Canine Myocardial Reperfusion Injury
Its Reduction by the Combined Administration of Superoxide Dismutase and Catalase

S.R. Jolly, W.J. Kane, M.B. Bailie, G.D. Abrams, and B.R. Lucchesi
From the Departments of Pharmacology and Pathology, The University of Michigan Medical School, Ann Arbor, Michigan

SUMMARY. Therapy directed against the toxic effects of reactive oxygen species may reduce the final extent of ischemic injury in otherwise viable tissue irreversibly injured by the abrupt reoxygenation of reperfusion. In four groups of dogs, superoxide dismutase plus catalase (groups I—III) or saline (controls) (group IV) was infused into the left atrium. Group I received the infusion for 2 hours, beginning 15 minutes before occlusion of the left circumflex coronary artery (90 minutes) and ending 15 minutes after reperfusion. Group II received the infusion for 1 hour starting 15 minutes before reperfusion. Group III received the infusion for 1 hour beginning 40 minutes after reperfusion. Dogs were killed the next day, and infarct size was determined by dissection and weighing, and confirmed histologically. Infarct size expressed as percent of the anatomic area at risk was: group I, 19.4 ± 5.0; group II, 21.8 ± 3.3; group III, 47.6 ± 10.3; group IV, 43.6 ± 3.5 (mean ± SEM). Analysis of variance followed by Duncan’s multiple range test showed that ultimate infarct size as assessed in groups I and II differed significantly (P < 0.05) from that observed in the control animals in group IV, whereas infarct size between groups III and IV did not differ significantly (P > 0.05). The percent of left ventricle at risk did not differ between the four groups. The beneficial effects of superoxide dismutase plus catalase could not be explained by hemodynamic differences. Similar protection of jeopardized myocardium in groups I and II suggest that potentially viable tissue is salvaged by scavenging free radicals during early reperfusion. Lack of protection in group III suggests that injury has occurred within the first 40 minutes of reperfusion. The results of this investigation demonstrate that the "primary" myocardial cellular damage due to ischemia is additive to the cardiac cell damage during the phase of reperfusion, and that the "secondary" effects are mediated by toxic metabolites of oxygen. (Circ Res 54: 277-285, 1984)

OXYGEN-FREE radicals such as superoxide anion ($O_2^-$), the hydroxyl radical ($\cdot$OH), and the reduced oxygen intermediate, hydrogen peroxide ($H_2O_2$), react with biological tissues and have been implicated in tissue injury from a variety of causes (Fridovich, 1978; McCord and Roy, 1982). Although ischemia is characterized in part by low tissue oxygen tensions, evidence from studies on the central nervous system (Demopoulos et al., 1980), the intestine (Granger et al., 1981), and the myocardium (Rao and Mueller, 1981; Meerson et al., 1982) suggests that reactive oxygen species contribute to the pathophysiology of ischemic injury. In the isolated heart, reoxygenation after a period of anoxia or hypoxia can lead to enzyme release and contracture which have been attributed to the reintroduction of molecular oxygen (Hearse, 1977). Likewise, reperfusion of ischemic myocardium is associated with accelerated and increased enzyme release and rapid development of electrophysiological evidence of irreversible tissue injury (Ganz et al., 1981).

With the specific free radical metabolizing enzymes, superoxide dismutase (superoxide oxidoreductase EC 1.15.1.1), and catalase ($H_2O_2$:$H_2O$ oxidoreductase EC 1.11.1.6), it has been possible to determine that protection against oxygen-free radicals is beneficial to ischemic myocardium which undergoes subsequent reperfusion. The effects of superoxide dismutase (SOD) plus catalase on the development of myocardial injury due to ischemia and reperfusion were examined by treating dogs throughout the ischemic period and during early reperfusion (group I), during the last 15 minutes of the 90-minute period of coronary artery occlusion, and continuing into the early reperfusion period (group II), and for 1 hour beginning 40 minutes after initiation of reperfusion (group III). Some of these results have been published previously in abstract form (Bailie et al., 1982) and—when taken together with our most recent observations—provide evidence that free radical scavengers can protect the ischemic heart against the destructive effects associated with reperfusion of the myocardium that has been subjected to a period of regional ischemia.

