

## THE PATHOGENESIS OF INFLAMMATORY DISEASE: SURGICAL SHOCK AND MULTIPLE SYSTEM ORGAN FAILURE

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### ABSTRACT

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Chronic inflammatory disease, embracing rheumatoid arthritis (RA), inflammatory bowel disease (IBD), hepatitis, asthma, atherosclerosis, multiple system organ failure (MSOF), etc., is mediated by reactive oxygen species (ROS). These ROS originate from activated neutrophils in infections and in immune and autoimmune reactions, from tissue deposits of ferritin, and from futile cycling of cytochrome P450 (CYP) following exposure to persistent chemicals, and may be perpetuated by the actions of complement, cytokines and eicosanoids. Acute inflammation is normally arrested by removal of ROS by tissue glutathione (GSH) and the antioxidant vitamins, A, C and E, all of which are regenerated by NADH and NADPH. Failure of this antioxidant defence system can lead to oxidative stress and to chronic inflammatory disease, including surgical shock and MSOF. The roles of oxidative stress and microcirculatory arrest in promoting MSOF, and of GSH, the antioxidant defence system, and fibronectin in preventing this, are reviewed in the light of recent experimental studies of surgical shock, including fasting, anaesthesia, hepatic ischaemia and reperfusion.

*Keywords:* chronic inflammatory disease, surgical shock, oxygen radicals, multiple system organ failure, rheumatoid arthritis, glutathione, leucocytes, cytokines, cytochromes P450 and toxic chemicals, eicosanoids, fibronectin and microcirculatory arrest, complement

### INTRODUCTION

Inflammation is a valuable defence mechanism, an aspect of the immune cascade which protects living animals against infections from invading organisms, antigens and foreign bodies. It involves vasodilatation, increased vascular permeability, tissue oedema, leucocyte migration and activation, leucocyte production of reactive oxygen species (ROS) [1] and release of lysosomal enzymes, the release of cytokines, and the formation of eicosanoids. Inflammation can progress from an acute, episodic phenomenon to a self-perpetuating chronic condition. The ultimate products of inflammation are ROS, which can have a cascade effect on the process, thereby augmenting and extending the phenomenon (Figure 1). Hence, the production of ROS by other mechanisms, e.g. redox cycling, futile cycling by the cytochromes P450 (Figure 2), prostanoid biosynthesis, and iron-promoted ROS damage [1], may similarly augment, or even initiate, inflammation.

ROS-mediated inflammation is well-known to be involved in the pathogenesis of infectious disease, including pneumonic plague and septic shock [2], in immune and autoimmune diseases, such as rheumatoid arthritis (RA) [1,3] and inflammatory

bowel disease (IBD) [4], and although its role is less well established, in diseases such as cancer [5-7], atherosclerosis [6,8,9], hepatitis [3,10], Alzheimer's dementia [11], AIDS [12], multiple system organ failure (MSOF) [13,14], and asthma and adult respiratory distress syndrome (ARDS) [15].

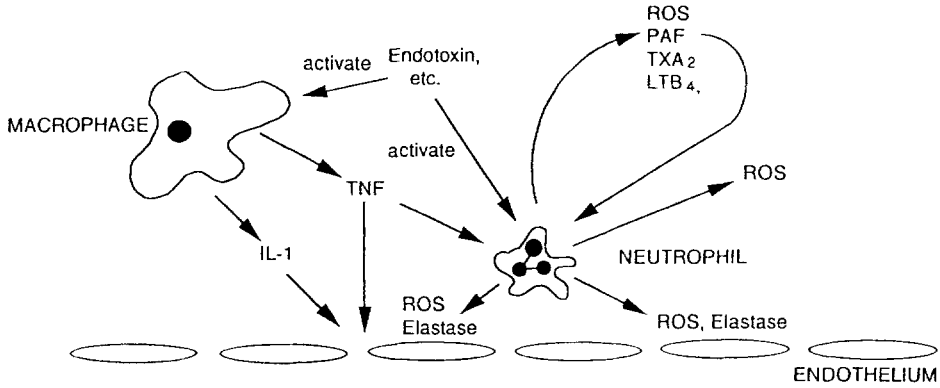


Figure 1. Activation of neutrophils and the cascade effect of ROS in inflammation. Endotoxin, bacteria, necrotic tissue, etc. activate neutrophils which adhere to endothelial cells, and activate macrophages to release IL-1 and TNF- $\alpha$ . The activated neutrophils release ROS and lysosomal enzymes (elastase), together with PAF, TXA<sub>2</sub>, and leukotrienes (LTB<sub>4</sub>, etc.) which, in turn, activate other neutrophils

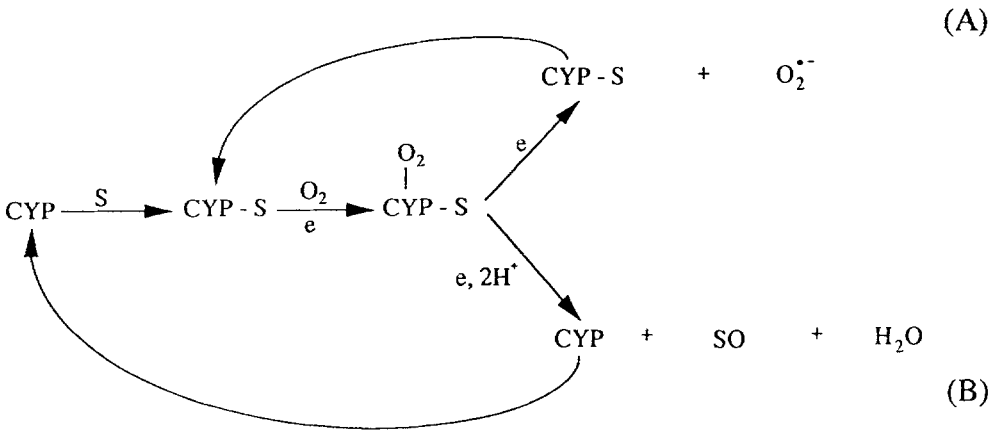


Figure 2. Futile cycling of cytochromes P450. Cytochrome P450 (CYP) interacts with a substrate (S) to give an enzyme-substrate complex, then with O<sub>2</sub> and an electron to give the oxygen-enzyme-substrate complex. If the substrate is readily oxygenated, this complex breaks down by pathway (B) to yield the oxygenated substrate and water, and regenerates the enzyme (mixed-function oxidation). If, however, the substrate is not readily oxygenated, the complex breaks down by pathway (A) to yield superoxide radical and the enzyme-substrate complex, and the process of reduction of O<sub>2</sub> to O<sub>2</sub><sup>•-</sup> continues cyclically via the oxygen-enzyme-substrate complex (futile cycling). From Reference [79] modified

## PATHOGENESIS OF INFLAMMATION

Over the past century, the complex mechanisms of the interactive multicomponent defence system, which initiates and maintains inflammation, have been the subject of numerous research investigations. Presumably, a primary objective has been to identify a critical mechanism that might be exploited to provide a treatment or prophylaxis. One component after another has been considered to be the critical self-promoting factor that prolongs the protective benefits of acute inflammation into the degenerative, chronic inflammatory disease states, such as RA, IBD, MSOF, etc. These have ranged from infecting micro-organisms [16] to complement [17], prostanoids [18], iron complexes [1], loss of glutathione [19], or energy (NADPH) to recharge the antioxidant defence mechanism [20]. This present review aspires to update this perplexing problem, and introduces additional new factors, namely the specific oxygen-activating enzymes, the cytochromes P450, which have been shown to be involved in oxidative stress, inflammation, tissue necrosis, surgical shock and MSOF [20].

