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OXYGEN-DERIVED FREE RADICALS MEDIATE LIVER DAMAGE IN RATS SUBJECTED TO TOURNIQUET SHOCK

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The placement of rubber band tourniquets upon rat hind-limbs for 5 h followed by reperfusion of the extremities results in a severe form of circulatory shock characterized by hypotension and death within 24 h of tourniquet release. Oxidative damage to muscle tissue is an early consequence of hind-limb reperfusion on tourniquet release, yet this local damage does not explain the lethal hypotensive shock state which evolves within the next 24 h. Multiple system organ failure (MSOF), of as of yet unknown causes, is usually described in relation to several shock states. It has been suggested that injured or necrotic tissue may activate neutrophils, platelets, and the coagulation system leading to embolization in remote tissues. Effective decreases in hepatic blood flow have been observed in several forms of sepsis which precedes the biochemical evidence consistent with an ischemic insult of the liver. In support of our original hypothesis, that organ failure has its genesis in a primary perfusion abnormality with secondary ischemic organ injury, herein we have assessed the possibility that oxygen-derived free radicals are generated in the liver of rats after reperfusion of their hind-limbs on release of the tourniquets. We report on the protective effects of allopurinol (ALLO) and a mixture of superoxide dismutase (SOD) catalase (CAT) and dimethylfulfoxide (DMSO) on liver free sulfhydryl content (SH), thiobarbituric acid-reactive substances (TBARS), and on the release of aspartic acid (AsT) and alanine aminotransferase (AlT) activities, and of alkaline phosphatase during a 5 h tourniquet period and after 2 h of reperfusion of the hind-limbs. During the hind-limb ischemic period hepatis tissue SH levels remained essentially constant during the first hour (6.02 ± 0.36 to 5.65 ± 0.20 μmoles/g wet tissue), and decreased significantly, over and above the normal circadian decrease of liver glutathione levels, to 4.02 ± 0.69 μmoles/g wet tissue after the third hour and remained lowered until tourniquet release. A further significant decrease (3.11 ± 0.49 μmoles/g wet tissue) was observed after 2 h of reperfusion. TBARS production remained constant during the 5 h hind-limb ischemic period (168.4 ± 37.3 μmoles/g wet tissue) and rose by 55% to 261.7 ± 55.8 μmoles/g wet tissue after 2 h of tourniquet release. ALLO, but not the SOD-CAT-DMSO combination, protected hepatic SH loss during the hind-limb ischemic insult, yet both offered protection after 2 h of tournoquet release. With regard to TBARS production, ALLO and the SOD-CAT-DMSO mixture had no effect on basal levels during the ischemic period, but both significantly reduced liver TBARS production after the two hour reperfusion period of hind limb reperfusion. Plasma AsT levels rose 8-fold from 99.4 ± 7.2 to 193 ± 17.0 U/L after the 5-hour tourniquet period, and to 844.8 ± 75.1 U/L two hours after hind-limb reperfusion. The plasma levels of AsT were significantly lower in both the ALLO and SOD-CAT-DMSO pre-treated animals. This was not the case with plasma AlT levels which increased 3-fold during the reperfusion period, but which could not be protected with these same pre-treatment protocols. Alkaline phosphatase plasma levels increased 2-fold during the same period. It is concluded that oxidative stress to the liver, as a result of himd-limb ischemia followed by reperfusion, is partly responsible for the MSOF which leads to circulatory derangements and death of rats subjected to this tourniquet shock model.

KEY WORDS: Oxidative stress, oxyradicals, liver, tourniquet shock, ischemia, reperfusion.

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INTRODUCTION

Reperfusion of rat hind limbs which have been subjected to the bilateral application of rubber tourniquets for 5 hours results in the release of lactic dehydrogenase isoenzymes,[1] and in a decreased oxygen consumption, loss of free sulfhydryl groups, and edema of the gastrocnemius muscle, as well as in the appearance of TBARS in the femoral veins after tourniquet release; all of which contribute to a shock state in which a 100% mortality rate is observed within 24 hours of hind-limb reperfusion.[2] Pretreatment of these animals with allopurinol, a xanthine oxidase inhibitor, resulted in significant protection.[3]

