Lex-O2-Con oxygen analyzer. Cardiac output was determined by a thermal dilution technique using a Columbus cardiac output computer. Duplicate determinations were made by injecting 2 ml of a room temperature solution of 0.9% NaCl into the right atrium via the jugular catheter and recording temperature changes with a thermistor tipped Swan-Ganz catheter positioned in the pulmonary artery. Heart rate was obtained from the electrocardiogram.

Quantification of Infarct Size

Unless otherwise noted, all dogs received a 500 mg intravenous dose of tetracycline hydrochloride one hour prior to sacrifice. The animals were sacrificed with an overdose of pentobarbital and the hearts were removed. In order to demonstrate that infarct size did not increase after 24 hours, dogs in group I were sacrificed either 24 hours or four days after coronary occlusion. Since after 24 hours of permanent occlusion delineation of the borders of an infarct may be incomplete, dogs in group II were sacrificed 48 hours after coronary occlusion, by which time the area of infarction has reached its full extent.2 Hearts were cut into transverse slices 1 cm thick. One slice was examined and photographed under ultraviolet light. This permitted visualization and recording of fluorescence due to the accumulation of tetracycline along the perimeter of the infarcted region.25,26 All slices were then incubated for 15 min at 35°C in a phosphate buffered solution of nitro-blue tetrazolium (NBT) as described by Nachlas and Shnitka.27 Incubation with NBT results in an intense blue staining reaction in undamaged regions of the heart while areas of ischemic injury appear as clearly delineated pale zones. The right ventricular free wall was excised from each slice and all remaining tissue was considered as left ventricle. After fixation in alkaline formalin, nonstaining portions of myocardium were dissected and weighed, and infarct size was calculated as a percentage of left ventricular weight. Hematoxylin and eosin stained sections were prepared from NBT staining and nonstaining regions of the myocardium to assess microscopic signs of injury.

Enzyme Depletion

Myocardial depletion of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were determined 24 hours after temporary LCX occlusion in 12 dogs from group I. These dogs did not receive tetracycline. The area perfused by the LCX was delineated by inserting a catheter at the site of occlusion and injecting 30 ml of 0.2% sodium fluorescein and 0.9% saline solution. The dyed tissue was dissected from the remainder of the heart under UV light.

Homogenates (10% w/v) were prepared at 4°C from normal and occluded regions in a sucrose homogenization medium.28 Further dilution with buffer (1:25, v/v) was made prior to centrifugation at 22,000 x g for 40 min. Protein was determined by the method of Lowry et al.29 with bovine albumin, fraction V used as a standard.

LDH activity was assayed spectrophotometrically by following the rate of disappearance of NADH at 340 nm at 27°C.30 Control and experimental cuvettes contained phosphate buffer, 0.2 M, pH 6.5; sodium pyruvate, 1.0 μM; and 0.05 to 0.2 ml of diluted spun homogenate (diluted 1:50 in the phosphate buffer). The reaction was started by addi-
tion of NADH (0.5 μM) to the experimental cuvette. Initial rate of disappearance of NADH was followed for 10 min. Total fluid volume was 3.0 ml. A millimolar extinction coefficient of 6.22 was used to express LDH activity as μM NADH consumed per minute per mg protein.

CPK activity was assayed spectrophotometrically by following the appearance of NADPH at 340 nm at 27°C by the method by Rosalki.31 Reconstituted lyophilized CPK reagent obtained from Calbiochem was added to control and experimental cuvettes. Prior to assay the spun homogenate was routinely diluted (1:800, v/v) with a buffer containing 0.2% bovine serum albumin and trihydroxymethylaminomethane (TRIS), 0.01 M, pH 7.4.28 The reaction was started by addition of 0.02 to 0.10 ml of diluted spun homogenate to the experimental cuvettes. The total fluid volume was 1.05 ml. The reaction rate was linear after six minutes and was followed for at least 10 minutes thereafter. The activity of CPK is given in international units using a temperature correction factor.31

Drug Administration

Methylprednisolone sodium succinate (Solu-medrol, Upjohn) was dissolved in sterile 0.9% NaCl solution 50 mg/ml and infused at a rate of 50 mg/min. Drug treatment was assigned on a random basis. Measurement of infarct size and histological examination were performed by persons unaware of the treatments assigned to each animal.

Statistical Analysis

All averaged data are expressed as a mean ± standard error of the mean. Student’s two tailed t-test29 was used to determine the statistical significance between mean values. Two tailed paired analysis was utilized for changes within a group.