### *Reactive oxygen species*

These comprise  $O_2^{\cdot-}$ , the superoxide ion radical;  $O_2^{2-}$ , the peroxide anion;  $\dot{O}H$ , the hydroxyl radical;  $O_2^1$ , singlet oxygen; and  $OCl^-$ , the hypochlorite ion, all of which are cytotoxic and are involved in the destruction of invading micro-organisms [9]. They are formed by one-electron reduction of molecular oxygen, and are produced by neutrophils and macrophages for the specific defensive role of microbial destruction, but are formed continuously in other tissues by a variety of metabolic processes, including electron leakage from mitochondrial membranes, transoxygenation in the biosynthesis of the eicosanoids, oxygen activation by the cytochromes P450 in anoxia, the action of xanthine oxidase in anoxia, futile cycling of cytochrome P450E1, and the redox cycling of quinones and carbon-centred radicals [21–23] (Figure 3). ROS have also been shown to be involved in the activation of carcinogens, the formation of neoantigens [3], DNA strand cleavage [24], and the initiation and promotion of carcinogenesis [25]. ROS may promote and extend inflammation by chemotactic attraction of neutrophils, and their activation to generate ROS, and by contributing to eicosanoid formation [22]; ROS also contribute to the regulation of prostacyclin ( $PGI_2$ ) synthesis and release during acute haemorrhage [26]. Biological systems are protected from the damaging effects of ROS by the antioxidant defence system (Figure 3), a complex integrated array of enzymes, antioxidants and radical scavengers, dependent largely on the intracellular redox buffer, glutathione (GSH), and involving GSH reductase, GSH peroxidase (GPx), phospholipid hydroperoxide GSH peroxidase (PHGPx) [27], superoxide dismutase (SOD), and catalase, the tocopherols (vitamin E), ascorbic acid (vitamin C), and retinoids (vitamin A) [27].  $\beta$ -Carotene and  $\alpha$ -tocopherol act synergistically, thereby enhancing the radical-trapping activity of vitamin E [28]. Retinoids are involved in limiting inflammation, inhibiting neutrophil generation of ROS, but with little effect on the xanthine oxidase system, and have been used in the treatment of inflammatory skin disease [29].

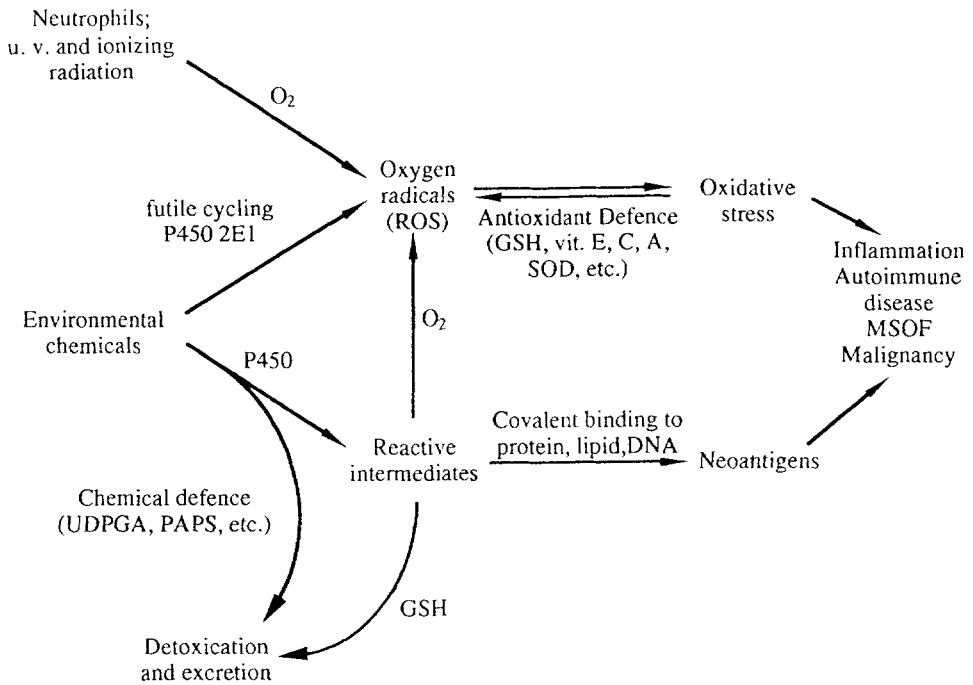


Figure 3. Oxygen radical toxicity and the antioxidant defence system. ROS are formed by the reduction of molecular oxygen, by radiation, CYP2E1, or cytochrome P450 futile cycling. Antioxidant defence depends largely on radical scavengers, including GSH, tocopherols (vit. E), ascorbate (vit. C), retinoids (vit. A), and on antioxidant enzymes, such as SOD, GPX, etc. Chemical defence depends largely on uridine diphosphate glucuronyl (UDPGA) and phosphoadenosine phosphosulphate (PAPS) transferases. Chemicals may also be metabolized to give rise to reactive intermediates, which bind to proteins to give neoantigens, or to DNA to initiate malignancy

Haem iron may also generate ROS, and may amplify and prolong the inflammatory reaction [30]. It has been postulated that, in RA, iron is released from accumulated ferritin deposits in inflamed joints, leading to the iron-catalysed production of ROS, and the consequent processes of chronic inflammation [1].

The free radical nitric oxide (NO) is involved in a wide range of physiological processes including vasodilatation; it is identical to endothelium-derived relaxing factor (EDRF) and contributes to the cardinal signs of inflammation [31]. It is an important cytotoxic molecule in the defence against malignant cells, fungi, protozoa, helminths and mycobacteria, but not against extracellular bacteria [32]. It is a neurotransmitter and neuromodulator in the central and peripheral nervous systems, and has a regulatory function on the immune system [31]. NO is generated from L-arginine by NO synthase; it inhibits osteoclast activity and NO synthase activity is decreased in old age and osteoporosis [1].

*Blood cells and complement*

Inflammation is characterized by migration of erythrocytes, platelets, neutrophils, and then macrophages and lymphocytes, from the vascular compartment into tissues, in response to the inflammatory stimulus of bacteria, endotoxin, necrotic tissue, etc. The leucocytes interact with target cells dependent on regulated adhesion molecules (integrins and selectins – glycoproteins and lectins from the immunoglobulin and other supergene families) which are essential for most aspects of cellular immunity and inflammation, including antigen presentation, leucocyte activation, and leucocyte migration to sites of inflammation [33]. Leucocytes possess an array of cell-surface receptors, mediating varying degrees of adhesion to a miscellany of adhesion molecules specific for lymphocytes, monocytes, platelets and endothelial cells, and for fibrinogen, collagen, fibronectin, complement, etc., facilitating chemotaxis, leucocyte migration, leucocyte adhesion and activation [33].