Reperfusion of tissues and organs, following periods of ischemia, leads to tissue injury and to shock by complex mechanisms which involve numerous processes acting in concert and which may result in cell death. Of the many factors which have been proposed as potential mediators of reperfusion injury, and to be involved in the outcome of shock, the most studied have been: oxygen free radicals;[4] calcium overload after reoxygenation;[5,6] or through a direct protein kinase C-dependent phosphorylation of calcium channels which results in an increased calcium influx;[7] activation of phospholipases followed by the production of arachidonic acid and eicosanoids;[8,9] loss of membrane integrity;[10] mitochondrial damage;[11] release of platelet activating factor;[12,13,14] activation of the complement system;[15] and neutrophil infiltration of tissues subjected to ischemia followed by reperfusion.[16,17]

Oxygen-derived free radicals have been implicated as mediators of the microvascular and parenchymal cell injury associated with reperfusion of ischemic tissues.[4,18] In the intestine, for example, ischemia-reperfusion leads to increased microvascular and mucosal permeability, interstitial edema, and impaired hydrolytic and absorptive functions.[19] Similar increases in vascular permeability and functional decrements are observed in skeletal muscle, although this tissue, unlike others such as the intestine, brain or heart, can withstand relative long periods of ischemia without significant injury.[20]

In an attempt to understand the underlining causes that lead to death in animals subject to burn, and other forms of shock, we postulated a model that suggested that local episodes of ischemia/reperfusion, in which oxygen-derived free radicals were generated, could result in the hypoperfusion of other organs, and that these would eventually be subjected to tissue injury through the generation of these same agents.[21] In agreement with this hypothesis are the results[22] which indicate that the severe form of circulatory shock, which results after splanchnic artery occlusion followed by reperfusion, leads to, among other pathophysiological events, hepatocellular injury, and that the extent of this injury could be ameliorated with SOD. Our hypothesis is also supported by the work of Schrimer *et al.*[23] who showed that femur fracture, associated with soft-tissue trauma, as opposed to that without soft-tissue injury, resulted in liver hypoperfusion.

Herein we present evidence that oxygen-derived free radicals are generated in the liver of rats 2 hours after reperfusion of their hind-limbs which have been subjected to 5 hours of ischemia with rubber band tourniquets, and that they are responsible, in part, for the oxidative stress observed in this organ after hind-limb reperfusion.

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MATERIALS AND METHODS

Reagents

SOD (EC 1.15.1.1, obtained from bovine erythrocytes containing 3,000 units per mg of protein), CAT (EC 1.11.1.6, obtained from bovine liver containing 10,000 to 30,000 units per mg of protein), ALLO, DMSO, 5,5'-dithiobis(2-nitrobenzoic) acid, thiobarbituric acid, butylated hychoxytoluene (BHT) and p-nitrophenyl phosphate were obtained from Sigma Chemical Co. (St. Louis). Plasma aminotransferase levels (AsT and AlT) were assayed with commercial kits (Boehringer, Mannheim GmbH).

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Experimental protocols

As previously reported,[1,2] rubber band tourniquets were applied for 5 h, under ketamine (10 mg/kg body wt) and xylazine (2.5 mg/kg body wt) anesthesia, to the hind-limbs of Sprague-Dawley rats of both sexes (250–300 g) with prior free access to food and water. Free SH and TBARS levels were determined in the right lateral lobes of livers which were excised, under ether anesthesia, at different time intervals during the ischemic period, and 2 h after blood circulation was restored to the hind limbs. In order to take into account the normal circadian decrease in liver glutathione (GSH) content during daylight hours, sham-operated control animals were subjected to the same surgical procedures, i.e. during tourniquet application and liver extraction, but differed from the experimental animals in that tourniquets were not applied, and were sacrificed at the same times as the experimental animals.

Animals involved in experiments involving anti-oxidant protective agents were divided into 4 groups. Group I rats correspond to the control animals discussed above. Group II animals received a single intraperitoneal injection of saline (0.5 ml) 2 h prior to the application of the rubber bands to their hind-limbs. Group III animals received two intraperitoneal doses of ALLO (40 mg/kg of body wt), one of which was administered 2 h before the application of the ligatures, and the second, 1 h before release of the same. Group IV animals were administered a mixture of SOD (40 mg/kg body wt), CAT (20 mg/kg body wt) and DMSO (1.25 ml/kg body wt) on the same schedules as group III rats.

