Free Rad. Res. Comms., Vol. 7, No. 3-6, pp. 315-324
Reprints available directly from the publisher
Photocopying permitted by license only

SPIN TRAPPING OF FREE RADICALS PRODUCED IN VIVO IN HEART AND LIVER DURING ENDOTOXEMIA*

DANIEL J. BRACKETT,[a,b] EDWARD K. LAI,[e] MEGAN R. LERNER,[a] MICHAEL F. WILSON,[a,c] and PAUL B. MCCAY[d,e]

Veterans Administration Medical Center[a], Departments of Anesthesiology,[b] Medicine,[c] and Biochemistry and Molecular Biology,[d] University of Oklahoma Health Sciences Center; Molecular Toxicology Group[e], Oklahoma Medical Research Foundation; Oklahoma City, OK

Studies using free radical scavengers and measurements of lipid peroxidation have suggested that free radicals are generated during endotoxemia. Conclusions from these studies have implied that free radicals may participate in the sequence of pathologic events following endotoxin challenge in the experimental animal. Current inferences of free radical generation and involvement have been derived from indirect evidence and are therefore inconclusive. To quantitate the generation of free radicals *in vivo* during endotoxemia this study employed the use of electron paramagnetic resonance spectroscopy (EPR) combined with spin trapping techniques. Five minutes before intraperitoneal endotoxin administration, trimethoxy-a-phenyl-t-butyl-nitrone [$(MeO)_3$ PBN] was administered intraperitoneally. Experimental animals were always matched with control animals receiving no endotoxin. At either five minutes or twenty-five minutes following endotoxin administration animals were decapitated and hearts and livers were rapidly taken for lipid extraction and EPR evaluation. Analysis of the EPR spectra revealed hyperfine splitting constants that indicated the presence of carbon-centered radical spin adducts in both organ tissues from animals exposed to endotoxin for twenty-five minutes. No signals were present in hearts and livers taken five minutes after endotoxin administration. EPR evaluation did not indicate spin adduct formation in control tissue. These data directly demonstrate that activation of processes *in vivo* involving free radical generation occur early during endotoxemia, but are not detectable immediately after the endotoxin challenge.

KEY WORDS: Endotoxemia, carbon-centered free radicals, spin traps, electron spin resonance, conscious animals.

INTRODUCTION

Free radicals are becoming recognized as possible mediators of pathological events occuring during endotoxemia. This concept is of clinical importance since elevated plasma levels of endotoxin have been documented in septic patients[1-3] and administration of antibodies to the core glycolipid region of endotoxin, the portion of the endotoxin molecule associated with toxicity, improves survival during human sepsis.[4,5] Much of the evidence accumulated associating free radical activity with endotoxemia has related to the use of radical scavengers, antioxidants, or trappers and their effect on actions known to be elicited by endotoxin. Superoxide dismutase,[6]

*Dedicated to the memory of Edward K. Lai, friend and colleague.

Send all correspondence to: Daniel J. Brackett, Research Service (151), VA Medical Center, 920 N.E. 13th Street, Oklahoma City, OK 73104.

a-tocopherol,[7] dimethyl sulfoxide,[8] and phenyl-t-butylnitrone[7,9] are reported to improve survival in rats following endotoxin. However it is also reported that there is not a survival effect by either superoxide dismutase[7] or a-tocopherol.[10] Endotoxin-induced lung injury is prevented by dimethyl sulfoxide in rats[8] but not by dimethylthiourea in sheep,[11,12] even though both are reported to be hydroxyl radical scavengers. Superoxide dismutase also had no effect on lung injury in sheep.[13] Disseminated intravascular coagulation produced by endotoxin in rats was prevented by superoxide dismutase,[14] catalase,[15] or vitamin E.[16]

Based on studies *in vitro* utilizing superoxide dismutase and catalase, it has been postulated that free radicals contribute to endotoxin-induced depression of myocardial sarcoplasmic reticulum Ca^{++} uptake rates and Ca^{++} stimulated ATPase activity consequenting in contractile failure and circulatory collapse.[17,18] In contrast the same scavengers had no effect on myocardial function or the course of endotoxemia in the intact dog.[19,20]

These controversies arising between different research groups implicating or eleminating free radicals as significant mediators during endotoxemia are all founded on exogenous intervention to adjust an endogenous imbalance between free radicals and the regulators of their generation and activity. The complexity of biologic free radical systems may preclude the effectiveness of these methods to make definitive conclusions regarding the presence or absence of free radical generation and require a different approach such as measurement *in vivo* to help elucidate the issue.