Methods
Occlusion-Reperfusion Model of Myocardial Infarction
Ischemic myocardial injury was produced in dogs by means of techniques detailed in previous publications
(Lucchesi et al., 1978; Jolly and Lucchesi, 1983). Male mongrel dogs (8–15 kg) were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and ventilated with room air via a Harvard respirator. Catheters for drug infusion and arterial pressure measurement were implanted in the left jugular vein and left carotid artery and exteriorized at the back of the neck. A thoracotomy was performed at the 5th left intercostal space, the heart suspended in a pericardial cradle, and the left circumflex coronary artery (LCX) isolated distal to its atrial branch and proximal to any major ventricular branches. An electromagnetic flow probe and a micrometer-driven coronary occluder (Hosko et al., 1977) were placed on the LCX. The flow probe was calibrated with whole blood and with the use of timed sample collections in a graduated cylinder. The lead II electrocardiogram and phasic arterial pressure were recorded continuously on a Grass model 7 polygraph. The coronary artery occluder was adjusted initially to constrict the circumflex coronary artery partially, to the point where resting flow was unchanged, but the peak flow increment (reactive hyperemic response), upon re-release of a 10-second complete occlusion, was decreased by more than 70% (critical stenosis). Five minutes later, myocardial ischemia was produced by adjusting the occluder so as to interrupt circumflex coronary artery flow completely for a period of 90 minutes. After 90 minutes of ischemia, flow was restored gradually over 30 minutes to the original set point of critical stenosis, which was retained for an additional 10 minutes.

The thoracotomy was closed and the animals were allowed to recover from the surgical procedure. On the next day, the electrocardiogram and arterial pressure were monitored for 1 hour with the dog resting quietly in a completely darkened room for a period of 90 minutes. After 90 minutes, the animals were reanesthetized, and the original thoracotomy incision was reopened to expose the heart. The heart was fibrillated electrically and rewarmed slowly to constrict the circumflex coronary artery partially, to the point where resting flow was unchanged, but the peak flow increment (reactive hyperemic response), upon re-release of a 10-second complete occlusion, was decreased by more than 70% (critical stenosis). Five minutes later, myocardial ischemia was produced by adjusting the occluder so as to interrupt circumflex coronary artery flow completely for a period of 90 minutes. After 90 minutes of ischemia, flow was restored gradually over 30 minutes to the original set point of critical stenosis, which was retained for an additional 10 minutes.

Treatment Groups

Four treatment groups were completed. Animals received either bovine serum superoxide dismutase (5 mg/kg (2900 U/mg, Sigma)) plus catalase (5 mg/kg (11,000 U/mg, Sigma)), or an equal volume of 0.9% sodium chloride solution. The superoxide dismutase employed in this study was not assayed for contamination by catalase. Dogs were assigned randomly to treatment or control groups on the day of surgery. Superoxide dismutase (SOD) plus catalase (CAT) were given by infusion into the left atrium to three groups. The atrial infusion route was chosen due to the short plasma half-life of SOD (Fridovich, 1983). Group I dogs received SOD plus CAT over a period of 2 hours, beginning 15 minutes before total occlusion of the left circumflex coronary artery, continuing through the ischemic period, and ending 15 minutes after reperfusion was begun. Group II animals received SOD plus CAT for 1 hour, beginning 15 minutes before reperfusion and continuing until 45 minutes after starting reperfusion. Group III animals received SOD plus CAT for 1 hour, beginning 15 minutes before reperfusion and continuing until 45 minutes after starting reperfusion. Group IV received no treatment. The time course of occlusion, reperfusion, and treatment for the three groups is shown in Figure 1.

