Deleterious Effects of Buthionine Sulfoximine on Cardiac Function During Continuous Endotoxemia (43893)

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Abstract. **Sepsis has been associated with reversible cardiac injury. To determine whether this injury is mediated by generation of reactive oxidants, tissue glutathione (GSH)—the major intracellular antioxidant—was depleted before endotoxemia. Basal values of cardiac contractile function, perfusion, and cardiac output were measured 5–7 days postsurgery. *Salmonella enteritidis* endotoxin was continuously infused at 3 μg/kg/hr iv via an osmotic pump (Alzet Corp). Endotoxemia significantly reduced myocardial glutathione content (394 ± 46) to 206 ± 9 μg/g), indicating oxidant stress during endotoxemia. Buthionine sulfoximine (BSO) pretreatment significantly reduced cardiac glutathione in sham pigs from 394 ± 46 to 199 ± 26 μg/g; and in endotoxemic pigs, BSO pretreatment significantly reduced cardiac glutathione to 106 ± 18 μg/g. Vehicle- and BSO-treated endotoxemic groups demonstrated similar cardiovascular responses to endotoxin challenge. Heart rate increases (122 ± 15 to 140 ± 17 bpm) and cardiac outputs decreases (1.50 ± 0.24 to 1.11 ± 0.35 l/min) were similar, indicating similar cardiovascular insults induced by endotoxemia. Percent short axis shortening and end-systolic pressure-diameter relation (ESPDR) were significantly reduced in BSO pretreated compared with vehicle-treated endotoxemic pigs. Results support a conclusion that endotoxemia-induced cardiac injury is mediated, in part, by free radical injury. This conclusion is based upon the finding that endogenous myocardial glutathione was depleted by continuous endotoxin infusion and that prior depletion of myocardial glutathione by buthionine sulfoximine exacerbated cardiac injury.** [P.S.E.B.M. 1995, Vol 209]

Significant cardiac impairment is a feature of human sepsis (1, 2). Continuous infusion of endotoxin (CET) in pigs has been demonstrated to induce cardiac injury (3, 4) and metabolic alterations (5, 6) similar to human sepsis. Proposed mechanisms for loss of contractile function in these and other models of sepsis include loss of myocardial perfusion (7), decreased adrenergic receptor sensitivity (8), increased ventricular stiffness limiting filling (9), metabolic deficits (10), loss of vascular integrity leading to large fluid and protein fluxes (11), and/or cardiotoxic effect of reactive oxidants (12, 13).

The concept that endotoxemia-induced cardiac injury may be mediated by uncontrolled generation of reactive oxygen metabolites has received wide support (10, 14–17). Endotoxin stimulates neutrophils, macrophages, and endothelial cells *in vivo* and *in vitro* to initiate inflammatory processes (18, 19) which produce reactive oxidants, cytokines, arachidonic acid metabolites, tumor necrosis factor, tissue procoagulant activity, chemotaxis factors, etc. (reviewed in Ref. 18 and 20). Oxidants are also produced as a by-product of metabolism. Thus, CET-induced increased myocardial metabolic rate due to elevated heart rate and, often,

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cardiac work during sepsis (1, 21) would generate an additional oxidant stress. Combined with the possibility of decoupling of oxidative phosphorylation during sepsis (22), would provide for an additional, intracellular, source oxidant stress to the myocardium. By these sources, endotoxin challenge could induce reactive oxidants in quantities sufficient to overwhelm antioxidant mechanisms to meditate cardiac injury (12).

This study tested the hypothesis that reactive oxidants mediate endotoxemia-induced cardiac injury. Continuous infusion of endotoxin into pigs was chosen as the experimental model (5–9). The hypothesis was tested by experimentally reducing endogenous myocardial glutathione before endotoxin challenge, reducing endogenous antioxidant defense capacity, and determining if cardiac injury was exacerbated. The glutathione-glutathione peroxidase system was chosen for experimental modulation because it is the most important cardiac antioxidant system due to having the largest antioxidant capacity, greater than superoxide dismutase or catalase (23). Reduced myocardial glutathione levels were reported during myocardial ischemia (24) and reperfusion (24, 25) of postischemic myocardium, a model which generates reactive oxygen intermediates (24–26). Sepsis has been reported to reduce tissue levels of glutathione (16) and increase levels of plasma glutathione disulfide (GSSH) (15) suggesting sepsis induces significant intracellular and extracellular oxidant stress. Cardiac glutathione was reduced by pretreatment with Buthionine sulfoximine (BSO), a potent inhibitor of γ-glutamylcysteine synthetase, the rate-limiting enzyme for glutathione synthesis (28). If this hypothesis is correct, experimentally reducing endogenous glutathione will exacerbate loss of cardiac inotropism previously observed (3, 4).