Methods

The liver SH content was estimated spectrophotometrically[24] after the addition of 5,5'-dithiobis(2-nitrobenzoic acid) to supernatants obtained after tissue homogenization with 5 vol of 10% trichloroacetic acid, and centrifugation at 2,500 rpm for 20 min. Lipid peroxidation was estimated as TBARS in 0.2 ml of liver homogenates using the thiobarbituric acid reaction (TBA) as described by Okhawa *et al.*[25] The livers were perfused through the portal vein with ice-cold 0.9% saline before homogenization. After washing with 0.9% NaCl, tissue homogenates were prepared in a ratio of 1 g of wet tissue to 9 ml of 1.15% KCl with a glass Potter-Elvehjen homogenizer. The reaction mixture contained 0.2 ml of sample, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of a 20% acetic acid solution adjusted at pH 3.5 with 10 M NaOH, 50 μl of BHT (to prevent autoxidation of the lipid fraction), and 1.5 ml of 0.8% aqueous solution of TBA. The mixture was made up to 4 ml with distilled water and heated at 100°C for 60 min. After cooling, the mixture was extracted by

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vigorously shaking with 1.0 ml of water and 5.0 ml of n-butanol-pyridine (15:1). The samples were left overnight at 4°C and centrifuged at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm. Alanine (AlT) and aspartic acid (AsT) aminotransferases were assayed in the same blood samples with commercial kits following the corresponding instructions. Serum alkaline phosphatase was determined as previously described.[26]

Statistics

The results are given as mean ± SE. Student's t-test. p values of less than 0.05 were considered significant.

RESULTS

Hepatic SH levels during the hind-limb tourniquet period and after hind-limb reperfusion

As shown in Figure 1, liver SH content does not change significantly during the first hour after the application of tourniquets when compared with sham-operated animals,

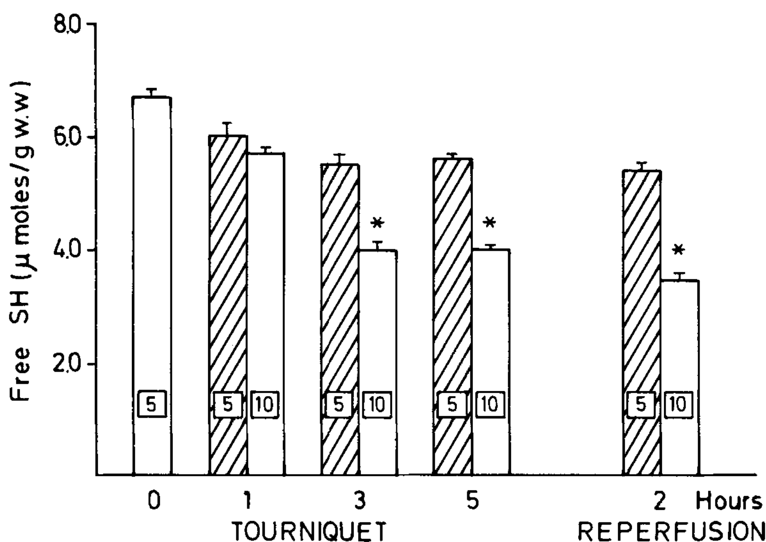

FIGURE 1 Liver SH contents during 5 h of bilateral application of hind-limb tourniquets and after 2 h of hind-limb reperfusion following tourniquet release compared to sham operated control animals sacrificed on the same time schedules described below. The bar labelled O reflects the free SH content of rats sacrificed at 10 a.m. and should be compared to the value obtained from the sham-operated control animals after 2 hours of reperfusion. The difference between these two values (about 20%) reflects the normal circadian decrease in liver GSH levels over this 7 h time period (10 a.m.–5 p.m.). The 1, 3 and 5 h tourniquet values (as well as from the sham-operated control) were obtained at 3 p.m. from animals which had their rubber bands applied at 2 p.m., 12 a.m., and 10 a.m., respectively. The 2 h reperfusion tourniqueted rats had their ligatures applied at 10 a.m. and were sacrificed at 5 p.m. The hatched bars represent the hepatic free SH levels of the sham-operated control animal. The open bars indicate the free SH content of rats subjected to bilateral tourniquets. The number of animals in each group is represented in the squares placed in the different columns. * $p < 0.001$. Three and 5 h ischemic animals (tourniquet) and the 2 h reperfusion group of animals vs their respective sham-operated controls.

