Antonie van Leeuwenhoek **63**: 77–83, 1993.

Effect of bacterial lipopolysaccharide on the content of lipid peroxidation products in lungs and other organs of mice

Dariusz Nowak, Tadeusz Pietras, Adam Antczak, Maciej Król & Grazyna Piasecka
Department of Pneumonology and Allergology, Medical University of Lódź, Kopcińskiego 22, 90–153, Poland

Received 21 July 1992; accepted in revised form 4 December 1992

Key words: lipopolysaccharide, endotoxin, lipid peroxidation, conjugated dienes, malondialdehyde, endotoxic shock

Abstract

The influence of lipopolysaccharide from *Escherichia coli* (LPS, 17 mg/kg body weight) on the lipid peroxidation process in organs of mice was studied. The content of conjugated dienes (CD), lipid peroxides (LP), malondialdehyde (MDA) (all three lipid peroxidation by-products), peroxidase (PO) activity and wet-to-dry weight ratio in lungs, heart, spleen, kidneys and liver were determined 1.5 h after intravenous injection of LPS. Animals observed at this time-point had reduced activity and decreased body temperature by about 2° C, however, all analysed organs did not reveal any changes of wet-to-dry weight ratio comparing to organs from mice injected with sterile, pyrogen free 0,9% NaCl. Only extracts from heart and lungs showed significant increase in the tissue level of at least two lipid peroxidation products. The heart content of CD, MDA, and LP was about 1.5-, 1.3-, and 2.4-fold higher than in control group. In lungs CD and MDA increased 3.3- and 1.3-times but in spleen only content of LP was elevated. In these organs the suppression of PO activity was also observed. Liver and kidneys did not reveal any convincing enhancement of lipid peroxidation process and alterations of PO activity. Since free radical reactions are involved in lipid peroxidation process and inactivation of PO these results suggest that heart, lungs and spleen are the organs mostly exposed to oxidative stress during the first 1.5 h after single injection of LPS in mice.

Abbreviations: CD – conjugated dienes; LP – lipid peroxides; LPS – lipopolysaccharide; MDA – malondialdehyde; PMNL – polymorphonuclear leukocytes; PO – peroxidase; TBA – thiobarbituric acid

Introduction

Lipopolysaccharide components (LPS) of Gram-negative bacteria (endotoxins) have been implicated in the pathogenesis of a wide variety of human and animal disorders. In particular, endotoxins can cause lung injury that consists of sequestration of polymorphonuclear leukocytes (PMNL) in the pulmonary microvasculature, damage of the capillary endothelium and enhanced plasma protein extravasation into the pulmonary interstitium (Brigham et al. 1986). Clinically it is called the adult respiratory distress syndrome (ARDS) and is characterised by diffuse bilateral pulmonary infiltrates, decreased lung compliance and hypoxemia. There are many pieces of evidence that oxygen radicals released from PMNL and subsequent lipid peroxidation are involved in the development of the endotoxin-induced lung injury (Demling et al. 1989; Ishizaka et al. 1988; Wong et al. 1984). LPS has been shown to

cause the release of oxygen radicals from PMNL and to enhance their adhesion to endothelium (Dahinden et al. 1983a; Dahinden et al. 1983b). LPS may also activate complement with subsequent generation of C5a, which in turn activates PMNL (Wilson 1985). In addition, tumour necrosis factor released from LPS-stimulated macrophages and monocytes, enhances degranulation, adherence and oxidative respiratory burst of PMNL (Gamble et al. 1985; Shalaby et al. 1987). These events lead to the enhanced lung lipid peroxidation which is manifested by increased pulmonary content of malondialdehyde (MDA) and conjugated dienes (CD) and correlates with the degree of increased plasma protein extravasation and of increased lung water after infusion of endotoxin (Demling et al. 1989; Ishizaka et al. 1988). Little is known, however, about lipid peroxidation in other organs during endotoxemia. Mizer and co-workers using phorbol ester-induced lung injury model in dogs reported accumulation of PMNL not only in lungs but also in other organs such as heart, liver and brain (Mizer et al. 1989). It suggests that enhanced lipid peroxidation may occur in these organs. On the other hand, systemic activation of complement system in rats produced acute pulmonary damage with increased content of CD in lung tissue, however, the rate of lipid peroxidation in other organs (liver, kidney, spleen) was unaffected (Ward et al. 1985). Taking above into consideration it remains unclear if endotoxin-induced lipid peroxidation is only limited to lung tissue or occurs in other organs. The aim of this study was to compare the lipid peroxidation in lungs and other organs (heart, kidneys, liver, spleen) in mice after single intravenous injection of LPS from Escherichia coli. By measuring the tissue level of CD, lipid peroxides (LP) and MDA we found that LPS at a dose that did not change the organ wet-to-dry weight ratio caused the highest increase in the lipid peroxidation in heart and lungs.