Materials and Methods

Preparation of Animals. Twenty-four Yorkshire pigs of either sex weighing between 20 and 30 kg, randomly distributed between groups, were preanesthetized with ketamine HCl (15 mg/kg) and acepromazine maleate (1 mg/kg) im before induction of anesthesia with sodium thiopental (10 mg/kg, iv). After intubation, surgical anesthesia was maintained with halothane (3%–5% induction, 1%–3% maintenance), nitrous oxide (65%), and oxygen (35%). Ventilation was adjusted to maintain arterial blood gases at physiologic levels. A heating pad was placed under the animal to maintain body temperature at 38°C during surgery. Sterile surgical procedures were conducted in an AAALAC approved aseptic surgical suite (described in Ref. 3 and 4). In brief, instruments to measure left ventricular pressure (P), internal short axis diameter (D), and coronary and pulmonary artery flow (CAQ and PAQ, respectively) were implanted and their leads exteriorized. A saline-filled, TEDMAC-heparin-treated catheter was placed into the left atrium and was used to infuse drugs.

Experimental Protocol. Pigs were instrumented on Day 0 and were allowed to recuperate for at least 5 days before their transport to the data collection laboratory for habituation to this new environment. After basal recordings were obtained through at least 5 postoperative days, osmotic pumps (Alzet Model 2 ML1, Palo Alto, CA) containing either *Salmonella enteritidis* endotoxin or sterile saline were implanted subcutaneously in the neck with its output connected to a teflon 0.9-mm i.d. tube inserted into the internal jugular vein. The output tube was cut to a volume such that endotoxin infusion was initiated at 8:00 AM ± 1 hr the next morning (3, 4).

Endotoxin Challenge Protocol. Twenty-four pigs were separated into four groups and studied during 5 hr of/endotoxin or sham challenge using the following protocols: (i) saline infusion only (n = 4); (ii) endotoxin only (CET: 3 μg/kg/hr; n = 8); (iii) pretreatment with BSO followed by endotoxin challenge (n = 8); (iv) pretreatment with BSO and saline infusion (n = 4).

Data Acquisition and Analysis. All recordings were made on a Gould TA-2000 recorder whose analog output was analyzed on an 386-25 personal computer (Dell Computer Corp., Austin, TX) via an A-D converter (Data Translation, Marlborough, MA) using software developed in this laboratory. Measured variables recorded were LVP, ECG, CAQ, PAQ, and short axis diameter (D). From these measured variables, the following were calculated: dP/dt maximum and minimum, stroke work was estimated by ($SW = \int \pi[D/2]^2 dP$), integrated CAQ and PAQ, work-to-flow ratio (SW/CAQ), instantaneous pressure-to-diameter ratio (E), heart rate (HR), peak LVP, end-diastolic diameter (EDD), and % diameter shortening (%DSh). Slope, intercept, and r values for end-systolic-pressure-diameter relationship (ESPDR) (29) was calculated. In each beat, end-systole was defined as left upper corner of the pressure-diameter loop. Values of P and D at end-systole (P_{es} and D_{es}, respectively) were stored and the line $P_{es} = m\,D_{es} + D_0$ was calculated by linear regression of all P_{es} and D_{es} values while afterload was increased 10–20 mm Hg via intra-atrial infusion of angiotensin (7).

Tissue Preparation and Measurement of Glutathione. Duplicate biopsies of myocardial tissue of approximately 200 mg were taken from each animal after 5 hr of endotoxin challenge. Tissue samples were blotted, weighted, and homogenized in five volumes of 1% aqueous picric acid to precipitate protein. Each sample was then centrifuged at 10,000g for 15 min, and the clear supernatant was immediately frozen at −70°C

until assay. Measurement of total glutathione was done according to the enzymatic method of Tietze (30). Tissue extracts were diluted (50 or 100 times) in phosphate buffer (0.1 *M*, ph 7.5 containing 5 mm EDTA). The following were combined in a cuvette: 0.2 ml 3 mm DTNB (5,5'-dithiobis-2-nitrobenzoate), 0.2 ml NADPH (1 mg/ml), 0.05–0.1 ml sample, 0.05 ml Glutathione reductase (25 units/ml), and phosphate buffer to a final volume of 1ml. Glutathione reductase was added last to initiate the reaction. Absorbance at 412 nm was recorded for 3 min at room temperature. Standard curves were generated by substituting known amounts of glutathione and equal amounts of picric acid in the sample. Internal standards, in which known amounts of glutathione were added to a tissue sample, were used to ascertain that tissue samples were free of substances which interfered with glutathione reductase activity.