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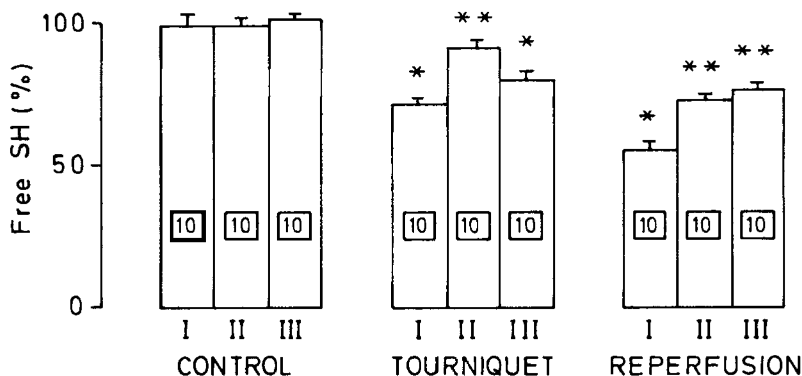

FIGURE 2 Effects of saline (bar I), ALLO (bar II), and SOD-CAT-DMSO (bar III) pre-treatments on rat liver SH contents (%) of sham-operated animals (control), of rats subjected to 5 h of bilateral tourniquets (tourniquet), and of rats 2 h after ligature release (reperfusion). The number of animals in each group is represented in the squares placed in the different columns. * $p > 0.01$ with respect to sham-operated rats (group I animals). ** $p < 0.01$ when compared with saline-treated rats (group II animals).

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but that after the third hour, and until tourniquet release two hours later, there is a significant decrease in tissue SH levels over and above the normal circadian daylight-hour decrease in liver glutathione (GSH) levels which amounted to an overall 20% decrease over the 7 h experimental period. There is a further significant drop in SH levels after the 2 hour hind-limb reperfusion period.

The protective effects of ALLO (bar II) and of the SOD-CAT-DMSO mixture (bar III) on hepatic SH levels is demonstrated in Figure 2. It can be seen (as also shown in Figure 1) that the SH levels of Group II animals decreased about 30% during the tourniquet period, when compared to the control animals (Group I), and by around 45% after the two-hour reperfusion period (bar I). ALLO, but not the SOD-CAT-DMSO mixture offers significant protection to liver SH loss during the 5 h tourniquet period, when compared with saline-treated animals (Group I), and that both treatments offer significant protection after the two-hour reperfusion period of the hind limbs.

Hepatic TBARS levels during the tourniquet period and after hind-limb reperfusion

Figure 3 shows that TBARS production does not change significantly during the five-hour tourniquet period, but that there is a significant increase of the same after two hours of hind-limb reperfusion. Figure 4 demonstrates that both the ALLO and the SOD-CAT-DMSO mixture significantly decrease TBARS formation after 2 h of hind-limb reperfusion, but that they have no effect on control animals (sham operated) nor on animals pre-treated with saline (bar I) during the 5 h tourniquet period.

Effects of tourniquet application and hind-limb reperfusion on hepatic aminotransferases and alkaline phosphatase levels in serum

Table I shows that there is a slight, but significant, increase in both AsT and AlT aminotransferases in serum during the hind-limb ischemic period, and that these

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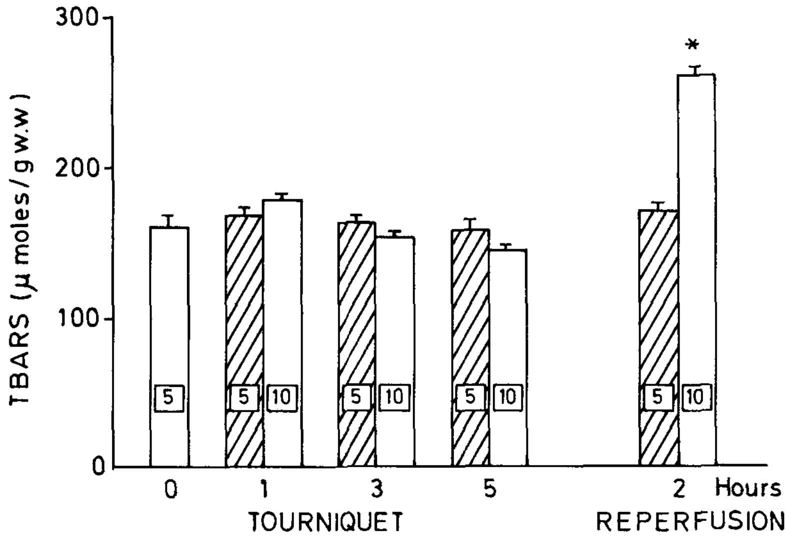