Postmortem Quantification of Infarct Size

Myocardial infarct size was quantified by an in vitro dual perfusion technique. Cannulas were inserted into the LCX immediately distal to the site of LCX occlusion, and into the aorta above the coronary ostia. The LCX coronary bed was perfused with 1.5% triphenyl tetrazolium hydrochloride (TTC) in 20 mM potassium phosphate buffer (pH 7.4, 38°C). The aorta was perfused in a retrograde manner with 0.5% Evans blue dye. Both the LCX region and the remainder of the heart were perfused with their respective saline solution in a graduated cylinder. The area of the left ventricle at risk of infarction due to its anatomical dependence on the LCX for blood flow was identified by the lack of Evans blue in this region. The region of infarcted myocardium within the area at risk was demarcated by the lack of staining of the tissue when perfused with TTC due to a loss of dehydrogenase enzymes which converts the colorless TTC to a red formazan. Transverse ventricular sections were traced carefully onto clear plastic overlays to provide a permanent record of infaract morphology and to allow planimetric confirmation of infarct size. Ventricular sections then were trimmed of right ventricular muscle, valvular, and fatty tissue. Total left ventricle, area at risk, and infarct were separated by careful dissection and weighed. In a previous study, the gravimetric analysis agreed very closely with determinations of infarct size obtained from planimetry of the overlay tracings (Jolly and Lucchesi, 1983). Infarct size was expressed as percent of the anatomic area at risk and as percent of the total left ventricular weight.

Histological Examination

Tissue blocks covering the full thickness of the ventricular wall were taken from the second section from the base of the heart in the region of the posterior papillary muscle, representing an area which is dependent on the LCX for its blood supply. A tissue block was also taken along the border of the Evans blue and TTC stains. Tissue blocks were coded, fixed in 10% formalin, paraffin embedded, and cut to a thickness of 5 to 7 μm. Sections were stained with hematoxylin and eosin, and were examined by an observer (G.D.A.) who was unaware of the treatment code. The presence of hemorrhage, the uniformity of necrosis from endocardium to epicardium, and the presence of leukocytic infiltrate were examined.
Statistics

All data are expressed as mean ± SEM. Differences were considered significant when \( P < 0.05 \). Differences among the four groups were analyzed by one-way analysis of variance. Differences within groups were analyzed by two-way analysis of variance. Statistical comparisons were made, using Duncan’s multiple range test (Duncan, 1955).

Results

In all, 48 dogs were used in the current series of experiments. Five dogs (two controls, one group I dog, and two from group II) died overnight and were not included because infarct-size data were not available. In addition, two dogs (one control and one group I) were excluded because infarct size was less than 1% of the area at risk, and these two animals failed to show regional cyanosis or ECG changes indicative of ischemia upon occlusion of the LCX. Of the remaining 41 experiments, six were terminated early due to intractable fibrillation between 1 and 4 hours after reperfusion (four group I and two group II). For this reason, groups I and II have been analyzed with and without inclusion of the dogs that died prior to completion of the 24-hour study protocol.

Reduction of Myocardial Infarct Size

No differences in total left ventricular mass or size of the anatomic risk region were observed among the four groups (Table 1). The overall area at risk for 35 experiments was 39.9% of the total left ventricle, with a standard deviation of ±6.0%. Due to the small variability in risk region size, the dependence of infarct size upon area-at-risk size, reported by Jugdutt et al. (1979), was not observed. It is also possible that reperfusion increases the variability of infarct size within the framework of area at risk. However, it can be concluded that differences in 24-hour infarct size could not be explained on the basis of different amounts of tissue at risk of infarction.