Experimental Depletion of Cardiac Glutathione. Depletion of myocardial glutathione was achieved by intramuscular injections of buthionine sulfoximine (BSO) (Chemalog, South Plainfield, NJ), a potent inhibitor of gamma-glutamylcysteine synthetase (28). One gram of BSO was dissolved in sodium chloride and sterilized by passing through a 0.22 μm Millipore filter and administered 12 hr and 1 hr at 20 mg/kg before the study.

Statistical Analysis. Basal values for each pig were obtained 5–7 days postsurgery. Ten to twenty observations taken on this day over 2–4 hr, when each pig was resting quietly in its cage, were used as "Basal" values. Because each animal served as its own control, variation between basal and endotoxemia induced changes in each cardiovascular variable were compared by analysis of variance (ANOVA) for repeated measures with $P < 0.05$ being considered significant. To determine which time point might be different from basal values, the Student-Newman-Keul test was performed (SigmaStat; Jandel Scientific, San Rafel, CA). To determine if BSO treatment caused significant changes between untreated and BSO pretreated endotoxemic pigs, two-way ANOVA for repeated measures was used (SigmaStat; Jandel Scientific). Myocardial glutathione levels from each treatment group were compared with nonendotoxemic control pigs by one-way ANOVA followed by Bonferroni *t* test comparison between groups.

Results

Figure 1 presents myocardial glutathione concentrations in all groups of pigs. BSO pretreatment of sham pigs significantly reduced myocardial glutathione from 394 ± 46 in control pigs to 199 ± 26 μg/g (mean ± SEM). Pigs pretreated with BSO and then subjected to endotoxin challenge exhibited a further reduction in myocardial glutathione to 106 ± 18 μg/g, significantly lower when compared with untreated, endotoxin challenged pigs (206 ± 8 μg/g glutathione). In this study, total glutathione concentration was measured because the ratio of reduced glutathione-to-oxidized glutathione (GSSG) ranged from 20:1 to 30:1. This confirmed previous findings (24) that total glutathione concentration was an accurate measure of reduced glutathione in these experimental conditions. In a separate series of experiments, measured wet:dry weight ratios of myocardial biopsies of control and CET pigs were found not to be statistically different suggesting that lowered glutathione levels were not artifacts due to myocardial edema.

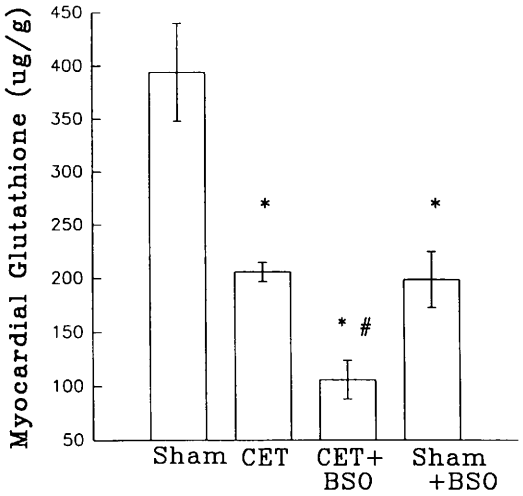

Figure 1. Myocardial glutathione content (μg/g myocardium, mean ± SEM) of samples obtained from pigs infused with saline only (Sham, $n = 4$), *S. enteritidis* endotoxin (CET, $n = 8$), BSO pretreatment and *S. enteritidis* endotoxin (CET + BSO, $n = 8$) or buthionine sulfoximine only (Sham + BSO, $n = 4$). $^{*}P < 0.05$ from sham pigs, $^{\#}P < 0.05$ from CET group (treatment groups are different $P < 0.001$, one-way ANOVA followed by Bonferroni *t* test.)

Each endotoxin challenged pig showed marked clinical signs and symptoms of endotoxemia. Prior to endotoxin challenge, each pig was transported to the data collection laboratory and monitored for up to 8 hr on successive days. During this time, pigs were quite inquisitive as to their surroundings, demanded food frequently, and rested quietly in their cages only after meals. The morning after implantation of an endotoxin loaded osmotic infusion pump, animals behaved similarly for 1–2 hr. It soon became quite apparent that endotoxin was beginning to enter the circulation, because they became quiescent and remained motionless for extended periods. Their heart rates and respiration rates rose noticeably as endotoxin challenge proceeded.

In sham pigs, BSO pretreatment did not induce any change in cardiac output or heart rate during 6 hr

of treatment (not illustrated), nor did any cardiovascular variable measured change including; stroke work, stroke volume, end-diastolic diameter, or peak left ventricular pressure. The load-independent assessments of cardiac inotropy, slope of ESPDR, and percentage of diameter shortening were also unchanged by BSO pretreatment in sham CET pigs.