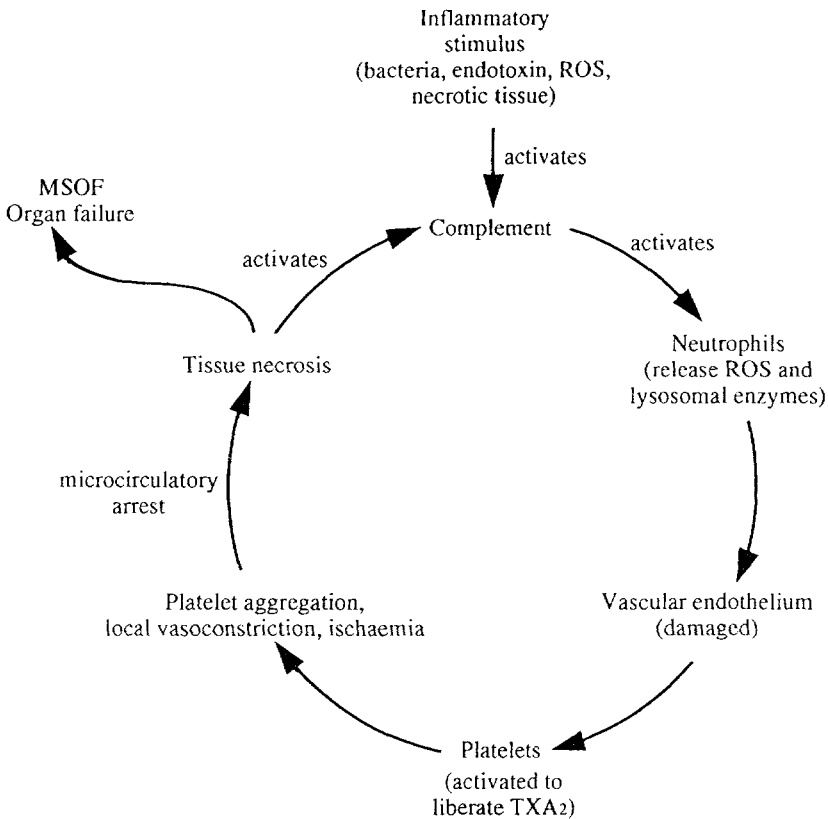


Figure 4. Role of complement in perpetuating inflammation. The activation of complement by endotoxin, ROS, necrotic tissue, and other inflammatory stimuli, in turn activates neutrophils, which release ROS and lysosomal enzymes, damaging the vascular endothelium. This activates platelets, liberating TXA<sub>2</sub>, resulting in platelet aggregation, local vasoconstriction and ischaemia, leading to microcirculatory arrest and tissue necrosis. In turn, the necrotic tissue again activates complement, thereby perpetuating this cycle of the inflammatory process. From Reference [35] modified

Complement is a cascade system of more than 20 proteins, which is concerned in the amplification of antigen-antibody reactions, and is also a mediator of the inflammatory response [17]. Complement activation markedly decreases liver perfusion, and the hepatic ischaemia associated with trauma and sepsis is probably mediated by complement activation. Complement is also a major determinant of eicosanoid release, eicosanoids acting as intermediate messengers mediating the response to bacteria, endotoxin, etc. [17,34]. It has been suggested by Schirmer and Fry [35] that complement may be a causative factor in the continuing activation of inflammation; bacteria, endotoxin and other inflammatory stimuli activate complement, which in turn activates neutrophils, releasing ROS and initiating a continuous cycle of inflammatory response [35] (Figure 4).

### *Cytokines*

Cytokines are regulatory proteins secreted by macrophages and other cells, which provoke numerous effects on cells of the immune system, and modulate the inflammatory response. Cytokines have high affinities for leucocytes and other cells, effecting immunoregulation and cell proliferation, and include the interleukins (IL-1 to IL-12), tumour necrosis factors (TNF $\alpha$  and  $\beta$ ) and the interferons.

IL-1 ( $\alpha$  and  $\beta$ ) are related interleukins with similar biological activity, produced mainly by activated mononuclear phagocytes. IL-1 is a major inflammatory hormone, causing leucocyte accumulation and activation, inducing fever and the acute phase response, and is also implicated in shock-induced ATP depletion [36]. IL-1 results in hypotension and tachycardia, mediated by increased PGI<sub>2</sub> and PGE<sub>2</sub> synthesis from several cell types, increased TXB<sub>2</sub> from macrophages and neutrophils, and increased LTB<sub>4</sub> from neutrophils. IL-1 induces many genes (including some oncogenes), activates regulatory G proteins and protein kinase C, and increases phosphorylation of certain cellular proteins [37]; it also increases muscle PGE<sub>2</sub> and muscle proteolysis.

Interleukin 6 (IL-6), synthesized by monocytes/macrophages, endothelial cells, fibroblasts, and other cells, regulates the induction of fever, the synthesis of acute phase proteins, T-cell proliferation, and induction of immunoglobulin production by activated B cells. The synthesis of IL-1 and IL-6 are promoted by ROS [38]. It has been suggested that dysregulation of IL-6, by chemical damage or viral infection, may play a role in the pathogenesis of autoimmune disease, leading to a self-perpetuating inflammatory process [39]. IL-6 is the main cytokine released after elective surgery, and tissue damage is a major determinant of the circulating levels of this cytokine [40].

Interleukin-2 (IL-2) is a T-cell growth factor and is a pivotal cytokine for generating effective immune response. It is associated with increased vascular permeability due to its increasing the adhesion of platelets and neutrophils to the endothelium [41]. Whereas the inflammatory cytokines, IL-1 and IL-6, decreased the activities of rat liver cytochrome P450-linked mono-oxygenase activities, IL-2 results in the induction of rat liver cytochrome P4502D (CYP2D) [42].

The cytokines, IL-3, IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF), synthesized and released from specific T-cells, have relatively selective activities in activating eosinophils, and are involved in the inflammatory process of asthma [43].

TNF, another cytokine elaborated by macrophages and other cell types, is one of the major endogenous mediators of endotoxic shock, resulting in increased eicosanoid production, hypotension and ischaemic necrosis of liver, lungs and bowel. It stimulates collagenase activity, PGE<sub>2</sub> formation in macrophages and fibroblasts, and increases endothelial PGI<sub>2</sub> synthesis mediating the hypotension.