Material and methods

Reagents

Lipopolysaccharide (LPS) from *Escherichia coli* was from Difco Laboratories (Detroit USA). Chloroform (for spectroscopy), heptane, pyridine, sodium dodecyl sulphate and thiobarbituric acid (TBA) were from Ubichem (England). Phenylmethylsulfonylfluoride (PMSF), peroxidase from horseradish type II (200 U/mg solid) and bovine serum albumin were from Sigma (St. Louis MO, USA). Butylated hydroxytoluene was from Aldrich (USA). All other reagents were obtained from POCH (Poland). LPS was dissolved in sterile pyrogen-free 0.9% NaCl to a concentration 2.5 mg/ml just before the intravenous injection.

Experimental protocol

Male mice Balb/c, weighing 26 to 31 g, 5–7 weeks of age were maintained in temperature-controlled room (18–21° C) and allowed free access to food and water. Animals were injected into a tail vein with LPS (17 mg/kg body weight) or with 200 µl 0.9% NaCl and were sacrificed by vertebral dislocation 1.5 h later. The thoracic and abdominal cavities were opened and lungs heart, liver, spleen and kidneys were excised. All organs were immediately washed with ice-cold phosphate buffered saline (PBS pH 7.4), then desiccated with lignin and stored (not more than 6 days) at –20° C under nitrogen until assay of lipid peroxidation products.

Lipid peroxidation products

Quantitative estimation of products of lipid peroxidation included assays for CD, LP, and MDA. The procedural details are described elsewhere (Buege et al. 1978; Ohakawa et al. 1976). Conjugated dienes – briefly, 250 mg of wet tissue was homogenized on ice with 1 ml of distilled water and then 0.5 ml of this homogenate was mixed with 7 ml of chloroform-methanol (1: 2 vol./vol.), shaked for 2 min and centrifuged (1500× g, 5 min). 5 ml of the lower (chloroform) layer was mixed with 2 ml of distilled water acidified with 0.1 N HCl to pH 2.5. The mixture was again shaked for 2 min and centrifuged as above. The chloroform layer was aspirated and dried under a flow of nitrogen gas. The residue was reconsti-

tuted with 1 ml of heptane and its absorbance was read against a heptane blank at 233 nm.

Malondialdehyde – weighed 250 mg portions of defrosted organs were homogenized on ice with 2 ml of 1.15% KCl and mixed with 4 ml of 0.25 N HCl containing 0.375%, wt/vol. TBA, 15%, wt/vol. trichloroacetic acid and 0.015%, wt/vol. butylated hydroxytoluene. After incubation at 100° C for 20 min, samples were centrifigued (1500× g, 5 min) and the absorbance of the supernatant was measured at 532 nm. An extinction coefficient of $1.56 \times 10^5 M^{-1}cm^{-1}$ was used to calculate micromoles of MDA per gram of wet organ. Lipid peroxides – 250 mg portions of wet organs were homogenized on ice with 2.25 ml redistilled water and then 0.1 ml of this homogenate was mixed with 0.1 ml 8.1% sodium dodecyl sulphate, 0.75 ml 20% acetic acid (pH 3.5), 0.75 ml 0.8% TBA and 0.3 ml distilled water. After 60 min incubation at 100° C, samples were cooled to room temperature, mixed with 2.5 ml of n-buthanol-pyridine (15: 1 vol./vol.) and centrifuged as above and absorbance of organic layer was measured against a blank containing PBS instead of homogenate at 532 nm.