The conscious, instrumented rat model of endotoxemia used in the study presented here results in disturbance of the cardiovascular system, altered metabolism, and tissue injury.[21-23] Electron microscopic evidence of endotoxin-induced cellular damage in the myocardium has been presented[24-26] that is similar to damage produced by free radical generation systems[27,28] and prevented by free radical scavengers during ischemia-reperfusion of cardiac tissue.[29-34] Further studies in this model have demonstrated that dimethyl sulfoxide prevented the myocardial injury elicited by endotoxin.[24,25] Dimethyl sulfoxide possesses properties that permit scavenging of the hydroxyl radical[35-40] and inhibition of superoxide anion radical release from the neutrophil.[41]

These data suggest that in this model free radicals may be significant mediators in the ultrastructural alterations occurring in the myocardium and possibly other tissue during endotoxemia. However, no work focusing on free radicals is relevant unless there is quantitative documentation that these processes occur in intact animals. The most powerful documentation is direct measurement and there is no evidence in the literature that this has been accomplished during endotoxemia. Free radical generation *in vivo* has been measured in hepatic tissue induced by radiation,[42] ethanol,[43] halothane,[44] carbon tetrachloride,[45] and ischemia-reperfusion[46] using electron paramagnetic resonance spectroscopy combined with spin trap techniques.[47,48] The above methodology has now been applied to this model of endotoxemia to detect endotoxin-induced free radical generation *in vivo* and we report here that this occurs in the myocardium and liver of the intact conscious rat.

METHODS

Experimental Protocol

Data obtained from ten male Sprague-Dawley rats weighing 300 $\pm$ 12 g are presented in this report. Intraperitoneal trimethoxy-a-phenyl-t-butyl-nitrone [$(MeO)_3$ PBN,

75 mg/kg) was given 5 minutes before 20 mg/kg of *E. coli.* endotoxin (0127:B8, Sigma) was administered by the intraperitoneal route. Twenty-five minutes or five minutes after endotoxin animals were killed by guillotine and the hearts and livers were quickly removed and immediately prepared for analysis. Each study always involved an animal challenged with endotoxin and an animal that received only saline as a matched control.

Tissue Preparation and Electron Paramagnetic Resonance Spectroscopy

After the organs were weighed each was homogenized in 15 vol of chloroform/methanol (2:1, vol/vol). To induce phase separation one-fifth volume of 0.9% NaCl was added. Following recovery of the chloroform layer and evaporation to dryness, samples of the lipid extracts were placed in quartz tubes and deoxygenated with N_2. Samples were placed in an IBM ER-300 EPR spectrometer equipped with an X-band microwave bridge and evaluated for the presence of spin adducts. All spectra were obtained at 25°C. The spectrometer settings are given in the figure legend.

RESULTS

Figure 1 illustrates an EPR spectrum that is typical of spectra generated from heart and liver tissue lipid extracts of rats challenged with an intraperitoneal injection of endotoxin (A and C respectively). The nitrogen and β-hydrogen splitting constants of these spectra ($a_N = 15.28$ G, $a_\beta^H = 2.00$ G) are consistent with the signals of $(MeO)_3$ PBN spin adducts of carbon centered radicals that have undergone ortho demethylation.[45] These adducts are similar to those found in the heart and liver lipid extracts of rats fed ethanol[43] and those observed during the hepatic metabolism of carbon tetrachloride[45] which is known to initiate lipid peroxidation. Spectra were not detectable for tissue taken five minutes after the endotoxin challenge. The absence of spectra at this early time point indicates that either no adducts were formed or that a sufficient amount to detect had not been produced. Heart and hepatic lipid extracts from rats given $(MeO)_3$ PBN but not challenged with endotoxin did not produce an EPR signal as demonstrated by typical spectra in B and D of Figure 1.

An additional six animals evaluated *in vivo* in this study (spectra not shown) using the spin trap 5,5 dimethyl-l-pyrroline-N-oxide (DMPO) produced EPR signals from heart and liver lipid extracts 25 minutes following intravenous endotoxin. Each spectrum yielded hyperfine splitting constants of $a_N = 14.80$G, $a_\beta^H = 20.27$G and $a_N = 15.00$G, $a_\beta^H = 23.04$G that are consistent with carbon-centered radical DMPO adducts. Tissue from matched control animals did not produce detectable spectra. However, we must consider these data to be inconclusive since, recently in our laboratory, tissue from untreated animals has produced EPR signals indicating weak DMPO adduct formation with splitting constants similar to those reported above, raising the possibility of artifacts.

DISCUSSION

Demonstration of the presence of carbon centered radicals during endotoxemia permits the following important conclusions. Direct evidence has been provided that