Figures 2 and 3 present cardiovascular and cardiac dynamics of endotoxin challenged animals. BSO pretreated CET pigs (n = 8) demonstrated a significant (one-way ANOVA, $P < 0.05$) increase in heart rate during the endotoxin infusion compared with the previous day. This response was similar to the untreated, endotoxin challenged group (two-way ANOVA). Peak LVSP showed no effects of treatment (vehicle or BSO) during endotoxin challenge. Endotoxin challenge reduced cardiac output significantly (one-way ANOVA) although there was no difference between treatments. Coronary artery blood flow (CAQ, ml/min) was not significantly affected by treatment during endotoxin challenge. Variation was high because dependent beds varied widely between pigs due to differences between coronary artery architecture requiring implantation of flow meters on widely divergent locations on the circumflex coronary artery. Thus, the perfusion distribution downstream of each flow meter could vary from 50% to 20% of the left ventricle. End-diastolic diameter (not illustrated) decreased nonsignificantly in both groups during endotoxemia.

Assessments of cardiac inotropy, slope of ESPDR, and percentage of diameter shortening, are illustrated in Figure 3. Values of the ESPDR slope whose $r^2 > 0.3$ are reported in Figure 3. Because these experiments were conducted on conscious, closed chest animals, variations of P_{es} due to respiratory motion added variation in the range of ±5 mm Hg causing low r^2 values compared with acute experiments. In untreated CET pigs, end-systolic pressure-diameter relationship (ESPDR) was unchanged (one-way ANOVA). In CET pigs pretreated with BSO, ESPDR was significantly reduced ($P < 0.001$, one-way ANOVA) with differences being significant at 30–120 min and 180–270 min (Student-Newman-Kuels test). Comparing CET and CET + BSO pigs, there was a significant effect of BSO treatment ($P < 0.001$, two-way ANOVA). Differences between these groups were significant at all times except 150 and 300 min ($P < 0.05$, Student-Newman-Kuels test). Percentage of diameter shortening was significantly reduced, compared with basal (0 time) in both CET groups ($P < 0.001$, one-way ANOVA). There was a statistical significant effect of BSO pretreatment on %DSh ($P <$

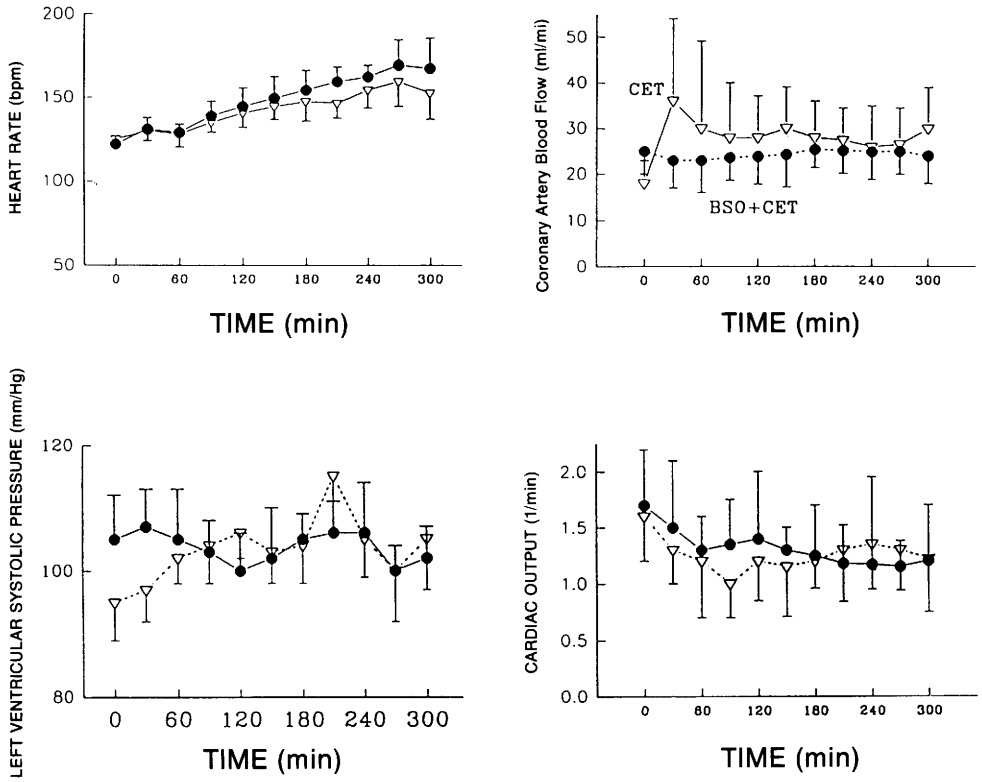

Figure 2. Cardiovascular response of pigs treated for 5 hr with endotoxin only (CET, n = 8 [open triangles]) or buthionine sulfoximine and endotoxin (BSO + CET, n = 8 [solid circles]). No significant changes in heart rate, peak left ventricular systolic pressure, or coronary artery blood flow were observed. Cardiac output was significantly decreased in all groups but no differences between groups were noted. Basal values (0) were obtained while each pig was resting the previous day. All data represented as means ± SEM.