In control dogs, mean infarct size was 43.6 ± 3.5% of the anatomic area at risk. No early deaths due to reperfusion arrhythmias were encountered. In group I (SOD plus CAT given over 2 hours, beginning 15 minutes before occlusion to 15 minutes after reperfusion), infarct size averaged 19.4 ± 5.0% of the area at risk. Infarct size was significantly different from controls, whether expressed as grams of infarcted tissue, percent of area at risk, or percent of total left ventricle infarcted (Table 1). Four animals died early in reperfusion, but the inclusion of these four animals in the data analysis does not change the interpretation of the results based on 24-hour survivors. Figure 2 shows photographs of heart sections from two control and two SOD plus CAT (group I)-treated dogs, showing the clear differences in the pattern of infarction as altered by the treatment regimen. In group II, SOD plus CAT given 15 minutes before initiating reperfusion until 45 minutes after reperfusion, infarct size averaged 21.8 ± 3.3% of the anatomical area at risk. As in group I, infarct size was significantly reduced by treatment with SOD plus CAT in the early reperfusion period, whether expressed in terms of infarcted tissue (g), percent of the anatomical area at risk, or percentage of the total left ventricle. Inclusion of two early deaths did not change interpretation of the results. In group III, SOD plus CAT given from 40 minutes to 100 minutes after reperfusion, infarct size had a mean value of 47.6 ± 10.3% of the anatomical area at risk; a value which did not differ significantly from that of saline-treated controls. On the other hand, mean infarct size in group III was significantly greater than the mean infarct sizes in groups I and II. Due to the delay in the initiation of treatment,

**Table 1**

Effect of Superoxide Dismutase Plus Catalase on 24-Hour Infarct Size in a Canine 90-Minute Occlusion—Reperfusion Model of Myocardial Infarction

<table>
<thead>
<tr>
<th>group</th>
<th>Infarct mass (g)</th>
<th>Risk region mass (g)</th>
<th>Total left ventricle mass (g)</th>
<th>Percent of area at risk</th>
<th>Percent of total left ventricle infarcted</th>
<th>Percent of total left ventricle at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>15</td>
<td>10.7 ± 0.9§</td>
<td>25.1 ± 1.2</td>
<td>65.2 ± 3.7</td>
<td>43.6 ± 3.5</td>
<td>17.0 ± 1.3</td>
</tr>
<tr>
<td>SOD plus catalasea*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7</td>
<td>4.5 ± 1.2‡</td>
<td>23.0 ± 1.4</td>
<td>57.6 ± 2.8</td>
<td>19.4 ± 5.0‡</td>
<td>7.8 ± 2.2‡</td>
</tr>
<tr>
<td>I</td>
<td>11†</td>
<td>6.2 ± 1.6‡</td>
<td>23.6 ± 1.2</td>
<td>60.3 ± 3.2</td>
<td>24.2 ± 5.9‡</td>
<td>9.8 ± 2.5‡</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>5.5 ± 1.0†</td>
<td>25.2 ± 2.8</td>
<td>60.5 ± 5.9</td>
<td>21.8 ± 3.3‡</td>
<td>9.7 ± 2.2‡</td>
</tr>
<tr>
<td>II</td>
<td>9†</td>
<td>6.6 ± 3.3†</td>
<td>26.1 ± 2.4</td>
<td>61.2 ± 4.4</td>
<td>25.5 ± 3.7†</td>
<td>11.3 ± 2.0</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>11.1 ± 2.4</td>
<td>24.0 ± 2.1</td>
<td>59.8 ± 4.0</td>
<td>47.6 ± 10.3</td>
<td>18.1 ± 3.7</td>
</tr>
</tbody>
</table>

* Superoxide dismutase plus catalase was administered by left atrial infusion. A total of 5 mg/kg of each enzyme was given (specific activity: superoxide dismutase, 2900 U/mg; catalase, 11,000 U/mg). Treatment regimens were as follows: infusion began 15 minutes before occlusion and ended 15 minutes after reperfusion has been initiated; group II infusion began 15 minutes before initiation of reperfusion and ended 45 minutes after; group III infusion began 40 minutes after initiation of reperfusion and ended 100 minutes after.
† Experiments that ended due to intractable fibrillation within 5 hours after reperfusion, have been added to the group.
‡ \( P < 0.05 \), compared to control, by analysis of variance, followed by Duncan’s multiple range test.
§ Data are expressed as mean ± 1 SEM.
FIGURE 2. Four 24-hour myocardial infarcts are shown delineated by triphenyltetrazolium hydrochloride. Panels A and B show control experiments. Panels C and D show experiments from the superoxide dismutase-plus-catalase group I (pretreatment, infusion beginning 15 minutes before ischemia and ending 15 minutes after reperfusion). In all four infarcts, the subendocardial surface shows extensive infarction. A reduction of the extension of myocardial infarction into midmyocardial and subendocardial tissue by treatment is suggested by comparison of panels A and B with C and D.

until 40 minutes after the start of reperfusion, SOD plus CAT was ineffective in reducing infarct size and serves as an internal negative control for groups I and II.