Lung lavage

The lungs were lavaged with 2× 1 ml phosphate buffered saline (PBS pH 7.4). The lavage fluids were centrifuged (400× g, 10 min at 4° C) and the cell pellet was resuspended in 200 µl PBS and counted in haemocytometer. Differential cell counts were performed on smears prepared from each sample using Giemsa stain.

Other techniques

Body temperature of animals was measured with thermistor thermometer (type Pu 391/1 Czechoslovakia). The wet-to-dry weight ratio of organs was calculated after weighing the freshly harvested organ, incubating at 60° C in a gravity convection oven for 96 h, and weighing the residuum, the weight of which was then constant. Total protein concentration and peroxidase (PO) activity were determined by the method of Bradford (Bradford 1976) and Henson (Henson et al. 1978) with some modifications (Nowak 1990) using bovine serum albumin and peroxidase from horseradish as standards, respectively. For these assays 1 g of wet organ was homogenized in 2 ml of ice cold PBS containing 0.3% Triton X-100 and 1 mM PMSF.

Statistical analysis

Results are expressed as the mean value ± the standard deviation. Individual protein and PO determinations were carried out on duplicates. Statistical significance was determined by Student t test. Changes in the body temperature were analysed using paired t-statistic.

Results

Preliminary experiments showed that after injection of LPS (17 mg/kg body weight) all mice had reduced activity and adopted a hunched posture. The body temperature declined by about 2° C (Table 1) and animals became lethargic. These changes in animal behaviour were already visible 1.5 h after LPS injection. The decrease in body temperature persisted for several hours and then diarrhoea occurred in all observed animals (n= 14). No deaths, however, were noted within 24 h of giving LPS. The dose of LPS as above was used in further experiments and the time-point 1.5 h was chosen for studying the changes in the lipid peroxidation in organs with reference to first visible alterations in animal behaviour after injection of endotoxin. All organs analysed at this time-point revealed no differences in wet-to-dry weight ratio comparing to organs of mice injected with 0.9% NaCl (Table 2). Similarly, total protein concentration 91± 25 µg/ml (n= 7) found in lung washings of mice treated with LPS did not differ from value 100± 39 µg/ml (n= 8) obtained in control group. It suggests that severe endothelial injury and increased vascular permeability did not develop during 1.5 h after LPS injection. Table 3 and 4 show the content of CD, MDA and LP in lungs, liver, spleen, heart and kidneys of mice after intra-

Table 1. Effect of LPS on body temperature of mice.

Treatment	Body temperature [°C]		
	A	B	p<
NaCl	36.5± 0.8	36.6± 1.1	NS
LPS	36.4± 1.0	34.3± 1.2	0,001

Mice were injected intravenously with 200µl 0.9% NaCl (n = 7) or LPS, 17mg/kg body weight (n = 14). Body temperature was measured in rectum before (A) and 1.5h after treatment (B).

Table 2. Wet-to-dry weight ratio in organs after injection of LPS.

Organ	Wet-to-dry weight ratio	
	A	B
Lungs	4.05± 0.11	3.99± 0.29
Heart	3.45± 0.11	3.59± 0.42
Liver	3.27± 0.15	3.24± 0.33
Spleen	3.42± 0.10	3.40± 0.53
Kidneys	3.62± 0.14	3.56± 0.24

Mice (n = 14) were injected intravenously with 200µl 0.9% NaCl (A) or LPS 17mg/kg body weight (B) and then after 1.5h organs were removed for determination the wet-to-dry weight ratio by gravimetric studies.

venous injection of LPS. Only extracts from heart showed significant increase in the tissue level of all measured lipid peroxidation by-products. The amount of CD, MDA and LP was about 1.5-, 1.3- and 2.4-fold higher in comparison with values obtained in animals treated with 0.9% NaCl. In lungs LPS induced 3.3- and 1.3-fold increase in CD and MDA while the content of LP was 2.2-times lower than that in control group. Other organs like liver, kidneys, spleen did not reveal any increase in CD. Moreover, it was significantly decreased in liver and kidneys after treatment with LPS. The content of MDA and LP was only increased in liver and spleen, respectively. Injection of LPS did not induce the increase in PO activity in organs, especially in lungs and lung washings (Table 5). Quite the reverse, in heart and spleen this activity was significantly lower than after injection of 0.9% NaCl. However, organs with relatively low basal PO activity (liver, kidneys) had no changes in this activity after LPS administration. Table 6 shows the 2.5-fold increase ($p < 0.001$) in the total number of cells in lung washings after injection of LPS. This increase was mainly due to the large influx of PMNL to the bronchoalveolar lining fluid. The absolute PMNL count of the lung washings increased 57-fold from $2 \pm 1 \times 10^3$ in the control group to $114 \pm 18 \times 10^3$ in the LPS group. PMNL accounted for only 2.5% of the cells in lavage fluid, whereas in mice treated with LPS, this increased 23-fold to 57%. The number of both lymphocytes and macrophages was also higher in LPS group, although this increase was not significant.