*Eicosanoids*

The eicosanoids (prostanoids) are oxidative metabolites of arachidonic acid, a family of vasoactive mediators of major importance in inflammation, regulation of the immune response, and circulatory shock [3,18,34,44]. Arachidonic acid, released from membrane phospholipids by phospholipase, is metabolized by the cyclo-oxygenase

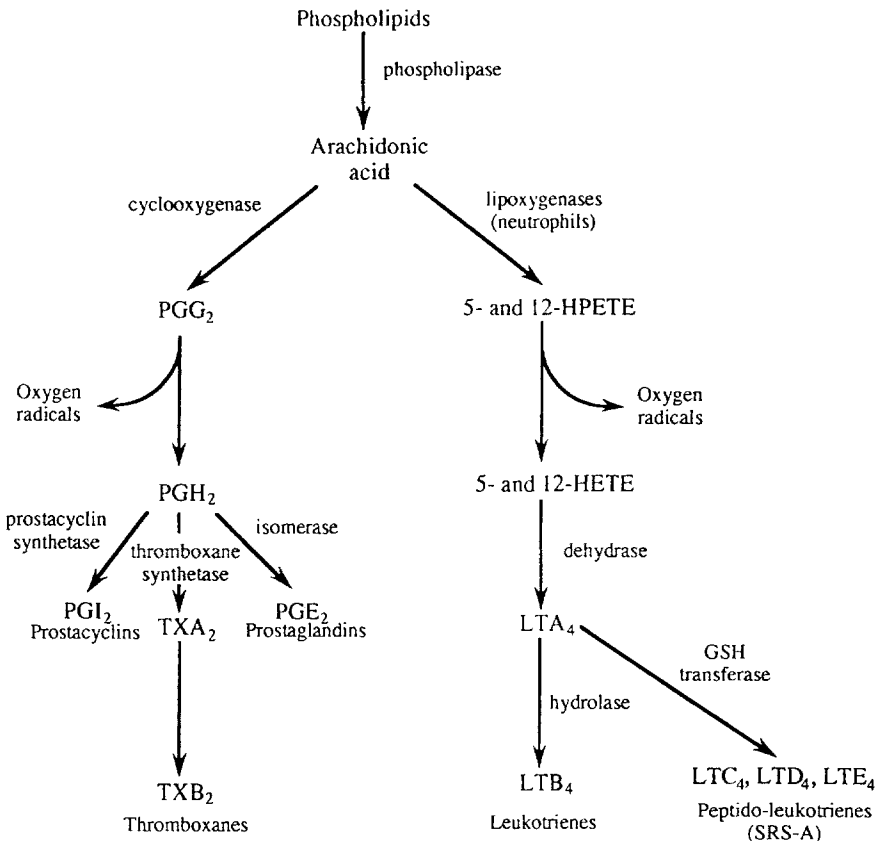


Figure 5. The eicosanoid cascade. Arachidonic acid released from membrane phospholipids is metabolized by cyclo-oxygenase to prostaglandins (PGH<sub>2</sub>, PGE<sub>2</sub>), prostacyclins (PGI<sub>2</sub>) and thromboxanes (TXA<sub>2</sub>, TXB<sub>2</sub>), or by lipoxygenases to hydroxyeicosatetraenoic acids (HETE) and leukotrienes (LTB<sub>4</sub>). Note the ROS released in the conversion of PGG<sub>2</sub> to PGH<sub>2</sub> and in the conversion of 5- and 12-HPETE to 5- and 12-HETE

pathway (platelets, endothelial cells) to form the prostaglandins, thromboxanes and prostacyclins, and by the lipoxygenase pathway (neutrophils) to yield the leukotrienes (Figure 5). Platelets synthesize large amounts of thromboxane ( $\text{TXA}_2$ ) but not prostacyclins ( $\text{PGI}_2$ ), whereas endothelial cells synthesize mainly  $\text{PGI}_2$ . The leukotriene,  $\text{LTB}_4$ , and to a lesser extent other leukotrienes, is formed in neutrophils and macrophages but not lymphocytes.

Eicosanoids are both pro- and anti-inflammatory. Prostaglandin E ( $\text{PGE}_2$ ) and prostacyclin  $\text{PGI}_2$  are potent vasodilating and hyperalgesic agents mediating the acute inflammatory response; they suppress the functions of inflammatory cells (mast cells, neutrophils, lymphocytes, macrophages) and suppress the production of  $\text{LTB}_4$  and the cytokines, IL-1 and IL-2.  $\text{PGE}_2$  inhibits neutrophil ROS production, suppresses B lymphocyte activity (antibody response) but enhances T-cell activity (cell-mediated immunity);  $\text{TXB}_2$  antagonizes the PGE compounds.  $\text{TXA}_2$  induces platelet aggregation;  $\text{PGI}_2$  inhibits platelet aggregation and is a vasodilator. The leukotrienes are potent mediators of inflammation; they increase vascular permeability, and endotoxin produces a marked increase in  $\text{LTE}_4$  metabolites in the bile.  $\text{LTB}_4$ , increased in rheumatoid synovial exudates, in psoriatic skin lesions, and in IBD, inhibits the leucocyte proliferative response, activates T lymphocytes to secrete interferon ( $\text{IFN-}\gamma$ ), and induces neutrophil ROS formation and monocyte-mediated cytotoxicity.

$\text{TNF}\alpha$  may initiate the eicosanoid cascade in endotoxaemia, and increased levels of  $\text{TXB}_2$  and its metabolites are seen in patients with advanced sepsis, severe burns, hepatic failure and MSOF. Renal failure is a prominent feature of the later stages of MSOF, and  $\text{LTD}_4$  and  $\text{LTC}_4$  have been implicated in acute renal injury.  $\text{LTD}_4$  causes marked splanchnic vasoconstriction and may be involved in stress-induced gastrointestinal ulceration.

Platelet-activating factor (PAF) is a phospholipid with diverse inflammatory actions, and, given intravenously, produces severe hypotension and shock. Eicosanoids, particularly  $\text{LTC}_4$  and  $\text{LTD}_4$ , and  $\text{TXB}_2$  are probably mediators of PAF action.

### *Cytochromes P450 and toxic chemicals*

The cytochromes P450 (CYP), a superfamily of mixed-function oxygenases [45], concerned in the oxygenation and metabolic detoxication of toxic chemicals and other xenobiotics [46], and in the elaboration of the steroid hormones, are also able to generate ROS, either directly (cytochrome P4502E1; CYP2E1) or by futile cycling (Table 1, Figure 2). In futile cycling, xenobiotic substrates (e.g. chlorodioxins) activate the P450-oxygen complex, but, because of the conformational difficulty of inserting oxygen into the substrate, oxidation does not occur; instead, superoxide anions are released and ROS generated (Figure 3). Many polyhalogenated, and other complex, molecules (e.g. TCDD; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) are known to initiate chronic inflammatory conditions because they:

- (a) Are lipophilic and become incorporated into tissue components (lipids) and tissues,



- (b) Activate the CYP system to continuously generate ROS, thereby initiating and perpetuating the inflammatory response, and
- (c) Are resistant to oxidative metabolism, detoxication and elimination, and persist in the tissues with half-lives well in excess of the life-span of the patient [3,46].

Two families of the cytochromes P450, namely CYP1A and CYP2E, metabolize xenobiotics to products with increased toxicity (reactive intermediates from CYP1A, and carbon-centred radicals plus ROS from CYP2E), and hence are involved in the activation of carcinogens and mutagens, and in the elaboration of immunotoxins and neoantigens [3,46,47] (Figure 3). Classic examples of the latter are the CYP autoantibodies of hepatitis [48], the endoplasmic reticulum autoantibodies of systemic lupus erythematosus [49], and the fatal hepatitis of tienilic acid antigens [50]. The CYP enzymes are particularly active in the liver, metabolizing nutrients and xenobiotics present in food; they are also present in most other tissues, including the leucocytes, where their function is as yet unknown [3].