Among the animals studied in groups I (n = 11) and II (n = 9), there were four in group I and two in group II which developed ventricular fibrillation within 5 hours of having undergone reperfusion of the LCX coronary artery. The results presented in Table 1 have been organized so as to show the analysis of the infarct size data, with and without inclusion of the animals that terminated prematurely, in the experimental protocol. Regardless of whether the early deaths are or are not excluded from the statistical analysis of the data, the end results are not affected. Ultimate infarct size expressed as a percent of the area at risk was significantly different from the values obtained in the saline control group (group IV) and also from group III. In the latter group, the administration of SOD plus CAT was delayed until after the initiation of reperfusion.

Hemodynamic Parameters

Hemodynamic parameters before placement of the critical stenosis are shown in Table 2. No differences in heart rate were observed among the four groups. The three SOD-plus-CAT treatment groups did not differ from saline controls with regard to initial mean arterial blood pressure. However, group I had a significantly lower control mean arterial blood pressure than did group II. The three SOD-plus-catalase treatment groups did not differ from saline-treated controls in the initial rate-pressure product. However, group I dogs again had a significantly lower rate-pressure product than did group II. To facilitate interpretation of electromagnetic flow values in the LCX-perfused region, flows have been divided by risk region mass, obtained from postmortem staining. For the 15 control experiments, normalized tissue flow has been calculated to be 0.97 ± 0.12 (mean ± se) ml/min per g. Mean left circumflex coronary artery flow in the SOD-plus-CAT treatment groups also did not differ from the control group. For this parameter, a difference between group II and group III was noted. The physiological significance of these baseline differences between groups is questionable. Changes in these parameters produced by coronary artery occlusion and reperfusion in 24-hour survivors are shown in Figure 3. No significant changes in heart rate were observed in any group. Blood pressure was highest
Initial Hemodynamic Results from Control and Superoxide Dismutase-Plus-Catalase Groups

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Mean left circumflex coronary flow (ml/min)</th>
<th>Rate x pressure product (Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>169 ± 7*</td>
<td>125 ± 6</td>
<td>23 3 ± 1.7</td>
</tr>
<tr>
<td>Superoxide dismutase plus catalase</td>
<td>7</td>
<td>136 ± 10</td>
<td>113 ± 6*</td>
<td>19 ± 3* 19 ± 1.5</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>164 ± 8</td>
<td>16 ± 2†</td>
<td>26 ± 1.9†</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>166 ± 13</td>
<td>131 ± 4</td>
<td>24 ± 2.5</td>
</tr>
</tbody>
</table>

*Mean ± SEM is given. No significant differences between the control and SOD-plus-catalase groups were observed after one-way analysis of variance, followed by Duncan’s multiple range test.

Before occlusion in all four groups and decreased significantly upon occlusion in all four groups. It increased somewhat thereafter, and then fell during the reperfusion period. For example, at 60 minutes after reperfusion, blood pressure was significantly lower ($P < 0.05$, compared to preocclusion) in all four groups. The rate-pressure product also fell significantly in all four groups upon LCX occlusion.

Amid the hemodynamic profiles of the four groups, the intergroup differences do not appear to be sufficient to account for variations in infarct size. As examples, no differences in heart rate were observed, and the highest mean arterial pressure before occlusion was observed in group II, which had a relatively small mean infarct size. One possible exception is the blood pressure change in group I.