Results

Mortality

Methylprednisolone sodium succinate treatment had no effect on survival after coronary occlusion. In group I, 18 of 35 (51%) control dogs and 22 of 42 (52%) steroid treated dogs survived at least 24 hours after coronary occlusion. There was no significant difference in mortality between the two steroid treatment schedules. The cause of death in 13 controls and nine treated animals was ventricular fibrillation during occlusion or upon reperfusion of the LCX. This mortality rate is similar to that observed by Reimer et al.28 who employed a similar procedure. The remaining deaths occurred overnight while the dogs were not monitored and cause of death could not be ascertained. In group II, 6 of 10 (60%) controls and 6 of 9 (67%) methylprednisolone sodium succinate treated dogs survived.

Infarct Size

Four of the 40 dogs in group I (three controls and one treated dog) were excluded from this study. These dogs exhibited no ST-segment elevation in the ECG upon occlusion, did not develop ventricular tachycardia and sustained negligible myocardial injury when measured by NBT staining or tetracycline accumulation. This observation is consis-

![Figure 1](http://circ.ahajournals.org/)  
**Figure 1.** Heart slice from a control dog sacrificed four days after 90 min LCX occlusion followed by reperfusion. Infarct size equals 43.6% of left ventricular weight. A) Viewed under UV light, a wide band of tetracycline fluorescence is demonstrated in the infarcted region surrounding the posterior papillary muscle. B) The same slice is shown after staining with NBT: the dark staining region represents normal myocardium; the pale, nonstaining region represents irreversibly damaged tissue and corresponds in area to the zone demarcated by tetracycline fluorescence.
cinate treatment did not appear to suppress leukocytic infiltration. In fact, it was impossible to distinguish any histological difference between infarcted regions from saline controls and drug treated animals.

Infarct size in group I did not increase after 24 hours as shown in figure 3. In five control dogs sacrificed 24 hours after coronary occlusion infarct size equalled 32.1 ± 4.2% of left ventricular weight. Infarct size for eight controls sacrificed four days after occlusion was 28.9 ± 4.9%. Nor did infarct size increase after 24 hours in pretreated dogs. Infarct size in nine pretreated dogs sacrificed at 24 hours was 30.6 ± 3.6% while at four days infarct size in five pretreated dogs averaged 29.8 ± 4.9%.

Since there was no difference in infarct size between the steroid pretreated and steroid posttreated animals, results from all methylprednisolone sodium succinate treated dogs in group I were pooled and compared with controls. This comparison is shown in figure 4. Methylprednisolone sodium succinate treatment produced no change in infarct size. In 13 controls, mean infarct size measured 30.4 ± 3.6% of left ventricular weight. In 19 steroid treated dogs infarct size averaged 30.0 ± 2.3%. This corresponds to average infarct weights of 16.0 ± 1.9 g in controls and 16.3 ± 1.3 g in treated dogs.

In dogs from group II, with permanent LAD occlusions, infarct size in six controls was 39.2 ± 1.6% of left ventricular weight. In six treated dogs infarct size measured 33.7 ± 3.4%, not significantly different from controls. Corresponding infarct weights were 23.3 ± 1.8 in controls and 18.0 ± 2.1 in treated dogs.

**Enzyme Depletion**

In six controls and six methylprednisolone posttreated dogs from group I, myocardial depletion of CPK and LDH were measured at 24 hours; these results are presented in figure 5. In controls, CPK activity in the area perfused by the LCX decreased by 26.5 ± 6.7% and LDH activity declined 26.4 ± 7.0%. CPK activity fell from 57.7 ± 4.6 IU in normal regions to 40.9 ± 4.8 IU in the LCX area. LDH activity fell from 3.77 ± 0.53 units to 2.73 ± 0.39. The

![Figure 2](image.png)

**Figure 2.** Heart slice from a control dog sacrificed 48 hr after permanent LAD occlusion. Infarct size equals 38% of left ventricular weight. A) Under UV light a narrow rim of tetracycline fluorescence is visible outlining an anterolateral infarct. Fluorescence is limited to the margins of the damaged area in contrast to the more extensive distribution of tetracycline seen in figure 1. B) After staining with NBT the nonstaining area again corresponds to the region delineated by tetracycline fluorescence.