TABLE 1  
Some families of the cytochromes P450

Cytochrome family	Inducers or substrates	Action
CYP1A1	Large planar molecules e.g. TCDD (dioxin)	Activates carcinogenic polycyclic hydrocarbons; metabolizes arachidonic acid to EETE and HETE
CYP1A2	Heterocyclic nitrogen compounds	Activates cooked-food mutagens; activates N-containing drugs to neoantigens
CYP2B	Non-planar chemicals e.g. phenobarbitone	Detoxication of drugs and chemicals
CYP2D	Induced by IL-2	Detoxication of tricyclic antidepressants
CYP2E1	Fasting (acetone), alcohol, halogenated solvents	Generates ROS; activates small molecules
CYP3A	Macrolides, steroids	Detoxicates large molecules e.g. cyclosporin
CYP4	Long-chain fatty acids	Fatty acid $\omega$ -oxygenation, H <sub>2</sub> O <sub>2</sub> production and peroxisome <sup>2</sup> proliferation
CYP7 ] CYP11 ] CYP17 ] CYP19 ] CYP21 ] CYP27 ]	Six families concerned with steroid metabolism	Cholesterol 7-hydroxylase Steroid 11-hydroxylase Steroid 17-hydroxylase Steroid aromatase Steroid 21-hydroxylase Steroid 25/27-hydroxylase

High rates of exposure to toxic chemicals and consequent heavy dependence on the chemical detoxication system, make large demands on the endogenous redox buffer, glutathione (GSH), both for the conjugation/detoxication of epoxide metabolites (potential carcinogens, immunotoxins) and for the reduction of quinone metabolites [51], thus depleting the GSH available for antioxidant defence and so enhancing the potential for oxidative stress (Figure 3). Furthermore, depletion of GSH decreases the integrity of lymphocytes and other cells of the immune system, resulting in impairment of T-cell and macrophage immune function, loss of immunocompetence [52] and inhibition of leucocyte differentiation [53].

The past few decades have seen the emergence of several widespread fatal chronic inflammatory syndromes associated with exposure to specific chemicals, namely, the toxic oil syndrome (TOS) associated with the ingestion of reprocessed adulterated rapeseed oil, the eosinophilia–myalgia syndrome (EMS) associated with the amino acid, tryptophan, and the acquired immunodeficiency syndrome (AIDS) associated with the use of nitrate vasodilators and other drugs. The toxic oil syndrome (TOS) that developed in Spain in 1981 followed the ingestion of fraudulently-marketed illicitly-recovered denatured rapeseed oil [54–57]. Over 20 000 cases were recorded, with symptoms of fever, rash, gastrointestinal pain, hepatomegaly, pulmonary and neurological disturbances, arthralgia and myalgia; over 300 died and many had persistent scleroderma-like symptoms for a decade or so [54]. Rapeseed oil, long known to be atherogenic due to its content of erucic acid, a natural mono-unsaturated fatty acid, is metabolized only with great difficulty. It results in hepatic peroxisomal proliferation, was associated with atherosclerosis, and was marketed as an industrial lubricant after denaturation by addition of aniline/pyridine and a brown dye to prevent human consumption. Illicit treatment to remove the aniline by steam distillation is thought to have resulted in the production of toxic acyl anilides [58]. Erucic acid is resistant to  $\beta$ -oxidation and is therefore metabolized by CYP4 and peroxisomal enzymes which are associated with peroxide formation and hepatotoxicity; anilides of fatty acids, which are even more resistant to  $\beta$ -oxidation, would accumulate, and could manifest immunotoxicity. Although not normally antigenic, fatty acids can be made immunogenic by chemical modification, and antibodies to several common fatty acids have been synthesized [59].

In 1989, a second such epidemic of chronic inflammatory disease, namely, eosinophilia–myalgia syndrome (EMS), associated with the ingestion of L-tryptophan, occurred in the USA and Canada [60,61]. The disease was marked by progressive neuromuscular, pulmonary and skin manifestations, resembling the symptoms of toxic oil syndrome, and although quinolinic acid and other metabolites of tryptophan were first indicted as possible causes, an impurity with the chemical structure of two tryptophan molecules joined by an ethylidene bridge – a manufactured impurity – was finally identified as the toxic entity [62].

The third example of these recent disastrous inflammatory diseases, namely, acquired immunodeficiency syndrome (AIDS), a disease of systemic inflammation and changed lymphocyte function, and generally believed to result from HIV infection, is now often considered to be the consequence of several interacting factors, including a viral-induced cysteine/glutathione deficiency [63] and the cytotoxicity of vasodilatory alkyl nitrites [64]. These chemicals are used as recreational drugs, and form S-nitrosoglutathione, depleting lymphocyte GSH and ATP, resulting in oxidative stress, DNA damage and cell death [64–66].

*Glutathione and metabolism*

The initial phase of critical illness (trauma, surgery, burns, infection), namely shock and hypovolaemia, is accompanied by decreased metabolic activity (raised blood glucose, decreased oxygen consumption, raised levels of adrenaline and other stress hormones), which is followed by a prolonged phase of hypermetabolism (increased oxygen consumption, lowered blood glucose, nitrogen loss, cytokine production). The degree of hypermetabolism is related to wound severity and the extent of associated infection.

In the absence of continuing external inflammatory stimuli (wounding, infection, antigens), ROS from activated neutrophils and other products of the primary inflammatory response are the major cause of progression and perpetuation of the acute inflammation to a chronic condition. Normally, the lipid-soluble  $\alpha$ -tocopherol, and the water-soluble ascorbic acid and glutathione, scavenge ROS and other radicals, limiting the oxidative stress and inflammatory response.  $\alpha$ -Tocopherol is the primary radical scavenger in biological membranes, and is continuously regenerated at the expense of ascorbate and glutathione oxidation (Figure 6), with the ascorbate and glutathione being continuously regenerated by NADH and NADPH in the presence of the relevant reductases [67,68]. Hence, the prevention of oxidative stress and progressive inflammation is highly dependent on sufficient provision of energy to regenerate glutathione and other antioxidants, and on adequate dietary provision of  $\alpha$ -tocopherol, ascorbic acid, sulphur amino acids, selenium, etc. to repair the daily