![Graph](attachment:image.png)

**Figure 3.** Hemodynamic parameters of heart rate, mean arterial pressure, and the rate pressure product for the four groups are shown. Open squares indicate the control group values. Open circles indicate group I, closed squares indicate group II, and closed circles indicate group III. High and low group values at control, 5 minutes after occlusion, and 24 hours after reperfusion are given for purposes of comparison. No significant differences between treatment groups, compared with controls for any parameter, were observed before occlusion by one-way analysis of variance followed by Duncan’s multiple range test.
which made the 5- and 15-minute postocclusion values significantly less than control. However, the short duration of the hypotension makes it unlikely that the modification of this parameter alone could have accounted for the marked sparing of myocardial tissue in the anatomical risk region, compared with group III-treated dogs, and with those animals in the saline-treated control group.

Pathological Examination

Gross examination of infarcts in control and SOD-plus-CAT groups revealed that the transmural extent of myocardial ischemic injury was reduced in treatment groups I and II, while most of the subendocardial surface bordered infarcted tissue in all groups. Second, SOD-plus-catalase-induced reduction of infarct size resulted in patchy infarcts of intermixed necrotic and viable myocardium, as judged by TTC staining (Fig. 2). Histological examination confirmed the presence of necrosis in tissue judged to be infarcted on the basis of the histochemical staining reaction. Infarcted tissue in all groups showed pyknotic nuclei, loss of nuclei, flocculent cytoplasm, and contraction bands. Some small areas of hemorrhage were detected in sections from all groups, as was infiltration of the ischemic area by polymorphonuclear leukocytes.

Discussion

These studies demonstrate that left atrial infusion of SOD and CAT may reduce the ultimate extent of ischemic injury in a canine occlusion-reperfusion model of myocardial infarction. A large body of evidence suggests that increasing myocardial oxygen supply into an ischemic region or reducing oxygen demand can be beneficial (Maroko and Braunwald, 1976) to reversibly injured myocardium (Schaper, 1979). The present results suggest that oxygen metabolites can also be detrimental to jeopardized, but still viable, heart muscle that has been rendered ischemic. SOD catalyzes the conversion of O$_2^-$ to H$_2$O$_2$ and molecular oxygen. Hydrogen peroxide is converted to water and molecular oxygen by catalase, as well as peroxidases. Protection of jeopardized myocardium by the combination of SOD plus CAT implicates reduced oxygen intermediates in ischemic processes and in reperfusion, but cannot distinguish which species, O$_2^-$, H$_2$O$_2$, or the hydroxyl radical (•OH) is most important. The observed differences in the measured hemodynamic parameters indicative of oxygen demand cannot account for infarct size modification. However, regional myocardial blood flow was not examined in this study, so possible improvements in this parameter after SOD plus CAT cannot be ruled out.

Free radical oxygen metabolites have been implicated in tissue injury due to irradiation (Petkau et al., 1978), pulmonary oxygen toxicity (Crapo and Tierny, 1974), and the cardiotoxic effects of doxorubicin (Doroshow et al., 1980). Free radicals also have been implicated in inflammatory models, such as degradation of synovial fluid in inflamed joints (McCord and Roy, 1982), and in endothelial cell injury dependent on neutrophils and complement (Sachs et al., 1978; McCormick et al., 1981). In most of these systems, free radical injury was implicated further by protection afforded by the administration of SOD. It has been suggested that, in the central nervous system (Demopoulos et al., 1970), and with intestinal tissue (Granger et al., 1981), oxygen-free radicals may participate in ischemic tissue injury. In isolated heart models, evidence suggests that oxygen may be detrimental to hypoxic or anoxic tissue subjected to reoxygenation (Hearse et al., 1975; Hearse, 1977; Ganote and Kaltenbach, 1979). Guarnieri et al. (1980) have demonstrated that anoxia produces loss of thiol groups and glutathione, giving rise to products of tissue membrane peroxidation, and leads to a reduction of myocardial SOD and glutathione peroxidase activities. These changes were exacerbated by reoxygenation. Thus, ischemia followed by reperfusion may cause free radical-induced injury and damage to the protective enzyme systems. Hess et al. (1981) have presented evidence suggesting that oxygen-free radicals contribute to ischemia-induced damage to the sarcoplasmic reticulum responsible for calcium transport. Lefer et al. (1981) have demonstrated protection of globally ischemic myocardium by MK477, a free radical scavenger, whereas Shlafer et al. (1982a, 1982b) have shown that SOD plus CAT, added to cardioplegic solutions, affords improved preservation of globally ischemic hearts which were perfused with either buffer or whole blood.