Discussion

Peroxidation of polyunsaturated fatty acids is a multistep process. The first initial step consists of hydrogen abstraction resulting in the formation of CD. The CD is a precursor of LP which then converts into lipid endoperoxide. Lipid endoperoxide can react with various compounds and finally it

Table 3. Effect of LPS on the content of conjugated dienes and malondialdehyde in organs of mice.

Organ	Conjugated dienes*			Malondialdehyde**		
	A	B	p<	A	B	p<
Lungs	0.12± 0.03	0.40± 0.04	0.001	5.6± 1.2	7.4± 1.3	0.05
Heart	0.29± 0.05	0.43± 0.04	0.001	4.9± 0.6	6.3± 0.9	0.02
Liver	0.39± 0.04	0.21± 0.04	0.001	4.1± 0.7	5.9± 0.4	0.001
Kidneys	0.47± 0.04	0.35± 0.07	0.01	5.6± 0.7	8.3± 3.2	NS
Spleen	0.35± 0.04	0.32± 0.03	NS	8.9± 0.9	9.0± 0.9	NS

Animals were injected with 0.9% NaCl (A) or LPS (B). Other details as for Table 2. *, ** Expressed as absorbance readings at 233nm and micromoles per gram of wet organ, respectively. Each value represents mean ±SD of at least 6 studies.

Table 4. Effect of LPS on the content of lipid peroxides in organs of mice.

Organ	Lipid peroxides*		
	A	B	p<
Lungs	0.22± 0.05	0.10± 0.03	0.001
Heart	0.13± 0.06	0.31± 0.07	0.001
Liver	0.11± 0.06	0.14± 0.03	NS
Kidneys	0.26± 0.07	0.24± 0.09	NS
Spleen	0.17± 0.05	0.26± 0.07	0.05

Animals were injected with 0.9% NaCl (A) or LPS (B). Other details as for Table 2. *Expressed as absorbance readings at 532nm per 0.25 gram of wet organ, respectively. Each value represents mean ±SD of at least 7 studies.

Table 5. Effect of LPS on peroxidase activity in organs of mice.

Organ	Peroxidase activity*		
	A	B	p<
Heart	98.6± 10.2	52.4± 16.7	0.001
Liver	5.6± 1.1	6.7± 1.6	NS
Kidneys	24.5± 4.5	29.2± 9.3	NS
Spleen	73.2± 11.7	39.2± 20.8	0.02
Lungs	104.8± 27.6	73.2± 19.6	NS
Lung washings	14.8± 8.3	7.8± 6.5	NS

Animals were injected with 0.9% NaCl (A) or LPS (B). Other details as for Table 2. Results obtained from at least 5 studies. *Expressed in μU per μg protein of organ homogenate.