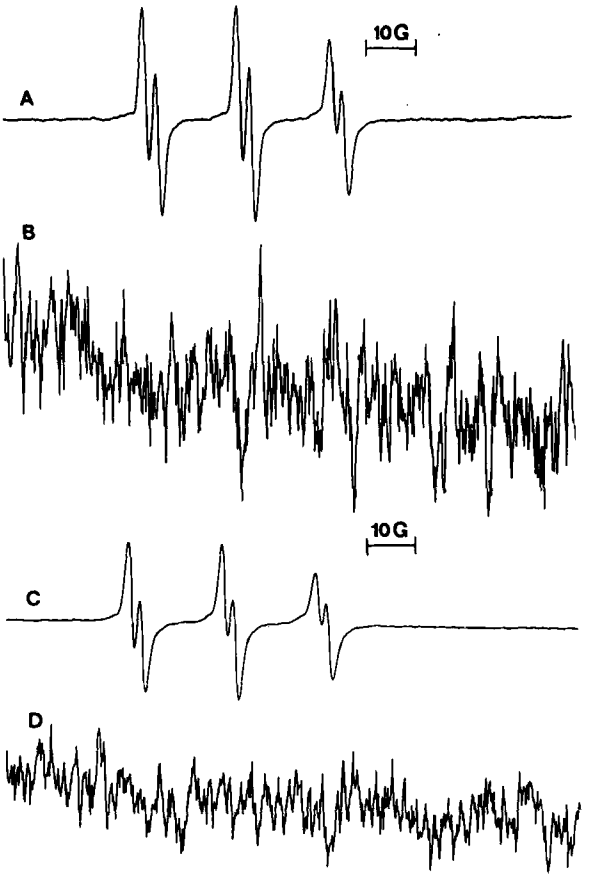

FIGURE 1 EPR signals of $(MeO)_3PBN$ radical adducts formed *in vivo* in the heart and liver of conscious rats. Rats were challenged intraperitoneally with either endotoxin or an equivalent volume of saline 5 minutes following intraperitoneal $(MeO)_3PBN$. Twenty-five minutes after the challenge the heart and liver were harvested for lipid extraction. Spectrometer settings for each spectrum, unless noted otherwise, were microwave power, 1.00e + 01 mW; modulation amplitude 0.997G, time constant, 1.28 ms; sweep width, 100.00G; sweep time, 335.544 s; gain, 1.00e + 06; scale, 17. (A) EPR spectrum observed in heart extract of endotoxin rat; modulation amplitude, 1.036G; gain, 1.00e + 5. (B) EPR spectrum from heart extract of control rat; modulation amplitude, 1.036G; gain, 1.00e + 5; scale, 11. (C) EPR spectrum from liver extract of endotoxin rat; modulation amplitude, 1.036G; gain, 1.00e + 5; scale, 18. (D) EPR spectrum from liver extract of control rat; modulation amplitude, 1.036G; gain, 1.00e + 5; scale, 12. Hyperfine splitting constants for heart and liver tissue from endotoxic rats were $a_n = 15.28G$, $a_\beta^H = 2.00G$

endotoxin has the capacity to stimulate an *in vivo* process involving free radical generation in the conscious rat and that this process occurs early during the development of endotoxemia. Furthermore, since the radicals produced are of the type that are formed during lipid peroxidation these results provide evidence that lipid peroxidation occurs in the heart and liver during endotoxemia. These data are compatible with previous reports providing evidence of lipid peroxidation elicited by endotoxin in experimental animals[6,10,11,49–53] as measured by the thiobarbituric acid assay for malondialdehyde. The presence of lipid peroxidation following endotoxin

using this methodology has been detected in the plasma, lungs, and liver of rats,[6,10,59,52,53] the liver of mice,[50] the blood of rabbits,[51] and the lungs of sheep.[11] This assay has also been applied to detect lipid peroxidation in the plasma of septic patients.[54] The latter observation is important since endotoxin has been shown to be present in the plasma of septic patients,[1-3] and it provides a common factor relating the experimental animal to the human septic patient involving endotoxin and possible tissue injury. This assay measuring an end product of lipid peroxidation is currently used as an index of free radical reactions and inferences concerning free radical generation are derived from results of the assay. However, the detection of malondialdehyde during a situation resulting in cellular damage and tissue injury does not necessarily provide conclusive evidence of free radical production and involvement; such products may occur as a consequence of cell injury and/or cell death. The free radical is a paramagnetic species due to the unpaired electron in the outer orbit and is measurable by electron paramagnetic resonance spectroscopy which is highly selective for paramagnetic species. Therefore, speculation concerning free radical production based on measurement of lipid peroxidation by-products can be confirmed by direct measurement using electron paramagnetic resonance spectroscopy. When combined with spin trapping techniques, electron paramagnetic resonance spectroscopy becomes a tool for the measurement of free radical generation in a biological system *in vivo*[55,56] as has been applied in this study. Even though the data presented here have demonstrated free radical generation *in vivo* during endotoxemia, conclusive evidence that free radicals are responsible for activation of the lipid peroxidation process and the subsequent cellular damage occuring during endotoxemia remains to be determined.

Possible sources of free radical production during episodes of elevated endotoxin plasma concentrations are abundant. Endotoxin is a potent activator of neutrophils[57-62] eventuating in the release of free radicals.[63-65] In addition to inducing the release of IL-1 and TNF from the macrophage to induce neutrophil activation,[58,66] endotoxin also primes the neutrophil for enhanced release of the superoxide anion as measured by superoxide dismutase-inhibiting reduction of cytochrome c.[67,68] During endotoxemia there are elevated plasma concentrations of cyclooxygenase products[59-71] and catecholamines.[72,73] Highly reactive species identified as free radicals or radical-like substances are generated during the cyclooxygenase pathway of the arachidonic acid cascade[74,75] and during the autooxidative metabolic breakdown of catecholamines.[76-80] Increased metabolic activity of both of these processes may contribute to reactions involving radicals that appear to occur during endotoxemia.