Meerson et al. (1982) recently have reviewed the evidence supporting the role of lipid peroxidation of membranes in the pathogenesis of ischemic myocardial injury and the critical importance of reperfusion injury secondary to the rapid reintroduction of molecular oxygen to previously ischemic heart muscle. The underlying mechanism leading to cellular injury is believed to be the step-wise reduction of molecular oxygen with the formation of activated oxygen species via acceptance of one, two, or three electrons by O$_2$. The products of this process are superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals. During myocardial ischemia, the process of oxygen utilization by the normal respiratory pathway is inhibited and may be switched to the oxygenase pathway, which gives rise to the formation of reactive oxygen species. These activated oxygen species can interact with endogenous substrates in the cell leading to incorporation of one atom or of the whole molecule into the oxidizing substrate, particularly endogenous membrane lipids. Even in the presence of severe ischemia, the lipid matrix of the cell membrane contains amounts of dissolved oxygen sufficient to support this process, an event which becomes "explosive" with the abrupt reintroduction of oxygen.
An accumulation of lipid peroxides has been reported to occur in myocardial tissue undergoing ischemia-induced injury, along with a simultaneous loss of myocardial SOD, CAT, and glutathione peroxidase activity (Rao and Mueller, 1981; Meerson et al., 1982). The loss of free radical protective enzymes from heart tissue subjected to reperfusion makes the once ischemic region highly vulnerable to the deleterious effects of the locally generated toxic oxygen species, as well as free radicals and hydrogen peroxide generated by leukocytes, which gain access, via reperfusion, to the once-ischemic region. It is of interest to note that mannitol has been reported to protect the ischemic-reperfused heart, an action believed to be related to the increase in the intravascular osmotic pressure whereby mannitol would prevent the ischemically injured tissues from becoming overloaded by fluid and electrolytes (Powell et al., 1976). It is now appreciated that mannitol is a scavenger of hydroxyl radicals (McCord and Fridovich, 1973), and its efficacy as a cardioprotective agent in the posts ischemia-reperfused heart may be attributed, in part, to its ability to prevent the deleterious effects of active oxygen species. The source of reactive oxygen metabolites in myocardial ischemia has not been determined, although there are several possible sources. Mitochondria generate superoxide anion and hydrogen peroxide during oxidative respiration and electron transport (Boveris and Chance, 1973). Xanthine dehydrogenase exists in two forms: type D requiring NADH, and type O requiring molecular oxygen, with the latter form capable of producing superoxide anion (McCord and Roy, 1982). In ischemic intestinal tissue, the conversion from type D to type O occurs spontaneously, and tissue damage can be ameliorated by superoxide dismutase (Granger et al., 1981). It has not yet been shown whether a similar phenomenon occurs in ischemic myocardium. Activated neutrophils, which produce superoxide anion, hydroxyl radical, and hydrogen peroxide (Weiss et al., 1978; Simchowitz and Ward, 1971; Pinkard et al., 1980) and metabolic products of arachidonic acid generated via the lipooxygenase pathway which are known to be chemotactic (Ford-Hutchinson et al., 1980). The role of the neutrophil as a mediator of myocardial injury has been emphasized recently in studies (Romson et al., 1983) in which neutrophil depletion was associated with a significant reduction in ultimate infarct size in the experimental animal.

Myocardial ischemic injury progresses with time from the subendocardium to subepicardium (Reimer et al., 1977; Reimer and Jennings, 1979), and early reperfusion within the first 3–6 hours after the onset of experimental ischemia is believed to salvage jeopardized, but as yet reversibly injured, myocardial tissue. The simultaneous occurrence of reperfusion injury resulting from rapid reoxygenation after a period of prolonged ischemia must be considered in light of evidence demonstrating the abrupt loss of myocardial enzymes, the development of contraction bands upon reperfusion (Hearse et al., 1975; Hearse, 1977; Ganote and Kaltenbach, 1979), and a loss of the myocyte’s ability to control intracellular volume (Whalen et al., 1974).