FIGURE 3 TBARS production by livers of rats subjected to different tourniquet times and after 2 h of hind limb reperfusion compared to that of sham operated control animals (bar 0). The experimental design is described in the legends to Figure 1. The number of animals in each group is represented in the squares placed in the different columns. * $p < 0.001$ with respect to sham-operated control animals.

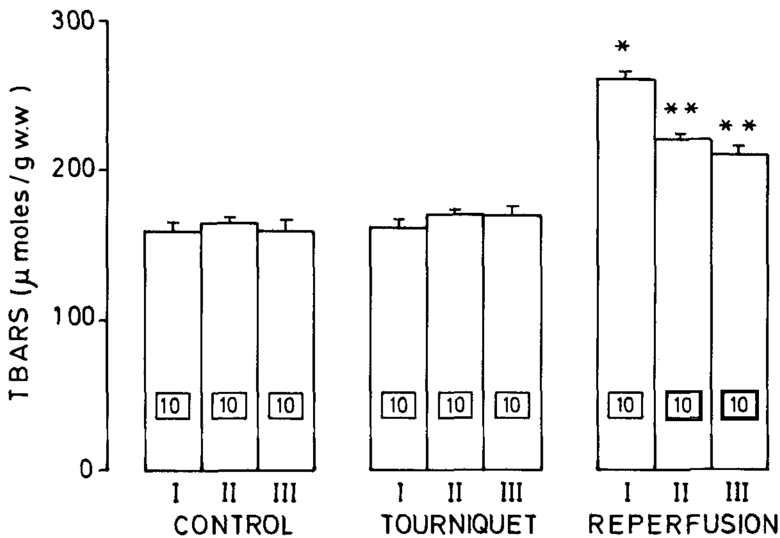

FIGURE 4 Protective effects of ALLO (bar II) and of the SOD-CAT-DMSO mixture (bar III) on rat liver TBARS production after 2 h of hind-limb reperfusion. The number of animals in each group is represented in the squares placed in the different columns. * $p < 0.01$ when compared to sham-operated control animals. ** $p < 0.01$ when compared to the saline-treated rats (group I reperfusion animals).

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TABLE I
Serum aminotransferase (AsT and AlT) and alkaline phosphatase levels in rats subjected to 5 h of himd-limb bilateral tourniquet and after 2 h of hind-limb reperfusion following release of rubber bands. * $p < 0.01$ and ** $p < 0.001$ when compared to sham-operated control animals

	n	AsT U/L	ALT U/L	Alkaline phosphatase U/ml
Control	5	99.4 ± 7.2	17.4 ± 0.8	87.9 ± 10.2
Tourniquet				
1 h	5	139.3 ± 17.9*	20.7 ± 0.9*	ND
3 h	5	182.6 ± 8.0*	24.4 ± 1.9*	ND
5 h	5	193.3 ± 16.1*	28.3 ± 0.9*	ND
Reperfusion				
2 h	5	844.8 ± 150.3**	95.4 ± 7.5**	157.4 ± 4.7*

increase 8-fold, and 3-fold, respectively, 2 h after the tourniquet release. Serum alkaline phosphatase levels increased about 2-fold from normal control levels after 2 h of hind-limb reperfusion. Both the ALLO and SOD-CAT-DMSO pre-treatments significantly decrease AsT serum levels (Figure 5), but not the AlT (Figure 6) levels after the 2 h reperfusion period. Note that number of animals increased from 15 to 25 in the results presented in Figure 6 as compared to those presented in Figure 5.