Inadequate tissue perfusion due to vasoconstriction, disseminated intravascular coagulation, and compromised cardiac output occurs during endotoxin shock. Episodes of compromised oxygen supply to the tissue creates two additional sources of free radical generation. Attenuation of oxidative phosphorylation subsequent to deficient oxygen supply and decreased activity of the electron transport chain triggers the xanthine oxidase-hypoxanthine mechanism for the production of the superoxide anion. Additionally when the concentrations of oxygen available to the mitochondrial electron transport chain are reduced, orderly flow of electrons is disrupted and free radicals normally generated at the flavo-protein and coenzyme Q levels[81,82] become disassociated and available for free radical reactions with molecular oxygen. Autooxidative/reductive cycling can then occur resulting in generation of the superoxide anion.[83-85]

The detection of free radical generation *in vivo* in the heart and liver during

endotoxemia is compatible with the literature in this area and confirms suggestions of free radical reactions derived from indirect methodology. Therefore during endotoxemia: 1) there are available sources for enhanced free radical generation; 2) processes that involve free radical reactions have been demonstrated; 3) substances known to interfere with free radical activity have demonstrated efficacy; and 4) with this report detection of free radical generation has been achieved *in vivo*. These present findings provide direct evidence for endotoxin-induced free radical generating processes and establish the significance of free radical reactions as a potential factor during known situations of elevated plasma concentrations of endotoxin such as sepsis,[1-3] cirrhosis of the liver,[86] hemorrhagic or traumatic shock,[87] heat stroke,[88] strenuous exercise,[89] acute hypoxia,[90] and compromised splanchnic blood flow.[91,92]

Acknowledgement

We are grateful to Drs. Edward G. Jansen and Coit M. Dubose for helpful discussions and analysis of EPR spectra, Drs. J. Lee Poyer and Sara A. Lloyd for scientific review of the manuscript, Theresa J. Lander for valuable technical support, and Carolyn K. Thorsen and Tracy R. Breslin for excellent transcription and editorial review of the manuscript. This work was partially supported by Medical Research Service, Veterans Administration and GM3612.

References

1. Levin, J., Poore, T.E., Zauber, N.P. and Oser, R.S. Detection of endotoxin in the blood of patients with sepsis due to gram-negative bacteria. *N. Engl. J. Med.*, **283**, 1313–1316, (1970).
2. van Deventer, S.J.H., Buller, H.R., TenCate, J.W., Sturk, A. and Pauw, W. Endotoxemia: An early predictor of septicemia in febrile patients. *Lancet*, **i**, 605–608, (1988).
3. Danner, R.L., Elin, R.J. Hosseini, J.M., Schlesinger, T., Roach, P. and Parrillo, J.E. Endotoxemia is associated with many clinical manifestations of septic shock (SS) in humans. *Clin. Res.*, **34**, 410A, (1986).
4. Ziegler, E.J., McCutchan, J.A., Fierer, J., Glauser, M.P., Sadoff, J.C., Douglas, H. and Braude, A.I. Treatment of gram negative bacteremia and shock with human antiserum to a mutant escherichia coli. *N. Engl. J. Med.*, **307**, 1225–1230, (1982).
5. Baumgartner, J-D., Glauser, M.P., McCutchan, J.A., Ziegler, E.J., van Melle, G., Klauber, M.R., Vogt, M., Muehlen, E., Luethy, R., Chiolero, R. and Geroulanos, S. Prevention of gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. *Lancet*, **ii**, 59–63, (1985).
6. Kunimoto, F., Morita, T. and Ogawa, R. The effect of oxygen free radical scavengers on experimental endotoxemia. In *Oxygen Free Radicals in Shock. Int. Workshop, Florence 1985*, edited by U. Novelli, pp. 114–118, Basel: Karger, (1986).
7. McKechnie, K., Furman, B.L. and Parratt, J.R. Modification by oxygen free radical scavengers of the metabolic and cardiovascular effects of endotoxin infusion in conscious rats. *Circ. Shock*, **19**, 429–439, (1986).
8. Breen, R.E., Connell, R.S. and Harrison, M.W. The effect of dimethyl sulfoxide on endotoxin induced pulmonary dysfunction: A biochemical and electron microscope study. *Ann. N.Y. Acad. Sci.*, **411**, 324–327, (1983).
9. Novelli, G.P., Angiolini, P., Martinim G. and Tani, R. Oxygen radicals scavenging in prophylaxis and treatment of experimental shock. In *First Vienna Shock Forum, Part A: Pathophysiological Role of Mediators and Mediator Inhibitors in Shock*, edited by G. Schlag and H. Redl, pp. 259–269, New York: Alan R. Liss, Inc, (1987).
10. Kunimoto, F., Morita, T., Ogawa, R. and Fujita, T. Inhibition of lipid peroxidation improves survival rate of endotoxemic rats. *Circ. Shock*, **21**, 15–22, (1987).
11. Damling, R.H., Lalonde, C., Jin., L-J., Ryan, P. and Fox, R. Endotoxomia causes increased lung tissue lipid peroxidation in unanesthetized sheep. *J. Appl. Physiol.*,**60**, 2094–2110. (1986).
12. Wong, C., Fox, R. and Demling, R.H. Effect of hydroxyl radical scavenging on endotoxin induced lung injury. *Surgery*, **97**, 300–306, (1985).