forms lipid hydroperoxide or MDA (Janero 1990; Ward et al. 1985). In this study we analysed changes in the tissue content of two first (CD, LP) and the one last (MDA) by-products of lipid peroxidation after injection of sublethal dose of LPS in mice. The obtained data suggest that LPS mostly enhances lipid peroxidation in heart, lungs and to some extent in spleen. Other organs, liver and kidneys did not reveal any convincing increase in the content of products of lipid peroxidation. If destruction of membrane lipids precedes the development of organ injury, our findings agree with opinion that endotoxemia apart from shock mainly induces severe lung injury (Brigham et al. 1986). On the other hand, one of the common symptoms observed in septic patients are reduced blood pressure and tachycardia that suggest the presence of heart dysfunction (Wendel 1991). In addition, sepsis produced by acute peritonitis in dogs caused transient heart insufficiency associated with myocardial PMNL infiltration and microcirculatory damage (Natanson et al. 1986). It should be noted, however, that our results are dependent not only on direct induction of lipid peroxidation but also on the balance between creation and interconversion of individual lipid peroxidation by-products. Thus the decline in lung level of LP may be a result of faster conversion of LP into MDA than conversion of CD into LP. The decrease of CD in liver and kidneys is difficult to explain. Other authors, however, also observed moderate decrease in CD in these organs after systemic complement activation in rats (Ward et al. 1985). It can not be excluded that some lipid peroxidation by-products were washed out with blood stream. Nevertheless, it seems that in our animal model LPS almost selectively induces enhanced lipid peroxidation in heart and lungs. The increased level of CD or MDA was reported to correlate with the severity of lung damage expressed as increased plasma protein leakage and tissue water (Demling et al. 1989; Ishizaka et al. 1988; Ward et al. 1985). In our animal model the increase in lung content of CD and MDA was similar or even higher than these found in studies cited above, however, no changes of lung water and protein concentration in

Table 6. Effect of LPS on number of cells found in lung washings.

Agent	Total cells	Macrophages	Lymphocytes	PMNL ($\times 10^3$)
NaCl (8)	79± 56	49± 16	26± 11	2± 1
LPS (7)	200± 43	56± 12	32± 8	114± 18

PMNL – polymorphonuclear leukocytes. Animals were injected with 0.9% NaCl or LPS. Other details as for Table 2. In parentheses number of mice in each group.

BAL fluids related to pulmonary damage were observed. Similarly the wet-to-dry weight ratio of heart was also unchanged. One explanation for this discrepancy could be that we analysed lungs at time in which LPS-induced migration of leukocytes from capillary lumens to interstitium (as expressed by increased number of PMNL in lung washings) preceded the increase in pulmonary vascular permeability (Brigham et al. 1979; Meyrick et al. 1983). PMNL are rich source of myeloperoxidase (MPO) a PO present in their azurophil granules (Rice et al. 1986). Some observations indicate the positive correlation between MPO activity in tissue, especially in lung tissue and the presence of PMNL influx (Goldblum et al. 1985; Welbourn et al. 1991). In our study we did not observe the increase in PO activity in all analysed organ homogenates and lung washings, which could be a result of PMNL influx and subsequent MPO burden. In opposite, the PO activity of heart and spleen homogenate from LPS-treated mice was significantly lower than that of one prepared from control animals. Even lungs and lung washings in which the number of PMNL increased several dozen times also revealed the 1.4- and 1.9-fold decrease in mean PO activity, however, probably due to the large variability it was not significant. MPO and other peroxidases could be inactivated by hypochlorous acid and H_2O_2 (Andrews et al. 1982; Van Zyl et al. 1990). In addition PMNL can lose about half of their MPO activity during ingestion of microorganism (Bradley et al. 1982) which is linked with increased production of reactive oxygen species (Babior 1984). It should be noted that the decrease in PO activity was found in organs that revealed enhanced content of products of lipid peroxidation. It additionally supports our conclusion that apart from lungs, heart and spleen are the organs mostly exposed to oxidative stress leading to lipid peroxidation during the first 1.5 h after single injection of LPS in mice.

Acknowledgement

This work was supported by grants 502 and 503 from Medical University of Lódź.

References

Andrews PC & Krinsky N (1982) A kinetic analysis of the interaction of human myeloperoxidase with hydrogen peroxide, chloride ions and protons. J. Biol. Chem. 257: 13240–13245

Babior BM (1984) Oxidants from phagocytes: agents of defence and destruction. Blood 64: 959–966

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254

Bradley PP, Christensen RD & Rothstein G (1982) Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood 60: 618–622

Brigham KL & Meyrick B (1986) Endotoxin and lung injury. Am. Rev. Respir. Dis. 133: 913–927

Brigham KL, Bowers R & Haynes J (1979) Increased sheep lung vascular permeability caused by E. coli endotoxin. Circ. Res. 45: 292–297

Buege JA & Aust SD (1978) Microsomal lipid peroxidation. In: Fleischer S, Packer L (Eds) Methods in Enzymology, Vol 52 (pp 303–310). Academic Press, New York