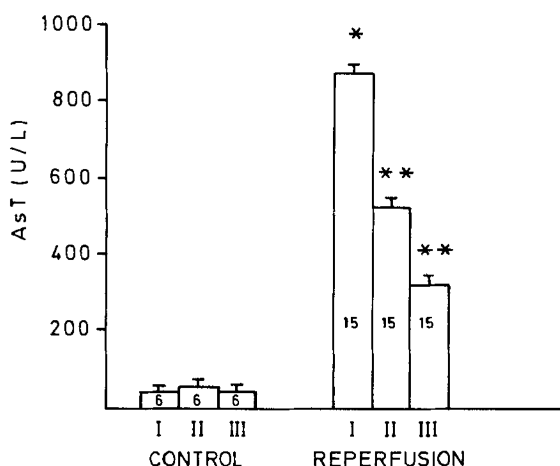

FIGURE 5 Serum aspartic acid aminotransferase (AsT) levels in saline (group I), ALLO (group II) and SOD-CAT-DMSO (group III) pretreated rats in control animals (sham operated) and in animals subjected to 5 h of bilateral hind-limb tourniquet application followed by 2 h of hind-limb reperfusion after ligature release (reperfusion) subjected to the same pretreatments. The number of animals in each group is represented in the different columns. * $p < 0.001$ when compared to sham-operated (control) animals. ** $p < 0.01$ when compared to saline-treated (bar I of reperfusion group) animals.

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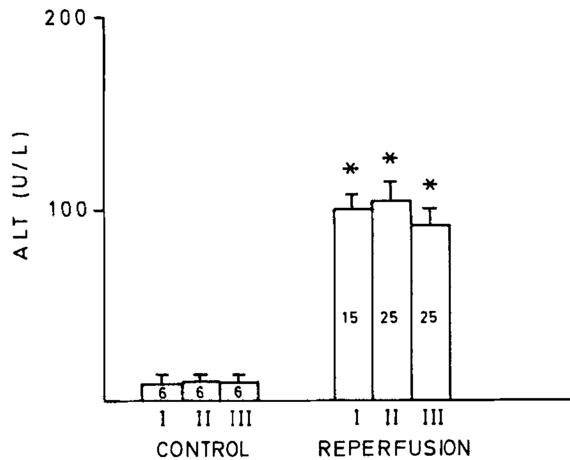

FIGURE 6 Serum alanine aminotransferase (AIT) levels in saline (group I), ALLO (group II) and SOD-CAT-DMSO (group III) pretreated rats in control animals (sham operated) and in animals subjected to 5 h of bilateral hind-limb tourniquet application followed by 2 h of hind-limb reperfusion after ligature release (reperfusion) subjected to the same pretreatments. * $p < 0.001$ when compared to sham-operated (control) animals. ** $p < 0.01$ when compared to saline-treated (bar I of reperfusion group) animals.

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DISCUSSION

A role for oxygen-derived free radicals in the pathogenesis of ischemia/reperfusion injury has been suggested for the liver and other organs,[4,17,27,28,29] yet the source of these radicals is still a debatable subject. In agreement with our results (Figure 2), it has been shown[28,30] that ALLO increases hepatic tolerance to ischemia/reperfusion injury. These authors have suggested that the oxy-radicals are generated as a result of the xanthine dehydrogenase/xanthine oxidase conversion during the ischemic period, which leads to the generation of superoxide anions when oxygen is again available to the liver after reperfusion. Although Siems *et al.*[31] have shown an increase in the ratio of oxidized to reduced glutathione in livers subjected to ischemia, and that ALLO prevented this transformation, we have no explanation as to why our rat liver SH levels should decrease, over and beyond that accountable by the normal daily circadian variation in GSH levels, during the tourniquet period (Figure 1); they are either being lost to bile or plasma in the form of glutathione (GSH), or alternatively are being oxidized to glutathione disulfide (GSSG), which is by far the most abundant SH compound in the hepatocyte. Our results show that ALLO confers significant protection to hepatic SH loss (Figure 2), not only during the hind-limb reperfusion stage—which is to be expected if the liver is hypoperfused after ligature release as discussed below—but strangely enough, also throughout the tourniquet period (Figure 2), during which time one would not expect the liver to be subjected to oxidative stress since its blood supply should be normal. Metzger and Lauterburg[32] have shown that plasma GSSG levels increase 8-fold after a 2 h partial hepatic ischemic period and that ALLO administered 18 h and 1 h prior to the onset of ischemia did not prevent the rise in plasma GSSG, nor did it alleviate the release of transaminases, and concluded that oxy-radicals generated by xanthine