13. Traber, D.L., Adams, T. Jr., Sziebert, L., Stein, M. and Traber, L. Potentiation of lung vascular response to endotoxin by superoxide dismutase. *J. Appl. Physiol.*, **58**, 1005–1009, (1985).
14. Yoshikawa, T., Murakami, M., Yoshida N., Seto, O. and Kondo, M. Effects of superoxide dismutase and catalase on disseminated intravascular coagulation in rats. *Thromb. Haem.*, **50**, 869–872, (1983).
15. Yoshikawa, T., Seto, O., Itani, K., Kakimi, Y., Sugino, S. and Kondo, M. Endotoxin-induced disseminated intravascular coagulation and shock, and their relationship with oxygen-derived free radicals. In *Oxygen free radicals in shock. Int. Workshop, Florence 1985*, pp. 125–136, Basel: Karger, (1986).
16. Yoshikawa, T., Murakami, M., Tanigawa, T., Sugino, S. and Kondo, M. Role of vitamin E on endotoxin-induced disseminated intravascular coagulation. In *Oxygen Free Radicals in Shock. Int. Workshop, Florence 1985,* edited by U. Novelli, pp. 137–148, Basel: Karger, (1986).
17. Manson, N.H. and Hess, M.L. Interaction of oxygen free radicals and cardiac sarcoplasmic reticulum: Proposed role in the pathogenesis of endotoxin shock. *Circ. Shock,* **10**, 205–213, (1983).
18. Hess, M.L., Thompson, J.A. and Kukereja, R.C. Myocardial failure and excitation-contraction uncoupling during the course of canine endotoxin and hemorrhagic shock. In *Pathophysiology of Heart Disease,* edited by N.S. Dhalla, P.K. Singal, and R.E. Beamish, pp. 325–334, Boston: Nijhoff, (1987).
19. Novotny, M.J., Laughlin, M.H. and Adams, H.R. Evidence for lack of importance of oxygen free radicals in Escherichia coli endotoxemia in dogs. *Am. J. Physiol,* **254**, H954–H962, (1988).
20. Laughlin, M.H., Smyk-Randall, E.M., Novotny, M.J., Brown, O.R. and Adams, H.R. Coronary blood flow and cardiac adenine nucleotides in E. coli endotoxemia in dogs: Effects of oxygen radical scavangers *Circ. Shock,* **25,** 173–185, (1988).
21. Brackett, D.J., Schaefer, C.F. and Wilson, M.F. The effects of H1 and H2 histamine receptor antagonists on the development of endotoxemia in the conscious, unrestrained rat *Circ. Shock,* **16**, 141–153, (1985).
22. Brackett, D.J., Schaefer, C.F., Tompkins, P., Fagraeus, L., Peters, L.J. and Wilson, M.F. Evaluation of cardiac output, total peripheral vascular resistance, and plasma concentrations of vasopressin in the conscious, unrestrained rat during endotoxemia. *Circ. Shock,* **17**, 273–284, (1985).
23. Wilson, M.F. and Brackett, D.J. Release of vasoactive hormones and circulatory changes in shock. *Circ. Shock,* **11**, 225–234, (1983).
24. Brackett, D.J., Lerner, M.R., Stiers, D.L., Schaefer, C.F. and Wilson, M.F. Dimethyl sulfoxide protects against tissue injury and improves the course of endotoxemia. *Cric. Shock,* **24**, 277, (1988).
25. Stiers, D.L., Brackett, D.J. and Wilson, M.F. Dimethyl sulfoxide protection of endotoxin-induced myocardial injury. In *Proc. of the 46th Ann Mtg. Electron Microscopy Soc. of Amer.*, edited by G.W. Bailey, pp. 320–321, (1988).
26. Stiers, D.L., Brackett, D.J., Schaefer, C.F. and Wilson, M.F. Ultrastructural evidence of progressive myocardial deterioration during endotoxemia. *Circ. Shock,* **24**, 285, (1988).
27. Feldman, D., Cavallo, G. and Rosenberger, L. Electron microscopic study of rat myocardial infarcts induced by a free radical generator system. In *Proceedings of the 45th Annual Meeting of the Electron Microscopy Society of America,* edited by G.W. Bailey, pp. 876–877, San Francisco: San Francisco Press Inc, (1987).
28. Basu, D.K. and Karmazyn, M. Injury to rat hearts produced by an exogenous free radical generating system. Study into the role of arachidonic acid and eicosanoids. *J. Pharmacol. Exp. Therapeu.*, **242**, 673–685, (1987).
29. McCord, J.M. Oxygen derived radicals A link between reperfusion injury and inflammation. *Fed. Proc.*, **46**, 2402–2406, (1987).
30. Simpson, P.J., Mickelson, J.K. and Lucchesi, B.R. Free radical scavengers in myocardial ischemia. *Fed. Proc.*, **46**, 2413–1421, (1987).
31. Simpson, P.J. and Lucchesi, B.R. Free radicals and myocardial ischemia and reperfusion injury. *J. Lab. Clin. Med.*, **110**, 13–30, (1987).
32. Anderson, P.G., Bishop, S.P. and Digerness, S.B. Transmural progression of morphological changes during ischemic contracture and reperfusion in the normal and hypertrophied rat heart. *Am. J. Pathol.*, **129**. 152–167, (1987).
33. Chambers, D.E., Parks, D.A. and Patterson, G. Xanthine oxidase as a source of free radical damage in myocardial ischemia. *J. Mol. Cell. Cardiol.*, **17**, 415–153, (1986).
34. Werns, S.W., Shea, M.J., Mitsos, S.E. *et al.* Reduction of the size of infarction by allopurinol in the ischemic-reperfused canine heart. *Circulation,* **73**, 518–524, (1986).
35. Repine, J.E., Eaton, J.W., Andes, M.W. Haidal, J.R. and Fox, R.B. Generation of hydroxyl radical by enzymes, chemicals, and human phagocytes *in vitro*. Detection with the anti-inflamatory agent, dimethyl sulfoxide *J. Clin. Invest,* **64**, 1642–1651, (1979).