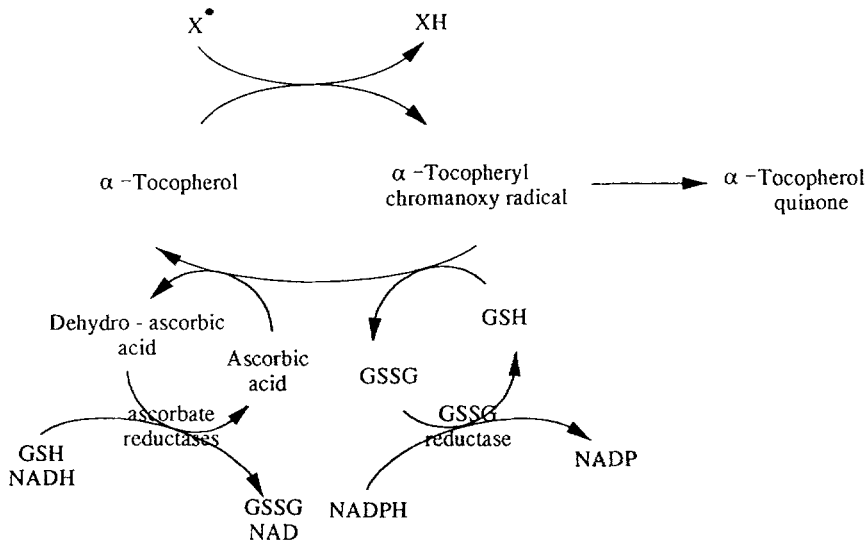


Figure 6. Antioxidant regeneration systems. Biological oxidants and ROS (represented by  $X^\bullet$ ) are reduced directly by the tocopherols. The oxidized tocopheryl chromanoxo radical is cyclically reduced to the tocopherols again by ascorbic acid or GSH. The dehydro-ascorbic acid is cyclically reduced back to ascorbic acid at the expense of GSH or NADH, and oxidized glutathione (GSSG) is reduced back to GSH by NADPH in the presence of glutathione reductase

losses of these vital chemicals and enzymes of the antioxidant defence system [19]. Chain oxidation of intracellular GSH to GSSG by superoxide-dependent production of  $H_2O_2$  is precluded by the presence of superoxide dismutase, so that the combination of GSH and SOD constitutes an integral component of cellular antioxidant defence [69]. Glutathione peroxidase (GPX), a selenoenzyme, is involved in protection against  $H_2O_2$  and other peroxides; hence the dietary need for selenium, deficiencies of which result in Keshan disease, a cardiomyopathy widespread in China [70].

Critical illness is associated with both immunosuppression and glutathione deficiency [52]; GSH deficiency decreases lymphocyte proliferation and production of tumour necrosis factor. Therapeutic or nutritional intervention to maintain GSH levels may enhance immunocompetence and improve the ability of patients to combat infection and recover from critical illness [52,63].

## SURGICAL SHOCK AND MULTISYSTEM ORGAN FAILURE

Surgical shock, for long a major cause of death following emergency amputation and similar elective surgery, was considered by the eminent 19th century surgeon, Joseph Lister, to be due to microbial infection, which was prevented by the use of antiseptic phenol sprays in the theatre. Subsequent surgeons considered that blood loss was the major cause of postsurgical fatality, and this led to fluid replacement by saline or blood transfusions. Later still, anaesthetics were believed to interfere with hepatic function, and postmortems on surgical fatalities revealed that hepatic necrosis and renal necrosis were frequent causes of death. Surgical shock is a major cause of death of critically ill and injured patients, particularly military casualties in time of war and road traffic accidents in peace time. Resuscitation of casualties to normal blood pressure proved to be inadequate, with many patients dying of renal failure, so that urine output became the factor to monitor for successful shock resuscitation in the 1950s. In World War 2, gastrointestinal haemorrhage, often fatal, was a major feature of shock in flying personnel suffering severe burns and shot down in combat. Following the correction of blood volume and renal function, pulmonary dysfunction became the next weak link, and, in the Vietnam war, 'shock lung' or Da Nang lung (acute respiratory distress syndrome; ARDS) was a major cause of fatalities. In the 1980s, medical technology had advanced to correct the various single organ dysfunctions, and multiple system organ failure (MSOF) emerged as a consequence [14]. Four major organ systems at risk have been identified, namely pulmonary failure, hepatic failure, renal failure and stress gastrointestinal bleeding. ARDS has been associated with massive effort at resuscitation, hepatic failure is associated with anaesthetic and drug toxicities, renal failure with drug toxicity and transfusion reactions, and hypovolaemic shock and uncontrolled infections have both been associated with all four organ failures. In those instances where any of these organ failures were studied in detail, inflammation with neutrophil invasion, ROS production, oxidative stress and eventual tissue necrosis, were characteristic features. Hence, MSOF may be considered to be a form of systemic inflammation, progressing from one organ to another, as modern medical technology is applied to rescue each individual system in the resuscitation of the patient.

*Fibronectin*

This is a high-molecular-weight glycoprotein, found in a soluble form in blood and lymph, and in an insoluble form in cells and cellular matrix; it has high affinity for cell surfaces, fibrin, collagen, bacteria, etc. Incorporation of fibronectin into tissues augments macrophage clearance of microaggregates of tissue debris, thus preventing microvascular injury from microemboli [71]. Fibronectin is synthesized in the liver, vascular endothelium, macrophages and fibroblasts; it has a fast turnover (plasma half-life of 30 h) and is increased in injury and shock. Plasma fibronectin is low in burn cases, infections and MSOF, and deficiency develops rapidly in starvation and trauma.

*Microcirculatory arrest*

Microaggregates of fibrin, fibrin degradation products, platelets, etc., circulating in the blood, are removed by the reticuloendothelial Kupffer cells in the liver, thereby preventing the formulation of microemboli, protecting the lungs and other organs from microcirculatory arrest. Blood-borne microaggregate debris in association with fibronectin deficiency may lead to leucocyte activation and sequestration, releasing ROS and proteases, resulting in endothelial injury, increased vascular permeability, oedema, tissue necrosis and organ failure [71]. In various forms of trauma, including crush injury, fractures and burns, necrotic tissue, fracture fragments and haematoma fragments serve as stimuli for continuing activation of inflammation. In instances of exposure to toxic chemicals activating the cytochromes P450 to give rise to futile cycling or neoantigen formation, numerous pathogenic mediators of inflammation are activated; these include cellular mediators (cytokines) and humoral mediators (complement, ROS, eicosanoids, kinins, etc.). This systemic inflammation is likely to be the primary trigger for widespread microvascular injury, microaggregation of tissue debris, and microcirculatory arrest, ischaemia and organ failure. MSOF is thus the likely consequence of generalized autodestructive systemic inflammation [35] (see Figure 4). Organs fail sequentially, rather than simultaneously. The sequence of organ failure is the clinical manifestation of the organ-specific responses to systemic inflammation, microcirculatory arrest, ischaemia, and tissue necrosis. Hepatic and pulmonary failure are the earlier manifestations of MSOF, probably because these tissues, unlike the kidney, are rich in tissue-fixed macrophages which release numerous inflammation-mediating factors, including IL-1, TNF $\alpha$ , PAF, eicosanoids, etc. Renal failure is usually terminal, since in the ultimate oxidative stress, ROS, lipid peroxides, and other toxic products of the ROS-mediated tissue lipid peroxidation, are excreted via the kidney, resulting in fatal damage, as has been reproduced in numerous animal models [72]. Because of the self-promoting nature of the systemic inflammation, removal of the original, initiating stimulus may do little to prevent the propagation of the inflammation, the organ ischaemia and organ failure, and eventual death.

*Reactive oxygen studies*

In an endeavour to elucidate in more detail the pathogenesis of surgical shock, studies in experimental animals were conducted to examine the roles of possible contributory factors, including (a) preoperation fasting, (b) anaesthesia, (c) duration of surgery, (d) blood loss and hepatic ischaemia, and (e) reperfusion [19,20,73–76].