![Figure 3](image.png)

**Figure 3.** Summary of infarct size data from group I dogs (90 min LCX occlusion followed by release). There is no significant difference between mean infarct size measured at 24 hrs and four days. There is no difference in infarct size between steroid pretreated and posttreated groups. Infarct size is expressed as a percent of left ventricular weight (mean ± SEM). Numerals at the bottom of each bar indicate the number of animals in each group.
decrease in both activities was significant \( P < 0.025 \). In steroid posttreated animals the fall in CPK activity was 28.1 ± 6.9% and LDH activity declined 30.7 ± 7.4%. In these dogs CPK fell from 54.0 ± 1.3 to 38.7 ± 3.6 IU \( (P < 0.025) \) and LDH fell from 3.46 ± 0.29 to 2.38 ± 0.3 units \( (P < 0.025) \). The loss of enzyme activities seen at 24 hours in methylprednisolone sodium succinate treated dogs was not statistically different from that observed in controls. Protein concentration in supernatants prepared from the normal regions of control dogs averaged 1.21 ± 0.07 mg/ml; in supernatants from occluded regions protein concentration averaged 1.22 ± 0.19. Protein concentration in supernatants prepared from normal regions of steroid treated dogs averaged 1.35 ± 0.08 mg/ml; in supernatants from occluded regions, protein concentration averaged 1.14 ± 0.07.

**Arrhythmias**

For five hours after coronary occlusion all premature ventricular beats in group I dogs were counted manually and tabulated as ectopic beats per minute. This tabulation is shown in figure 6. The two steroid treated groups tended to have more premature beats than did controls in every time period. During the last 30 minutes of occlusion the mean ectopic rate in steroid posttreated dogs of 4.0 ± 1.6 beats was significantly higher than the ectopic rate of 1.1 ± 0.3 observed in controls \( (P < 0.025) \). Upon reperfusion of the coronary arteries all dogs experienced periods of ventricular tachycardia lasting from 30 minutes to an hour. One hour after reperfusion the ectopic rate in steroid posttreated dogs was 51.6 ± 16.6, significantly higher than the control ectopic rate of 18.6 ± 6.4 \( (P < 0.05) \). The ectopic rate declined in all dogs from one to two hours after reperfusion and then increased steadily throughout the remainder of the observation period.

**Hemodynamics**

Methylprednisolone sodium succinate pretreatment was followed by a slight drop in mean arterial pressure of 6 ± 3 mm Hg. This change was not statistically significant. Mean arterial pressure dropped in all dogs by 30 min after coronary occlusion. The mean fall in pressure for controls measured 8.0 ± 3 mm Hg \( (P < 0.01) \). Dogs in the steroid posttreatment group, which had not yet received drug at this time demonstrated a fall of 11 ± 3 mm Hg \( (P < 0.005) \). This change was not significantly different from that seen in controls. Blood pressure in the pretreated group fell 16 ± 3 mm Hg during the first 30 min of occlusion \( (P < 0.001) \). The fall in pressure for the treated dogs was significantly greater than the fall seen in controls \( (P < 0.05) \). During the 90 min of occlusion there was no further significant change in blood pressure in the control or pretreated animals. In contrast, the posttreated dogs, which received drug 30 min after occlusion, sustained a further drop in blood pressure in the subsequent 30 min period. Mean pressure fell 11 mm Hg from 112 ± 7 to 101 ± 6 \( (P < 0.025) \).
No consistent differences in heart rate were observed between dogs assigned to the different treatment schedules in group I. Nor did coronary occlusion cause consistent changes in heart rate within treatment schedules. After reperfusion, heart rate gradually increased throughout the four-hour observation period for dogs in each schedule. Heart rate in control dogs increased from 155 ± 5 beats/min before reperfusion to 184 ± 7 beats/hour after reperfusion (P < 0.005). In the same time period, heart rate increased from 148 ± 4 to 166 ± 7 beats/min (P < 0.01) in the steroid pretreated group, and from 145 ± 5 to 185 ± 10 (P < 0.005) in the posttreated group.

The degree of ischemia induced in group II dogs produced only moderate hemodynamic changes. By 30 min after occlusion mean arterial pressure had fallen from 115 ± 9 to 95 ± 6 mm Hg in controls. This change was not statistically significant. In treated dogs pressure fell from 116 ± 13 to 102 ± 12 mm Hg (P < 0.05) after 30 min of occlusion. By four hours pressure had returned to near control values in both groups. Heart rate tended to increase throughout the four-hour observation period in both groups, rising from 143 ± 7 to 168 ± 9 beats/min in controls and from 154 ± 10 to 185 ± 18 in treated dogs. Neither increase proved to be statistically significant. Cardiac output fell from 2.1 ± 0.3 to 1.3 ± 0.1 L/min in control dogs after two hours of occlusion (P < 0.01). In the treated dogs cardiac output declined slightly from 1.9 ± 0.2 to 1.6 ± 0.4 L/min, a statistically insignificant decrease. Coronary occlusion produced no consistent changes in arterial oxygen content or A-V O2 difference in controls or treated animals. During these experiments mean arterial oxygen content ranged from 13.3 to 14.7 volumes percent in controls and from 15.0 to 17.5 volumes percent in treated dogs. Mean A-V O2 difference ranged from 4.2 to 6.9 volumes percent in controls and from 5.6 to 7.2 volumes percent in treated dogs. At no time were the hemodynamic values measured in steroid treated dogs significantly different from those measured in controls.