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oxidase during reperfusion of ischemic livers do not contribute significantly to ischemic injury. These latter findings are supported by the recent work of Kunz *et al.*[33] whose electron-spin-resonance studies demonstrated an increase in oxygen radicals during early reperfusion of rat livers subjected to 60 min of warm ischemia and showed that an SOD/CAT mixture afforded protection, as did deferoxamine, but that ALLO showed no beneficial effect, i.e. the xanthine dehydrogenase to xanthine oxidase conversion during the ischemic period is not an important initial step in radical generation; in consequence, the protective effect observed by us could be due to allopurinol's free radical scavenging properties and not to its known role as a xanthine oxidase inhibitor. Our rationale for using the SOD-CAT-DMSO antioxidant mixture was to ensure, as far as possible, the detoxification of all reactive oxygen species, whether generated in the extracellular or intracellular compartments. SOD and CAT are normally used to accelerate the detoxification of the superoxide anion and hydrogen peroxide, thus preventing the generation of the far more reactive hydroxyl radical, and it is generally assumed that these enzymes protect against an intracellular oxidant stress, yet it is highly unlikely that significant amounts of these enzymes, administered into the peritoneum, penetrate the hepatocyte. Furthermore, the half-life of SOD administered intravenously is only six minutes, and that of the superoxide anion, should it be postulated to leave the cell, is in the microsecond range. Consequently, and as suggested by Jaeschke,[34] only superoxide anions and hydrogen peroxide generated in the extracellular compartment would be effectively scavenged by these enzymes, or by the high molecular weight extracellular heparin-binding SOD B found attached to the endothelium of rat blood vessels, and which can be released into serum by heparin.[35] On the other hand, DMSO distributes rapidly to all body fluid compartments and is non toxic even at high doses[36] and would thus scavenge hydroxyl radicals wherever they might be generated. The protection afforded by the SOD-CAT-DMSO mixture, but not by SOD alone (results not shown), also supports the hypothesis that reactive oxygen species, i.e. superoxide anion, hydrogen peroxide, hypochlorous acid, and/or the hydroxyl radical, are involved in hepatocellular injury, since liver SH levels (Figure 2) and TBARS production (Figure 3) were much closer to control values after 2 h of hind-limb reperfusion. Yet this combination of antioxidants does not seem to protect liver loss of SH during the 5 h that the tourniquets were in place, though it has been shown[27,37] that plasma GSH and GSSG levels do not change during hepatic ischemia, and that both increase after reperfusion. In order to further assess liver oxidative stress after hind-limb reperfusion, we measured the production of red 532 nm-absorbing pigment formed by the thiobarbituric acid (TBA) reaction of peroxidized lipids which is regarded as an excellent method for evaluating the degree of lipid oxidation,[38] though it must be kept in mind that the assay is neither specific nor does it fully reflect the extent of polyunsaturated fatty acid oxidation. Other more specific methods are available to measure the formation of aldehydes which better reflect the decomposition of lipid hydroperoxides.[39] Our results show that liver TBARS production does not seem to change during the 5 h tourniquet period (Figure 3) which suggests that there is no membrane associated peroxidative damage taking place during this period; yet the significant increase in TBARS during hind-limb reperfusion (Figure 3), and the protective effects of the antioxidant treatments (Figure 4) also supports the hypothesis that oxygen-derived free radicals are generated in the liver during the reperfusion period of the extremities, and that they are responsible for hepatocellular injury. The hypothesis is further supported by the finding of very high levels of liver