Dahinden C & Fehr J (1983b) Granulocyte activation by endotoxin II: Role of granulocyte adherence, aggregation, and effect of cytochalasin B, and comparison with formylated chemotactic-peptide-induced stimulation. J. Immunol. 130: 863–867

Dahinden C, Galanos C & Fehr J (1983a) Granulocyte activation by endotoxin I: correlation between adherence and other granulocyte function, and role of endotoxin structure on biologic activity. J. Immunol. 130: 857–862

Demling R & LaLonde C (1989) Relationship between lung injury and lung lipid peroxidation caused by recurrent endotoxemia. Am. Rev. Respir. Dis. 139: 1118–1124

Gamble JR, Harlan JM, Klebanoff SJ & Vadas MA (1985) Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumour necrosis factor. Proc. Natl. Acad. Sci. USA 82: 8667–8671

Goldblum SE, Wu KM & Jay M (1985) Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbits. J. Appl. Physiol. 59: 1978–1985

Henson PM, Zanolari B, Shwartzman NA & Hong SR (1978) Intracellular control of human neutrophil secretion I. C5a-induced stimulus-specific desensitisation and the effects of cytochalasin B. J. Immunol. 121: 851–855

Ishizaka A, Stephens KE, Tazelaar HD, Hall EW, O'Hanley P & Raffin T (1988) Pulmonary edema after Escherichia coli peritonitis correlates with thiobarbituric-acid-reactive materials in bronchoalveolar lavage fluid. Am. Rev. Respir. Dis. 137: 783–789

Janero DR (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9: 515–540

Meyrick B & Brigham KL (1983) Acute effects of E. coli endotoxin on the pulmonary microcirculation of anesthetised sheep: structure, function, relationships. Lab. Invest. 48: 458–470

Mizer LM, Weisbrode SE & Dorinsky PM (1989) Neutrophil accumulation and structural changes in nonpulmonary organs after acute lung injury induced by phorbol myristate acetate. Am. Rev. Respir. Dis. 139: 1017–1026

Natanson C, Cunnion RE & Barrett DA (1986) Reversible myocardial dysfunction in a canine model of septic shock is associated with myocardial microcirculatory damage and focal neutrophil infiltration. Clin. Res. 34: 639A

Nowak D (1990) Hydrogen peroxide release from human polymorphonuclear leukocytes measured with horseradish peroxidase and o-dianisidine. Effect of various stimulators and cytochalasin B. Biomed. Biochim. Acta 49: 353–362

Ohakawa H, Ohishi O & Yagi K (1976) Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351–358

Rice WG, Kinkade JM & Parmley RT (1986) High resolution of heterogeneity among human neutrophil granules: physical, biochemical and ultrastructural properties of isolated fractions. Blood 68: 541–555

Shalaby MR, Palladino MA, Hirabayashi SE, Eessalu TE, Lewis GD, Shepard HM & Aggarwal BB (1987) Receptor binding and activation of polymorphonuclear neutrophils by tumour necrosis factor-alpha. J. Leukoc. Biol. 41: 196–204

Van Zyl JM, Basson K & Van der Valt BJ (1990) The oxyferrous adduct of myeloperoxidase (compound III) and its reactivity with chloride. S. Afr. J. Sci. 86: 199–203

Ward PA, Till GO, Hatherill JR, Annesley TM & Kunkel RG (1985) Systemic complement activation, lung injury and products of lipid peroxidation. J. Clin. Invest. 76: 517–527

Welbourn CRB, Goldman G, Paterson IS, Valeri CR, Shepro D & Hechtman HB (1991) Neutrophil elastase and oxygen radicals: synergism in lung injury after hindlimb ischemia. Am. J. Physiol. 260: H1852–H1856

Wendel A (1991) Reactive oxygen species, antiproteases and cytikones in sepsis. Klin. Wochenschr. 69: 969–974

Wilson ME (1985) Effects of bacterial endotoxins on neutrophil function. Rev. Infect. Dis. 7: 404–418

Wong C, Flynn J & Demling RH (1984) Role of oxygen radicals in endotoxin-induced lung injury. Arch. Surg. 119: 77–82