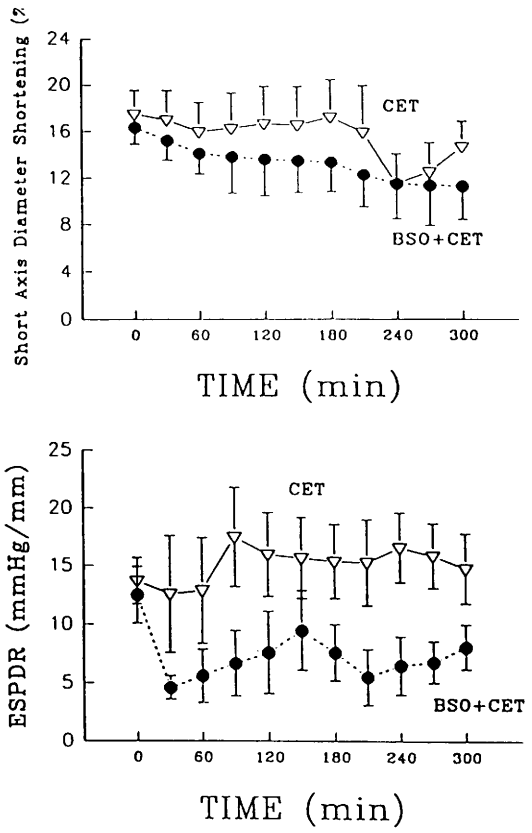

Figure 3. Cardiac dynamic response of pigs treated for 5 hr with endotoxin only (CET, $n = 8$ [open triangles]) or pretreated with buthionine sulfoximine and endotoxin (BSO + CET, $n = 8$ [solid circles]). In untreated CET pigs, end-systolic pressure-diameter relationship (ESPDR) was unchanged. In CET pigs pretreated with BSO, ESPDR was significantly reduced with differences being significant at 30–120 min and 180–270 min. Comparing CET and CET + BSO pigs, there was a significant effect of BSO treatment; differences between these groups were significant at all times except 150 and 300 min. Percent diameter shortening was significantly reduced in both CET groups. Comparing CET and CET + BSO pigs, BSO pretreatment significantly reduced %DSh; differences were significant at 150, 180, and 210 min. See text for complete review of statistical methods. Basal values (0) were obtained while each pig was resting the previous day. All data are represented as means ± SEM.

0.001, two-way ANOVA). %DSh was significantly reduced, compared with CET pigs at 150, 180, and 210 min ($P < 0.05$, Student-Newman-Kuels test). Basal values (0) were obtained while each pig was resting the previous day.

Discussion

Several previous studies have suggested that sepsis or endotoxemia induce cardiac injury that is mediated by toxic effects of reactive oxygen species (2, 17, 19, 27). This study sought to explore whether endotoxemia-induced generation of reactive oxidants could mediate cardiac injury. Such a causal relationship was tested by determining if depleting intracellular antioxidant defenses before endotoxin challenge would exacerbate cardiac injury during endotoxemia. Because glutathione-glutathione peroxidase is the major cardiac intracellular oxidant scavenging system (23), myocardial glutathione was depleted prior to endotoxin challenge by pretreatment with buthionine sulfoximine. BSO was selected because it has been reported to have no toxic side effects (28), and, indeed, no alterations in cardiovascular parameters were induced by BSO treatment of sham pigs.

Results of this study support a conclusion that endotoxemia-induced cardiac injury is mediated, in part, by generation of reactive oxygen species. That endotoxin challenge involves an oxidant stress was demonstrated by reduced myocardial glutathione content in untreated pigs (Fig. 1) (10, 16, 17). When intracellular glutathione was depleted before endotoxin challenge by BSO, degree of cardiac injury was exacerbated. This is illustrated in Figure 4, which plots myocardial glutathione content of sham, endotoxin-challenged (CET), and endotoxin-challenged (CET) pigs pretreated with BSO versus percentage diameter shortening at 300 min. Endotoxemic pigs pretreated with BSO demonstrated greater injury associated with greater glutathione reduction. Note that although BSO-treated sham pigs exhibited reduced myocardial glutathione levels with normal cardiac mechanical function (not illustrated), only during endotoxin challenge when oxidants were presumably produced did reduction in intracellular antioxidant defenses make the myocardium more sensitive to toxic effects of oxidants.