36. Kharasch, N.T. and Thyagarajan, B.S. Structural basis for biological activities of dimethyl sulfoxide. *Ann. N.Y. Acad. Sci.*, **411**, 391–402, (1983).
37. Ashwoood-Smith, M.J. Current concepts concerning radioprotective and cryoprotective properties of dimethyl sulfoxide in cell free systems. *Ann. N.Y. Acad. Sci.*, **243**, 246, (1975).
38. Dorfman, L.M. and Adams, G.E. Reactivity of the hydroxyl radical in aqueous solutions. Washington, D.C.: U.S. Department of Commerce, National Bureau of Standards. NSRDS-NBS Publication No. 46, (1973).
39. Lagercrantz, C. and Forshult, S. Trapping of short-lived free radicals as nitroxide radicals detectable by ESR spectroscopy. The radicals formed in the reaction between OH-radicals and some sulphoxides and sulphones. *Acta Chem. Scand.*, **23**, 811, (1969).
40. Ward, P.A., Till, G.O., Kunkel, R. and Beauchamp, C. Evidence for role of hydroxyl radical in complement and neutrophil dependent tissue injury. *J. Clin. Invest.*, **72**, 789–801, (1983).
41. Beilke, M.A., Collins-Lech, C. and Sohnle, P.G. Effects of dimethyl sulfoxide on the oxidative function of human neutrophils. *J. Lab. Clin. Med.*, **110**, 91–96, (1987).
42. Lai, E.K., Crossley, C., Sridhar, R., Misra, H.P. Janzen, E.G. and McCay, P.B. In vivo spin trapping of free radicals generated in brain, spleen, and liver during radiation in mice. *Arch. Biochem. Biophys.*, **244**, 156–160, (1986).
43. Reinke, L.A. Lai E.K., DuBose, C.M. and McCay, P.B. Reactive free radical generation *in vivo* in heart and liver of ethanol-fed rats. Correlation with radical formation *in vitro*. *Proc. Natl. Acad. Sci.*, **84**, 9223–9227, (1987).
44. Poyer, J.L. and McCay, P.B. In vivo spin trapping of radicals formed during halothane metabolism. *Biochem. Pharmacol.*, **30**, 1517–1519, (1981).
45. McCay, P.B., Lai, E.K. and Poyer, J.L. Oxygen and carbon-centered free radicals formation during carbon tetrachloride metabolism. Observation of lipid radicals in vivo and in vitro. *J. Biol. Chem.*, **259**, 2135–2143, (1984).
46. Bolli, R., Patel, B.S., Jeroudi, M.O., Lai, E.K. and McCay P.B. Demonstration of free radical generation in "stunned" myocardium of intact dogs with use of the spin trap alpha-phenyl-n-tert-butylinitrone.*J. Clin. Invest.*, **82**, 476–485, (1988).
47. McCay, P.B. and Poyer, J.L. General mechanisms of spin trapping in virto and in vivo. CRT Press, (in press).
48. Janzen, E.G., Stronks, H.J., Dubose, C.M., Poyer, J.L. and McCay, P.B. Chemistry and biology of spin-trapping radicals associated with halocarbon metabolism in vitro and in vivo. *Environ. Health Perspect.*, **64**, 151–170, (1985).
49. Takeda, K., Shimada, Y., Amano, M., Sakai T., Okada, T., Sasaki, S. and Yoshiya, I. Lipid peroxidation in septic rats. *Anesthesiology*, **59**, A142, (1983).
50. Peavy, D.L. and Fairchild II, E.J. Evidence for lipid peroxidation in endotoxin poisoned mice. *Infect. Immun.*, **52**, 613–616, (1986).
51. Murai, T., Ogawa, Y., Kawasaki, H. and Kanoh, S. Physiology of the potentiation of lethal endotoxin shock by streptococcal pyrogenic exotoxin in rabbits. *Infect. Immun.*, **55**, 2456–2460, (1987).
52. Yoshikawa, T., Takemura T., Tanigawa, T., Miyagawa, H., Yoshida, N., Sugino, S. and Kondo, M. Role of lipid peroxidation and free radical scavengers on endotoxin shock. *Bioelectrochem. Bioenergetics*, **18**, 295–300, (1987).
53. Feeley, T.W., Minty, B.D., Scudder, C.M., Jones, J.G., Royston, D. and Teng, N.N.H. The effect of human antiendotoxin monoclonal antibodies on endotoxin-induced lung injury in the rat. *Am. Rev. Respir. Dis.*, **135**, 665–670, (1987).
54. Takeda, K., Shimada, Y., Amano, M., Sakai, T. and Yoshiya, I. Lipid peroxidation in critically ill patients. *Anesthesiology*, **57**, A117, (1982).
55. Janzen, E.G. Spin trapping. *Accts, Chem. Res.*, **4**, 31–40, (1971).
56. Janzen, E.G. A critical review of spin trapping in biological systems. In *Free Radicals in Biology Volume 4*,edited by W.A. Pryor, pp. 116–154, New York: Academic Press, (1980).
57. Movat, H.Z. Tumor necrosis factor and interleukin-1: Role in acute inflammation and microvascular injury. *J. Lab. Clin. Med.*, **110**, 668–681, (1987).
58. Movat, H.Z., Cybulsky, M.I., Colditz, I.G., Chan, M.K.W. and Dinarello, C.A. Acute inflammation in gram negative infection: endotoxin, interleukin 1, tumor necrosis factor, and neutrophils. *Fed. Proc*, **46**, 97–104, (1987).
59. Colditz, I.G. and Movat, H.Z. Chemotactic factor-specific desensitization of skin to infiltration by polymorphonuclear leukocytes.*Immunol. Lett.*, **8**, 83–87, (1984).
60. Dinarello, C.A. Interleukin 1 *Rev. Infect. Dis.*, **6**, 51–95, (1984).
61. Grisham, M.B., Everse, J. and Janssen, H.F. Endotoxemia and neutrophil activation in vivo. *Am. J. Physiol.*, **254**, H1017–1022, (1988).