American surgeons in the First World War considered that anaesthetic toxicity and consequent liver damage were primary causes of battle fatalities and found that transfusion with 5% glucose/saline for 24 h greatly decreased the mortality rate [74,77]. Diethyl ether was the major anaesthetic in use at the time, and was known to cause damage to the liver and kidneys, especially after fasting and fluid loss [74,78]. Indeed, from these early observations by Philadelphiaian surgeons nearly eighty years ago, came the first understandings of chemical detoxication and of the susceptibility of the liver to chemical toxicity, the protective effects of good nutrition, especially sulphur amino acids, and the role of hepatic glutathione [78]. In recent studies, rats exposed to fasting (20 h) and light ether anaesthesia showed marked evidence of oxidative stress including:

- a) Increased exhalation of alkanes in the expired air,
- b) 4-Fold increases in the chemiluminescence and thiobarbituric acid-reacting (TBAR) material of liver and kidney cytosols,
- c) A 70% loss in the total cytochromes of the rat liver and kidney, and
- d) A 140% increase in cytochrome P4502E1 (CYP2E1) activity [74].

The effects of the pre-anaesthetic fasting, and the effects of the light ether anaesthesia, were about equal, and were additive [19,74]. Hence, it was concluded that both food deprivation and ether anaesthesia result in ROS generation, lipid peroxidation and oxidative stress, probably by the induction of CYP2E1, a cytochrome P450 which is known to be highly active in futile cycling and the generation of ROS [75]. Thus, in addition to the generation of ROS by infections and by immune reactions, we now have the possibility of ROS generation from the cytochromes P450 following exposure to certain chemicals. Like microbial infections and immune reactions, the CYP2E1 generation of ROS may also lead to inflammation. In subsequent studies, tissue glutathione (GSH) and total radical-trapping activity (TRAP) (ascorbic acid, tocopherols, retinoids, glutathione, etc.) were determined, together with specific cytochrome P450 (CYP) activities; these showed that GSH, TRAP, total CYP, CYP1 and CYP2B decreased progressively with increasing exposure to ether, in both liver and kidney, while TBAR and CYP2E progressively increased [76]. This is considered to provide quantitative evidence of the effects of the duration of ether anaesthesia on CYP2E induction, its generation of ROS, tissue lipid peroxidation, and organ necrosis. Although diethyl ether was the anaesthetic used in all these experiments, other volatile anaesthetics, particularly the halogenated anaesthetics, e.g. halothane, enflurane, and other substrates/inducers of CYP2E, are likely to exhibit the same ROS-generating effects.

In parallel experiments, in a rat liver ischaemia–reperfusion model to examine the molecular pathology of hypovolaemic shock [20], it was found that liver ischaemia alone resulted in slight oedema, a slight increase in TBAR substances (lipid peroxidation), neutrophil infiltration, and decreases in liver GSH and TRAP, all indices



TABLE 2  
The effects of anaesthesia, liver ischaemia and liver reperfusion on hepatic oxidative stress in the rat

Treatment	Liver parameters				
	GSH	GSSG	GSH/GSSG ratio	TRAP	TBAR
Fed					
Anaesthesia only	3.3	0.21	16	13.7	0.3
Anaesthesia + sham surgery	3.0	0.22	14	12.9	1.2
Anaesthesia + ischaemia	2.6	0.18	14	8.2	1.8
Anaesthesia + ischaemia + 30 min reperfusion	1.8	0.24	7.5	5.0	2.4
Anaesthesia + ischaemia + 60 min reperfusion	1.1	0.29	3.8	3.9	4.2
Anaesthesia + ischaemia + 90 min reperfusion	0.8	0.23	3.4	1.9	5.9
Fasted					
Anaesthesia + ischaemia	1.5	0.21	7.0	5.5	2.4
Anaesthesia + ischaemia + 30 min reperfusion	0.8	0.25	3.2	3.5	3.1
Anaesthesia + ischaemia + 60 min reperfusion	0.6	0.23	2.6	2.4	5.2
Anaesthesia + ischaemia + 90 min reperfusion	0.2	0.21	1.0	1.3	6.5

Liver reduced glutathione (GSH) is expressed as nmol/mg tissue; oxidized glutathione (GSSG) is expressed as nmol GSH equiv/mg tissue; total radical trapping activity (TRAP) is nmol O<sub>2</sub> uptake per min per mg tissue; thiobarbituric acid-reactive substance (TBAR) is expressed as nmol tetraethoxypropane equiv/mg tissue protein. Six rats per group were studied and mean values are given; SEM values were 20% of the mean values or less. Data is taken from references [19,20]



of hepatic inflammation. After 60 minutes of liver reperfusion, liver oedema was increased by 40%, neutrophil infiltration into the liver was increased 40-fold, TBAR substances were increased 20-fold and were maximal; liver GSH was 95% decreased, TRAP was 90% decreased, and both these were at a minimum [20]. All parameters of hepatic oxidative stress (inflammation) were exacerbated by 24-h starvation; liver GSH closely paralleled the TRAP, and ischaemia alone depleted both by 30% in fed rats and 50% in fasted rats. The oxidative stress and lipid peroxidation were associated more with the period of reperfusion than with the initial ischaemia and more with the later neutrophil infiltration than with the initial ROS generation by the xanthine oxidase system [20]. After 90 min of liver reperfusion, neutrophil infiltration was observed also in the rat lung, indicating that inflammation of the liver could generate circulating mediators, possibly interleukins (IL-1, IL-6), platelet-activating factor (PAF), and leukotrienes (LTB<sub>4</sub>), that provoked sympathetic inflammation in the lungs. Hence, the phenomenon of ARDS following surgical shock or MSOF is explained as a manifestation of systemic inflammation, and possible mechanisms are illustrated in Figure 7.

In a study of the role of tissue (liver/kidney) GSH in the prevention of surgical shock, it was found that fasting of rats alone decreased tissue GSH by 50% and increased TBAR by 100%; fasting plus 30 min ether anaesthesia decreased tissue GSH by 80% and increased TBAR by 600% [19]. The oxidation of tissue glutathione increased progressively with fasting, anaesthesia, ischaemia, and ischaemia followed by reperfusion (Table 2) [20]. A complex series of molecular mechanisms has been advanced to account for the pathogenesis of surgical shock and MSOF, and these include:

- a) Energy and GSH depletion,
- b) Induction of CYP2E1 activity by fasting and anaesthesia,
- c) Generation of ROS,
- d) Cytokine release,
- e) Oxidative stress and lipid peroxidation,
- f) Neutrophil activation and sequestration, and
- g) Oxidative stress, cell death and tissue necrosis [19].

Hence, it would appear that the protective acute inflammatory response may progress to destructive chronic inflammatory disease states, such as rheumatoid arthritis, peptic ulceration, hepatitis and MSOF, by progressive systemic oxidative stress associated with a variety of factors, including: tissue iron [30], infection [2], starvation [27], cytochrome P4502E1 [10], complement [17], or loss of tissue GSH [19,20]. It is known that loss of GSH from increased ROS formation, and greater demands for chemical detoxication [51], also has adverse effects on lymphocyte function and immune competence [52,53,66]. Thus, high levels of exposure to environmental chemicals may constitute yet another causative mechanism of inflammatory disease; and epidemiology confirms this. Chemically-degraded rapeseed oil in TOS, a manufactured aberrant of tryptophan as the causative agent in EMS, alkyl nitrites and AIDS, anaesthetics in surgical shock, and drug-associated lupus and similar autoimmune diseases, are some of the many examples of inflammatory disease now recognized as being chemically-induced [3,49,50]. This may indeed prove an explanation of why, in this age of chemical industry and environmental pollution, inflammatory disease is now most prevalent.