Reductions of tissue glutathione has been interpreted as an indicator of significant oxidant stress (15,

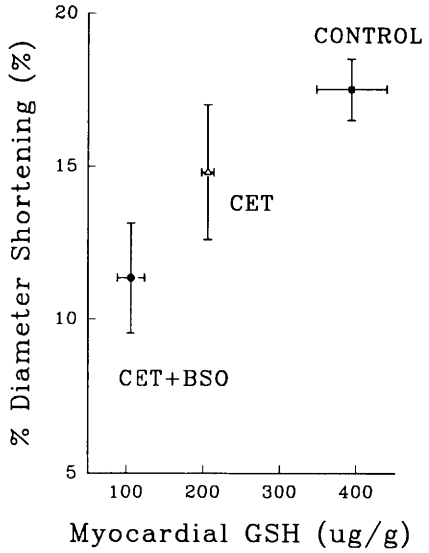

Figure 4. Relationship between percentage of diameter shortening (an indicator of cardiac intropism) at 300 min of endotoxin challenge and myocardial glutathione content. Glutathione levels were significantly lower in each group as was percentage of diameter shortening. %DSh was significantly lower than sham in both CET groups and CET + BSO was significantly lower than CET pigs ($P < 0.05$, one-way ANOVA, Bonferroni t-test). were This correlation between myocardial glutathione content and cardiac inotropism in these groups implies that cardiac injury was associated with mediated reactive oxidant stress.

16, 24–26). Glutathione is a tripeptide thiol which acts as an important myocardial antioxidant (23). Its antioxidant capacity resides in its ability to donate hydrogen atoms from its thiol group to most biologically generated radicals (31). Hydrogen peroxide is reduced to water by glutathione peroxidase using glutathione as an electron donator. Oxidized glutathione, glutathione disulfide (GSSG), is reduced back to glutathione by glutathione reductase in a reaction using NADPH as a co-factor. If an insufficient amount of NADPH is available, GSSG, a highly cytotoxic compound, is actively transported from the cell. In preliminary experiments for this study, glutathione:GSSG ratios did not fall below 20:1 even when total glutathione levels were halved. In fact, it has been reported that endotoxin challenge caused circulating levels of GSSG to be elevated (15) and tissue glutathione levels to be reduced (16). Decreased myocardial glutathione levels observed can be due to a combination of increased metabolic use and leakage of GSSG from the myocardium. Regardless of cause, its depletion will make myocardium more vulnerable to oxidant-induced injury (23, 24). Such observations have been made in reperfused post-ischemic myocardium (6, 24, 25, 32). Thus, reduced glutathione levels in endotoxin challenged pigs would suggest that CET induced significant oxidant stress to the myocardium.

Considerable evidence suggests reactive oxidant generation occurs during endotoxemia and may mediate observed cellular and systemic organ injury (13, 15, 16). The origin of these reactive oxygen species remains incompletely understood but appears to arise from sources intrinsic and extrinsic to the myocardium. A major intrinsic source consists of the xanthine oxidase pathway (27), whereas activated neutrophils have been implicated as an important extrinsic source (18, 19). There is increasing evidence that oxidants may be a key agent for promotion of cellular injury leading to irreversible sepsis (reviewed in Ref. 18 and 20). Drugs shown to inhibit xanthine oxidase-and/or neutrophil-derived oxidant generation in other circulatory disorders should ameliorate responses to endotoxin, if the latter depend on reactive oxidant generation for cardiovascular pathogenesis. Despite speculation in favor of this relationship, data to support a conclusion has not been convincing. Hess *et al.* (22) described basic similarities between endotoxin-induced and leukocyte-induced disorder of Ca^{++} uptake in cardiac sarcoplasmic reticulum. However, that study was based on phorbol myristate-activated leukocytes *in vitro*. It is not known whether leukocytes activated during endotoxemia exert similar effects in *in vivo* heart.