62. Dahinden, C., Galanos, C. and Fehr, J. Granulocyte activation by endotoxin. I. Correlation between adherence and other granulocyte functions, and role of endotoxin structure on biologic activity. *J. Immunol.*, **130**, 857–862, (1983).
63. Weissman, G., Smolen, J.E. and Korchak, H.M. Release of inflammatory mediators from stimulated neutrophils. *N. Engl. J. Med.*, **303**, 27–34, (1980).
64. Babior, B.M. The respiratory burst of phagocytes, *J. Clin. Invest.*, **73**, 599–601, (1984).
65. Green, M.J., Hill, H.A.O. and Tew, D.G. The rate of oxygen consumption and superoxide anion formation by stimulated human neutrophils. *Fed. Eur. Biochem. Soc.*, **216**, 31–34, (1987).
66. Figari, I.S., Mori, N.A. and Palladino, Jr., M.A. Regulation of neutrophil migration and superoxide production by recombinant tumor necrosis factor -α and -β: Comparison to recombinant interferon- and interleukin 1α. *Blood*, **70**, 979–984, (1987).
67. Johnston, R.B. Jr., Guthrie, L.A. and McPhail, L.C. Priming of neutrophils for enhanced oxidative metabolism by bacterial endotoxin. In *Oxy radicals and their scavenger systems. Volume II: Cellular and Medical aspects*, edited by R.A. Greenward and G. Cohen, pp. 69–73, Elsevier Science Publishing Co., (1983).
68. Guthrie, L.A., McPhail, L.C., Henson, P.M. and Johnston, R.B. Jr. Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *J. Exp. Med.*,**160**, 1656–1671, (1984).
69. Cook, J.A., Wise, W.C. and Halushka, P.V. Elevated thromboxane levels in the rat during shock: Protective effects of imidazole, 13-azaprostanoic acid, or essential fatty acid deficiency *J. Clin. Invest.*, **65**, 227–230, (1980).
70. Anderson, F.L., Jubiz, W., Tsgaris, T.J. and Kuida, M. Endotoxin induced prostaglandin E and F release in dogs. *Am. J. Physiol.*, **223**, 410–414, (1975).
71. Bult, H., Beetens, J., Vercrvysse, P. and Herman, A.G. Blood levels of 6-keto-PGF-1α, the stable metabolite of prostacyclin during endotoxin-induced hypotension. *Arch. Int. Pharmacodyn. Ther.* **236**, (1978).
72. Jones, S.B. and Romano, F.D. Plasma catecholamines in the conscious rat during endotoxicosis. *Circ. Shock*, **14**, 189–201, (1984).
73. Nykiel, F. and Glaviano, V.V. Adrenal catecholamines in E. coli endotoxin shock. *J. Appl. Physiol.*, **16**, 348–350, (1961).
74. Kuehl, F.A. Jr., Humes, J.L., Torchiana, M.L., Ham, E.A. and Egan, R.W. Oxygen-centered radicals in inflammatory processes. In *Advances in Inflammation Research*, edited by G. Weissmann, pp. 419–430, New York: Raven Press, (1979).
75. Kuehl, F.A. Jr., Humes, J.L., Ham, E.A., Egan, R.W. and Dougherty, H.W. Inflammation: The role of peroxidase-derived products. In *Advances in Prostaglandin and Thromboxane Research*, edited by B. Samuelsson, P.W. Ramwell, and R. Paoletti, pp. 77–86, New York: Raven Press, (1980).
76. Heikkila, R.E. and Cohen, G. 6-hydroxydopamine: Evidence for superoxide radical as an oxidative intermediate *Science*, **181**, 546–457, (1973).
77. Misra, H.P. and Fridovich, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, **247**, 3170–3175, (1972).
78. Cohen, G. and Heikkila, R.E. The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6 hydroxydopamine, dialuric acid, and related cytotoxic agents. *J. Biol. Chem.*, **249**, 2447–2452, (1974).
79. Graham, D.G. Tiffany, S.M., Bell, W.R. Jr. and Gutknecht, W.F. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6 hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro *Mol. Pharmacol.* **14**, 644–653, (1978).
80. Sachs, C. and Jonsson, G. Mechanisms of action of 6 hydroxydopamine *Biochem. Pharmacol.*, **24**, 1–8, (1975).
81. Demopoulos, H.B., Flamm, E.S., Seligman, M.L., Mitamura, J.A. and Ransohoff, J. Membrane perturbations in central nervous system injury: Theoretical basis for free radical damage and a review of the experimental data. In *Neural Trauma*,edited by A.J. Popp, R.S. Bourke, L.R. Nelson, and H.K. Kemelberg, pp. 63–67, New York: Reven Press, (1979).
82. Ruzicka, F.J., Beinert, H., Schepler, K.L., Dunham, W.R. and Sands, R.H. Interaction of ubisemiquinone with a paramagnetic component in heart tissue. *Proc. Natl. Acad. Sci. USA*, **72**, 2886–2890, (1975).
83. Fridovich, I. The biology of oxygen radicals. *Science*, **201**, 875–880, (1978).
84. Kellog, E.W. and Fridovich, I. Liposome oxidation and erythrocyte lysis by enzymatically generated superoxide radical and hydrogen peroxide. *J. Biol. Chem.*, **252**, 6721–6727, (1977).
85. Demopoulos, H.B. The basis of free radical pathology. *Fed. Proc.*, **32**, 1859–1869, (1973).
86. Lumsden, A.B., Henderson, J.M. and Kutner, M.H. Endotoxin levels measured by a chromogenic