**Discussion**

In recent years investigators have reported that a variety of drugs may favorably influence the course of myocardial injury resulting from coronary occlusion.38-40 End points used to measure the efficacy of interventions have included electrocardiographic ST-segment changes, myocardial enzyme release, and early hemodynamic and metabolic dysfunction. The only definitive conclusions that can now be made about cell viability and its pharmacological modification must be based on actual measurements of ultimate infarct size.41 Evidence from the present study indicates that 24 hours after permanent or temporary coronary occlusions the NBT method accurately delineates areas of myocardial necrosis. Microscopic examination demonstrated that histological signs of myocardial necrosis were found consistently and exclusively in those regions where the NBT reaction did not develop. These findings are in complete agreement with those of Nachlas and Shnitka.42

The second measure used to assess cell viability was depletion of myocardial enzymes. Although it is difficult with this method to quantitate precisely infarct size, enzyme depletion is probably a useful index of tissue damage.43 When using enzyme depletion measurements after coronary reperfusion, care must be taken that extensive hemorrhagic infarction has not occurred.44 Otherwise, increased protein concentration in homogenates from the infarcted region may invalidate calculations of enzyme activity. Histological examination indicated that reperfusion did not produce extensive hemorrhage in these experiments. The focal hemorrhage observed in reperfused animals was no greater than that seen in dogs with permanent occlusions. Likewise, protein assay demonstrated that protein concentrations were not significantly different in supernatants from normal and occluded regions. Since results obtained for LDH depletion are nearly identical to those observed for CPK, little LDH activity was contributed by extracardiac sources.

There is no evidence from these experiments that methyl-
predictnisolone sodium succinate exerts any protective effect on ischemic myocardium. Infarct size was not decreased nor was myocardial enzyme activity preserved after 24 hours. These results are in distinct contrast to effects observed in the first six hours of coronary occlusion. Spath et al. have observed that methylprednisolone sodium succinate pre-treatment or posttreatment prevents myocardial CPK depletion five hours after coronary occlusion. Shatney et al. have reported that methylprednisolone sodium succinate decreases apparent infarct size by 23% at six hours. One explanation for this discrepancy is that the glucocorticoids may delay release of enzymes from ischemic myocardium by virtue of the drug’s membrane stabilizing properties but without enhancing cell viability. After coronary occlusion there is an apparently exponential loss of enzyme activity which does not reach its full extent for at least 24 hours. Thus, at a single intermediate time point, delay in enzyme release due to steroid treatment would appear as an absolute increase in enzyme activity when compared to controls; but when depletion had reached its ultimate extent this difference would no longer be apparent. This hypothesis is consistent with the observations of Hearse and Humphreys that methylprednisolone sodium succinate prevents loss of enzyme activity after temporary anoxia but does not maintain contractile activity upon reoxygenation. On the other hand, high glucose concentrations or a cardioplegic solution preserve both enzyme activity and function. Using an isolated heart model, Naylor and Sealra-Gomes have recently shown that methylprednisolone sodium succinate may delay enzyme release without decreasing total enzyme loss.

The NBT method also depends on enzyme loss from dead cells. As the previous discussion would suggest, at least 24 hours is required before nonstaining areas reach maximum size and correspond with the full extent of histologically observable damage. Likewise, Malek et al. have pointed out that early after coronary occlusion NBT staining can still be observed within the area of tetracycline accumulation, but at 24 hours the nonstaining region has expanded to coincide with that of tetracycline fluorescence. Under these circumstances if the glucocorticoid steroids simply delay enzyme release an apparent decrease in infarct size would be observed in the first several hours after occlusion. This apparent decrease would not persist once the infarct had reached its ultimate size. It is clear that ultimate infarct size was reached in our experiments since mean infarct size did not increase after 24 hours and the absence of NBT staining coincided exactly with regions of tetracycline fluorescence.