In vivo studies suggest that reactive oxidants may mediate injury, although there may be differences between circulatory beds. Traber *et al.* (11) studied intact sheep and reported SOD enhanced pulmonary vascular damage resulting from endotoxin suggesting that H_2O_2 and $HO\cdot$, rather than superoxide anion itself, were responsible for tissue damage associated with endotoxemia. Kunimoto *et al.* (17) indicated that SOD or SOD and catalase were effective against endotoxemia. In contrast, McKechnie *et al.* (10) found that similar treatment of endotoxin rats was not efficacious. Deutschman *et al.* (33) reported that although endotoxin shock in the pig cause alterations in cerebral microvascular responses, no O_2^- was produced. These inconsistencies in previous endotoxin studies may reflect differences in experimental design and different pharmacokinetic characteristics of oxidant scavengers themselves. For example, differences between length of pretreatment schedule may effect outcomes. That is, continuous intravenous infusion of a scavenging agent will provide more effective treatment than a single bolus injection before endotoxin challenge. It is doubtful that the latter would provide adequate protection during a study lasting several hours or days.

If oxidant generation occurs during endotoxemia and mediates observed cellular and systemic alterations, augmentation of endogenous antioxidant defenses should be protective in endotoxemia. N-acetylcysteine (NAC) has been used to support cellular levels of glutathione because it acts to transport cysteine across cell membranes for intracellular synthesis of glutathione. In longer duration experiments, Peristeris *et al.* (34) administered NAC to LPS-treated rats and reported that it reduced LPS-induced lethality. In that study, NAC also inhibited tumor necrosis factor (TNF) production, a major mediator of pathogenesis of endotoxin shock. However, NAC did not inhibit production of other cytokine mediators of shock, such as interleukin-6 (IL-6), IL-1a or corticosterone. Zhang *et al.* (35) pretreated dogs with NAC 30 min before infusion of *Escherichia coli* endotoxin and reported it has significant protective effects. NAC pretreatment provided improved oxygen extraction capabilities, enhanced glutathione peroxidase activity and inhibited TNF production. These findings suggest that the *in vivo* pharmacology of NAC may be more complex than simply acting as a transmembrane transporter for cysteine.

Results support a conclusion that endotoxemia-induced cardiac injury is mediated, in part, by free radical injury. This conclusion is based upon the finding that endogenous myocardial glutathione was depleted by continuous endotoxin infusion (CET) and that prior depletion of myocardial glutathione by buthionine sulfoximine exacerbated cardiac injury.