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aminotransferases in serum after the reperfusion period (Table I). As expected, both ALLO and SOD-CAT-DMSO administration significantly reduced AsT plasma levels after the 2 h reperfusion period (Figure 5), yet this was not the case with AlT (Figure 6) which is a liver-specific enzyme, with only very low amounts found in the heart and skeletal muscle, as opposed to AsT which is also released in high amounts from these tissues. Obviously it is possible that both ALLO and the oxyradical scavenging mixture could be protecting the release of AsT from the hind-limbs after reperfusion, as we have shown to be the case with lactic dehydrogenase isozyme release from these tissues.[1] Due to the fact that in some experiments we could demonstrate protection of AlT release and that in others no protection was apparent, we repeated the experiments over and over again. The results presented in Figure 6 represent the pooled findings of several experiments (25 animals in all) some of which showed significant protection with both the ALLO and the SOD-CAT-DMSO mixture after the reperfusion period, and some in which no protection seemed evident. The final outcome is disconcerting since it would signify that the pre-treatments used in our experimental protocols were ineffective in protecting the release of this highly specific hepatic enzyme (Figure 6). This latter result, but not the former, would seem to disagree with those reported previously[28,37] in *in vivo* perfused rat livers subjected to periods of ischemia followed by reperfusion in the presence of ALLO or and SOD-CAT mixture, where it was shown that these antioxidants significantly decreased the release of glutamic oxaloacetic (SGOT) and glutamic pyruvic (SGPT) transaminases during the reperfusion period. Nevertheless, hepatic tissue injury does seem to occur during the hind-limb ischemic period, albeit to a much lesser extent, as suggested by the release of these aminotransferases during this period (Table I). This latter result disagrees with the data presented by Jaeschke[27] which indicated that plasma AlT levels did not increase during 90 min of hepatic ischemia. Clearly this was not the case with our results. Alkaline phosphatase is found chiefly in the membranes of bile canaliculi and in bile. Its activity in blood is often increased in liver disease because any local or general obstruction to the flow of bile, i.e. cholestasis, increases its synthesis in the obstructed canaliculi, and increased amounts of enzyme then reflux into blood. Although we have not followed up on the matter, the 2-fold increase in plasma alkaline phosphatase (Table I) could be a reflection of the above, either as a result of liver hypoperfusion as postulated by us,[21] to edema formation and swelling, or to straightforward oxidative damage to the canalculi membranes. It was recently shown[40] that xanthine oxidase is released from the liver during the reperfusion period and the authors suggested that it could be a relevant source of extracellular reactive oxygen formation. If this is the case, it might explain the protective effect which we have observed with ALLO, yet the lack of evidence in isolated blood-free perfused livers for intracellular reactive oxygen radical formation, sufficient to cause direct cell damage through thiol oxidation and lipid peroxidation, led Jaeschke and Farhood[37] to test the hypothesis that Kupffer cells and infiltrating neutrophils were responsible for oxy-radical formation and that they contributed to ischemia-reperfusion injury of the liver. These authors concluded that Kupffer cells are the predominant source of reactive oxygen formed during the initial reperfusion period and that these cells contribute to reperfusion injury of the liver; consequently, the protective effect of our antioxidant mixture could be due to the scavenging of these extracellular oxy-radicals generated by these cells.

As a consequence of severe shock, many patients develop organ dysfunction, variously referred to as multiple system organ failure (MSOF), adult respiratory

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distress system (ARDS) or "shock lung syndrome", often of lethal consequences. During this pathophysiological process, various cellular and humoral 'mediator systems' are activated in a complex interacting sequence in which phagocytic and endothelial cells are involved. The causes of this remote organ failure are still unknown, though several hypotheses have been put forward. It has been suggested that injured or necrotic tissue, in our case in the extremities due to the prolonged ischemic period, may activate neutrophils, platelets, and the coagulation system leading to embolization in remote tissues.[41] As discussed by Blaisdell,[42] restoration of circulation to an extremity, which has had its blood supply obstructed, is not without risk. Morbidity and mortality relate directly to the duration and the degree of ischemia and the mass of tissue involved. Long-term ischemic periods (6 to 8 h) result in death of tissue, and reperfusion at this points leads to a washout into the systemic circulation of dead and devitalized tissue which produces intravascular coagulation and a diffuse inflammatory response with systemic vascular permeability. Respiratory distress syndrome develops after severe ischemic injury, and if patients are not adequately monitored and blood volume maintained, renal failure and multiple organ failure will ensure. Pulmonary failure and death within 24 hours has been demonstrated in a dog model[43] following four hours of arterial occlusion and restoration of blood flow to the lower extremities. Fibrin platelet aggregates were demonstrated in the inferior vena cava blood of samples obtained after removal of the aortic occluding clamps, yet no lung damage was observed when the inferior vena cava blood was directed through the liver prior to reaching the lung by portocaval transposition, demonstrating that the liver is a potent filtering and detoxifying site for by-products of ischemia.[44] These results, together with the findings that there is a reduction in the effective hepatic blood flow, which precedes the biochemical evidence consistent with an ischemic insult of the liver,[45,46] in both a murine peritonitis model and in femur fracture associated with soft tissue injury,[23] support our original hypothesis[21] that organ failure has its genesis in a primary perfusion abnormality with secondary ischemic organ injury.

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