In the experimental model employed in this study, great care has been used in slowly reperfusing the previously ischemic myocardium while maintaining a critical stenosis on the coronary artery—and thereby preventing the abrupt increase in coronary blood flow (reactive hyperemia) which otherwise is observed upon reperfusion of the ischemic heart. We have reported previously (Lucchesi et al., 1978) that this approach prevents the development of hemorrhagic infarctions and reduces the high incidence of ventricular fibrillation seen when reperfusion of the heart is instituted abruptly. Similar observations regarding the importance of maintaining a critical stenosis during reperfusion of the ischemic heart have been reported by Sheehan and Epstein (1982). These observations emphasize the importance of the manner in which molecular oxygen is reintroduced to the once ischemic heart. If reperfusion injury is an important component to the overall development of ultimate infarct size, proper attention to the manner in which oxygen is reintroduced and/or the proper timing of an intervention for the prevention of lipid peroxidation of membranes via reactive species of oxygen could further enhance the extent to which salvage of jeopardized (but yet reversibly injured) myocardium might be achieved.

The ideas expressed in the above discussion are supported by the results obtained in our group II animals in which treatment with SOD plus CAT was initiated 75 minutes after the onset of the ischemic event and just 15 minutes before the start of reperfusion. The intervention was targeted to the early reperfusion period in which the component of injury related to the formation of reactive oxygen species is believed to be maximally operative. The marked protection by SOD and CAT in the group II
animals, in contrast to the failure of the intervention to protect the animals in group III, would provide further evidence that reperfusion injury does occur when the previously ischemic heart is reexposed to molecular oxygen, and that the administration of appropriate free radical scavengers can protect against the further development of irreversible myocardial injury and ultimate myocardial necrosis. These conclusions are supported by our recent studies (Romson et al., 1983) which led us to speculate about the important contribution of leukocyte infiltration into the reperfused myocardium and the ability of the phagocytic cells to produce free radicals that would lead to the further destruction of otherwise viable myocardial tissue.

It is difficult to extrapolate results from the experimental animal to the clinical situation in which ischemic myocardium is subjected to reperfusion. SOD has long been used in veterinary practice for the management of arthritic inflammatory states (Huber and Saifer, 1977), and has been used experimentally in humans for similar purposes (Beckmann and Flohe, 1981). The results of the present investigation provide strong evidence for the ability of reperfusion to sacrifice potentially viable tissue when oxygenated blood is permitted to perfuse a previously ischemic region of heart muscle. Since reperfusion decreases ultimate infarct size, it was believed that reperfusion only hastened the evolution of changes associated with cell death in a given population of cells considered to be irreversibly injured (Ganz et al., 1981). This does not appear to be the case. Attempts to prevent reperfusion injury by the previous administration of free radical scavengers may afford an opportunity to salvage greater amounts of myocardial tissue than heretofore realized. Contraction band necrosis has been found to localize in myocardium supplied by widely patent bypass grafts, which may represent deleterious effects of reperfusion in humans (Bulkley and Hutchins, 1981). The present results may be applicable to current efforts to salvage ischemic myocardium through the application of thrombolytic therapy, and/or percutaneous transluminal coronary angioplasty, for the restoration of regional myocardial blood flow in patients with evolving myocardial infarction. Simultaneous use of SOD plus CAT as an adjunct may prevent that component of tissue injury associated with the reintroduction of molecular oxygen and the subsequent generation of toxic oxygen species capable of causing lipid peroxidation of membranes. If the ultimate goal of thrombolytic therapy is to salvage myocardial tissue and restore contractile function, then the above concepts warrant further detailed studies.

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Address for reprints: Brudet R. Lucchesi, M.D., Department of Pharmacology, M6322 Medical Science Building, The University of Michigan Medical School, Ann Arbor, Michigan 48109

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S R Jolly, W J Kane, M B Bailie, G D Abrams and B R Lucchesi

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