assay in portal, hepatic and peripheral venous blood in patients with cirrhosis. *Hepatology*, **8**, 232–236, (1988).
87. Rush, B.F. Jr., Sori, A.J., Murphy, T.F., Smith, S., Flanagan, J.J. and Machiedo, G.W. Endotoxemia and bacteremia during hemorrhagic shock. The link between trauma and sepsis *Ann. Surg.*, **207**, 549–554, (1988).
88. Gathiram, P., Gaffin, S.L., Brock-Utne, J.G. and Wells, M.T. Time course of endotoxemia and cardiovascular changes in heat-stressed primates. *Aviat. Space Environ. Med.*, **58**, 1071–1074, (1987).
89. Bosenberg, A.T., Brock-Utne, J.G., Gaffin, S.L., Wells M.T.B. and Blake, G.T.W. Strenuous exercise causes systemic endotoxomia. *J. Appl. Physiol.*, **65**,106–108, (1988).
90. Gaffin, S.L., Brock-Utne, J.G., Zanotti, A. and Wells, M.T. Hypoxia-induced endotoxemia in primates: role of reticuloendothelial system function and anti-lipopolysaccharide plasma. *Aviat. Space Environ. Med.*, **57**, 1044–1049, (1986).
91. Gathiran, P., Gaffin, S.L., Wells, M.T. and Brock-Utne, J.G. Superior mesenteric artery occlusion shock in cats: Modification of the endotoxemia by antilipopolysaccharade antibodies (Anti LPS). *Circ. Shock*, **19**, 231–237, (1986).
92. Zanotti, A.M. and Gaffin, S.L. Prophylaxis of superior mesenteric artery occlusion shock in rabbits by antilipopolysaccharide (Anti LPS) antibodies. *J. Surg. Res.*, **38**, 113–115, (1985).

Accepted by Prof. T.F. Slater