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1. Parker MM, Shelhamer JH, Bacharach SL, Green MV, Natanson C, Frederick TM, Damske BA, Parrillo JE. Profound but reversible myocardial depression in patients with septic shock. Ann Intern Med **100**:483–490, 1984.
2. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, Parrillo JE. The cardiovascular response of normal humans to the administration of endotoxin. N Engl J Med **321**:280–287, 1989.
3. Lee KJ, van der Zee H, Dziuban SW, Luhman K, Goldfarb RD. Left ventricular function during chronic endotoxemia in swine. Am J Physiol **254**:H324–H330, 1988.
4. Lee KJ, Dziuban SW, van der Zee H, Goldfarb RD. Cardiac function and coronary flow in chronic endotoxemia pigs. Proc Soc Exp Biol Med **189**:245–252, 1988.
5. Fish RE, Burns AH, Lang CH, Spitzer JA. Myocardial dysfunction in a non-lethal, non-shock model of chronic endotoxemia. Circ Shock **16**:241–252, 1985.
6. Fish RE, Spitzer JA. Continuous infusion of endotoxin from an osmotic pump in the conscious, unrestrained rat: A unique model of chronic endotoxemia. Circ Shock **12**:135–149, 1984.
7. Goldfarb RD, Nightingale LM, Kish P, Weber PB, Loegering DJ. Left ventricular function during lethal and sublethal endotoxemia in swine. Am J Physiol **251**:H364–H373, 1986.
8. Breslow MJ, Miller CF, Parker S, Walman AT, Traystman RJ. Effect of catecholamines on organ blood flow during endotoxin shock in the pig. Crit Care Med **12**:246, 1984.
9. Natanson C, Fink MP, Ballantyne HK, MacVittie TJ, Conklin JJ, Parrillo JE. Gram-negative bacteremia produces both severe systolic and diastolic cardiac dysfunction in a canine model that simulates human septic shock. J Clin Invest **78**:259–270, 1986.
10. McKechnie K, Furman BL, Parratt JR. Modification by free radical scavengers of the metabolic and cardiovascular effects of endotoxin infusion in conscious rats. Circ Shock **19**:429–439, 1986.
11. Traber DL, Adams T, Sziebert L, Stein M, Traber L. Potentiation of lung vascular response to endotoxin by superoxide dismutase. J Appl Physiol **58**:1005–1009, 1985.
12. Manson NH, Hess ML. Interaction of oxygen free radicals and cardiac sarcoplasmic reticulum: Proposed role in the pathogenesis of endotoxin shock. Circ Shock **10**:205–213, 1985.
13. Youn YK, LaLonda C, Demling R. Use of antioxidant therapy in shock and trauma. Circ Shock **35**:245–249, 1991.
14. Bernard GR, Lucht WD, Niedermeyer ME, Snapper JR, Ogletree ML, Brigham KL. Effect of N-acetylcysteine on the pulmonary response to endotoxin in the awake sheep and upon *in vitro* granulocyte function. J Clin Invest **73**:1772–1784, 1984.
15. Chang SW, Lanterburg BH, Voehel NF. Endotoxin causes a neutrophil independent oxidant stress in rats. J Appl Physiol **65**:358–367, 1988.
16. Chen MF, Chen LT, Boyce HW. Effect of endotoxin on the rat colon glutathione level. Biochem Biophys Res Comm **151**:844–850, 1988.
17. Kunimoto F, Morita T, Ogawa R, Fujita T. Inhibition of lipid peroxidation improves survival rate of endotoxemic rats. Circ Shock **21**:15–22, 1987.
18. Movat HZ, Cybulsky MI, Colditz IG, Chan MKW, Dinarello CA. Acute inflammation in gram-negative infection: Endotoxin, interleukin-1, tumor necrosis factor, and neutrophils. Fed Proc **46**:97–104, 1987.
19. Wilson ME. Effects of bacterial endotoxins on neutrophil function. Rev Infect Dis **7**:404–418, 1985.
20. Raetz CRH, Ulevitch RJ, Wright SD, Sibley CH, Ding A, Nathan CF. Gram-negative endotoxin: An extraordinary lipid with profound effects on eukaryotic signal transduction. FASEB J **5**:2652–2660, 1991.
21. Cunnion RE, Shaer GL, Parker MM, Natanson C, Parrillo JE. The coronary circulation in human septic shock. Circulation **73**:637–644, 1986.
22. Hess ML, Thompson JA, Kureja RC. Myocardial failure and excitation-contraction uncoupling during the course of canine endotoxin and hemorrhagic shock. In: Dhalla NS, Singh PK, Beamish RE, Eds. *Pathophysiology of Heart Disease*. Boston: Nijhoff, pp 325–334, 1987.
23. Simmons TW, Jamall IS. Relative importance of intracellular glutathione peroxidase and catalase *in vivo* for prevention of peroxidation to the heart. Cardiovasc Res **23**:774–779, 1989.
24. Singh A, Lee KJ, Lee CY, Goldfarb RD, Tsan MF. Relation between myocardial glutathione content and extent of ischemia-reperfusion injury. Circulation **80**:1795–1804, 1989.
25. Cochrane CG, Schraufstatter IU, Hyslop P, Jackson J. Cellular and biochemical events in oxidant injury. In: Halliwell B, Ed. Oxygen Radicals and Tissue Injury. Bethesda: FASEB, pp 49–56, 1988.
26. Curello S, Ceconi C, Bignoli C, Ferrari R, Albertini A, Guarnieri C. Changes in cardiac glutathione status after ischemia and reperfusion. Experientia **41**:42–43, 1985.
27. McCord JM. Oxygen-derived free radicals in post-ischemic tissue injury. N Engl J Med **312**:159–163, 1985.
28. Griffith OW. Mechanism of action, metabolism and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. J Biol Chem **257**:13704–13712, 1985.
29. Sagawa K. The ventricular pressure-volume diagram revisited. Circ Res **43**:677–687, 1978.
30. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Anal Biochem **27**:502–522, 1969.
31. Meister A, Anderson ME. Glutathione. Annu Rev Biochem **52**:711–760, 1983.
32. Robinson MK, Rounds JD, Hong RW, Jacobs DO, Wilmore DW. Glutathione deficiency increases organ dysfunction after hemorrhagic shock. Surgery **112**:140–147, 1992.
32. Werns SW, Fantone JC, Ventura A, Lucchesi BR. Myocardial glutathione depletion impairs recovery of isolated blood perfused hearts after global ischemia. J Mol Cell Cardiol **24**:1215–1220, 1992.
33. Deutschman CS, Kirsch JR, Breslow MJ, Miller CF, Traystman RJ. Failure of endotoxic shock to elicit superoxide anion production in pig brain. Circ Shock **31**:149–158, 1990.
34. Peristeris P, Clark DB, Gatti S, Faggioni R, Mantovani A. N-acetyl-cysteine and glutathione as inhibitors of tumor necrosis factor. Cell Immunol **140**:390–392, 1992.
35. Zhang H, Spapen H, Nugyen DN, Benlabed M, Buurman WA, Vincent, J-L. Protective effects of N-acetyl-L-cysteine in endotoxemia. Am J Physiol **266**(Heart Circ Physiol 35): H1746–H1754, 1994.