There is another possibility which might account for the inability to observe a protective glucocorticoid effect. Since reperfusion after coronary occlusion is known to cause some additional cellular damage, it might be argued that methylprednisolone sodium succinate would not be expected to decrease infarct size under these circumstances. But several investigators have found that reperfusion results in a significant salvage of myocardium. If methylprednisolone sodium succinate truly increases cell viability, the period of reversible cellular injury should be prolonged and reperfusion should be followed by an increased salvage of myocardium. The reperfusion model is a sensitive test for protective agents since other treatment regimens such as propranolol, intra-aortic counterpulsation, and myocardial cooling can significantly decrease signs of myocardial necrosis after temporary coronary occlusion followed by reperfusion.

It is clear that reflow occurred in these experiments since reperfusion was accompanied by marked arrhythmias and an increased penetration of tetracycline, similar to the pattern seen with Tc-phyrophosphate after reperfusion. The pattern of tetracycline accumulation also suggests the presence of a reoxygenation phenomenon since the central areas of infarction consistently lacked tetracycline fluorescence. The reperfusion model thus may simulate the human clinical situation in which the ischemic insult at the time of an acute myocardial infarction is seldom associated with a complete deprivation of blood flow to the area.

Permanent coronary occlusions like those used by Shatney et al. were also used in this study and failed to demonstrate glucocorticoid protection. Although the number of animals used was not large, calculations from the observed standard deviations indicate that a decrease in infarct size of 22% could have been detected in these experiments, a figure similar to the apparent decrease in infarct size reported by Shatney et al. In addition, when results from the larger number of animals used by these investigators are analyzed by the two-tailed t-test the reduction seen in infarct size after methylprednisolone sodium succinate treatment is not statistically significant.

The lack of any striking hemodynamic changes with corticosteroid treatment is consistent with the observations of others. The only significant change we observed after steroid administration was a greater drop in blood pressure during coronary occlusion in treated dogs from group I. Since blood pressure in steroid pretreated dogs did not drop significantly until after coronary occlusion this may denote that the fall in blood pressure is not a direct drug effect but reflects a decreased tolerance to coronary occlusion after drug administration. The only other study which reports a similar change in blood pressure is that of Osher. Two similarities between these two studies which might account for the decrease in blood pressure include a larger dose of drug than is often employed (50 mg/kg vs 30 mg/kg) and a relatively higher point of occlusion than that used by others.

The only other difference between treated animals and controls observed in this study was the increase in arrhythmias in methylprednisolone sodium succinate treated dogs. Increased arrhythmias in animal models of myocardial infarction after glucocorticoid treatment have not been reported previously. Nonetheless, this observation is consistent with the increase in arrhythmias seen by Roberts et al. after repeated administration of methylprednisolone sodium succinate to patients with myocardial infarction. In the latter study, increased arrhythmias were associated with increased infarct size, as measured by serum CPK determinations. No evidence suggests that the increase in arrhythmias observed in the present study was associated with increased infarct size. Another mechanism which can explain steroid induced arrhythmias is an increase in blood flow after drug treatment. An increase in flow to severely ischemic cells may allow aberrant conduction to occur in
regions that were previously electrically silent. It is likely that such a mechanism also accounts for the arrhythmias normally seen upon reperfusion.48

In conclusion, because methylprednisolone sodium succinate did not decrease infarct size or limit myocardial enzyme depletion 24 hours after coronary occlusion, it is unlikely that this treatment significantly enhances cell viability during ischemia. This suggests that the primary steroid effect during myocardial ischemia is to delay the disruptions in membrane structure responsible for enzyme loss. It is also possible that during coronary occlusion and upon reperfusion glucocorticoids cause an increase in coronary flow which is sufficient to restore electrical activity to some border zone cells but is insufficient to bring about a significant reduction in infarct size. This study underscores the limitations of enzyme depletion and NBT staining when these methods are used to estimate cell viability in the early stages of infarction. The protective benefits of the glucocorticoids or any other agents will remain unproven until a reduction in infarct size and an improvement in cardiac performance can be demonstrated on a long-term basis.

References
41. Kloner RA, Ganote CE, Whalen DA, Reimer KA, Jennings RB: Effect of a transient period of ischemia on myocardial cells. II. Fine structure during the first few minutes of reflow. Am J Pathol 74: 399, 1974
47. Kloner RA, Ganote CE, Jennings RB: The "no reflow" phenomenon following temporary coronary occlusion in the dog. J Clin Invest 54: 1496, 1974
48. Warren JV: Di Si Dolce Morire: It may be safer to be dead than alive. Circulation 50: 415, 1974
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