## REFERENCES

1. Winrow VR, Winyard PG, Morris CJ, Blake DR. Free radicals in inflammation: second messengers and mediators of tissue destruction. *Br Med Bull.* 1993;49:506–22.
2. Welbourn CRB, Young Y. Endotoxin, septic shock and acute lung injury: neutrophils, macrophages and inflammatory mediators. *Br J Surg.* 1992;79:998–1003.
3. Parke AL, Ioannides C, Lewis DFV, Parke DV. Molecular pathology of drug–disease interactions in chronic autoimmune inflammatory diseases. *Inflammopharmacology.* 1991;1:3–36.
4. Babbs CF. Oxygen radicals in ulcerative colitis. *Free Rad Biol Med.* 1992;13:169–81.
5. Trush MA, Kensler TW. An overview of the relationship between oxidative stress and chemical carcinogenesis. *Free Rad Biol Med.* 1991;10:201–9.
6. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. *Proc Natl Acad Sci USA.* 1993;90:7915–22.
7. Witz G. Active oxygen species as factors in multistage carcinogenesis. *Proc Soc Exptl Biol Med.* 1991;198:675–82.
8. Piotrowski JJ, Hunter GC, Eskelson CD, Dubick MA, Bernhard VM. Evidence for lipid peroxidation in atherosclerosis. *Life Sci.* 1990;46:715–21.
9. Halliwell B. Free radicals and antioxidants: A personal view. *Nutr Rev.* 1994;52:253–65.
10. Elliot RH, Strunin L. Hepatotoxicity of volatile anaesthetics. *Br J Anaesth.* 1993;70:339–49.
11. Evans PH. Free radicals in brain metabolism and pathology. *Br Med Bull.* 1993;49:577–87.
12. Baruchel S, Wainberg MA. The role of oxidative stress in disease progression in individuals infected by the human immunodeficiency virus. *J Leuk Biol.* 1992;52:111–14.
13. Marshall JC, Cristou NV, Meakins JL. The gastrointestinal tract. The undrained abscess of multiple organ failure. *Ann Surg.* 1993;218:111–19.
14. Fry DE. Multiple system organ failure. In: Fry DE, ed. *Multiple system organ failure.* St Louis: Mosby Year Book; 1992:3–14.
15. McLean JS, Byrick RJ. ARDS and sepsis – definitions and new therapy. *Can J Anaesth.* 1993;40:585–90.
16. Babior BM. Oxygen dependent microbial killing by phagocytes. *N Engl J Med.* 1978;298:659–68.
17. Clevenger FW, Fry DE. Complement. In: Fry DE, ed. *Multiple system organ failure.* St Louis: Mosby Year Book; 1992:167–77.
18. Reines HD, Cook JA. Prostaglandins. In: Fry DE, ed. *Multiple system organ failure.* St Louis: Mosby Year Book; 1992:123–41.
19. Liu PT, Ioannides C, Symons AM, Parke DV. Role of tissue glutathione in prevention of surgical trauma. *Xenobiotica.* 1993;23:899–911.
20. Liu PT, Symons AM, Howarth JA, Boulter PS, Parke DV. Studies in surgical trauma: oxidative stress in ischaemia-reperfusion of rat liver. *Clin Sci.* 1994;86:453–60.
21. Parke DV, Ioannides C. The effects of nutrition on chemical toxicity. *Drug Metab Rev.* 1994;26:739–65.
22. Sussman MS, Schiller HJ, Buchman TG, Bulkley GB. Oxygen free radicals. In: Fry DE, ed. *Multiple system organ failure.* St Louis: Mosby Year Book; 1992:143–65.
23. Bagchi M, Hassoun EA, Bagchi D, Stohs SJ. Production of reactive oxygen species by peritoneal macrophages and hepatic mitochondria and microsomes from endrin-treated rats. *Free Rad Biol Med.* 1993;14:149–55.
24. Kukielka E, Cederbaum AI. DNA strand cleavage as a sensitive assay for the production of hydroxyl radicals: role of cytochrome P4502E1 in the increased activity after ethanol treatment. *Biochem J.* 1994;302:773–9.
25. Parke DV. The cytochromes P450 and mechanisms of chemical carcinogenesis. *Environ Health Persp.* 1994;102:852–3.
26. Myers SI, Hernandez R. Role of oxygen-derived free radicals on rat splanchnic eicosanoid production during acute haemorrhage. *Prostaglandins.* 1992;44:25–36.
27. Parke DV. The importance of diet and nutrition in the detoxication of chemicals. In: Parke DV, Ioannides C, Walker R, eds. *Food nutrition and chemical toxicity.* London: Smith–Gordon; 1993:1–15.
28. Palozza P, Krinsky NI.  $\beta$ -Carotene and  $\alpha$ -tocopherol are synergistic antioxidants. *Arch Biochem Biophys.* 1992;297:184–7.
29. Yoshioka A, Miyachi Y, Imamura S, Niwa Y. Anti-oxidant effects of retinoids on inflammatory skin diseases. *Arch Dermatol Res.* 1986;278:177–83.
30. Trenam CW, Winyard PG, Morris CJ, Blake DR. Iron-promoted oxidative damage in rheumatic diseases. In: Lauffer VB, ed. *Iron and human disease.* Boca Raton: CRC Press; 1992:395–417.
31. Vallance P, Moncada S. Nitric oxide – from mediator to medicines. *J R Coll Phys (Lond).* 1994;28:209–19.

32. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacol Rev*. 1991;43:109-42.
33. Adams DH. Leucocyte adhesion molecules and alcoholic liver disease. *Alcohol Alcoholism*. 1994;29:249-60.
34. McGiff JC. Cytochrome P450 metabolism of arachidonic acid. *Ann Rev Pharmacol Toxicol*. 1991;31:339-69.
35. Schirmer WJ, Fry DE. Microcirculatory arrest. In: Fry DE, ed. *Multiple system organ failure*. St Louis: Mosby Year Book; 1992:73-85.
36. Pellicane JV, De Maria EJ, Abd-Elfattah A, Reines HD, Vannice JL, Carson KW. Interleukin-1 receptor antagonist improves survival and preserves organ adenosine-5'-triphosphate after haemorrhagic shock. *Surgery*. 1993;114:278-84.
37. Kracht M, Shiroo M, Marshall CJ, Hsuan JJ, Saklatvala J. Interleukin-1 activates a novel protein kinase that phosphorylates the epidermal-growth-factor receptor peptide T669. *Biochem J*. 1994;302:897-905.
38. Ala Y, Palluy O, Favero J, Bonne C, Modat G, Dornand J. Hypoxia/reoxygenation stimulates endothelial cells to promote interleukin-1 and interleukin-6 production. Effects of free radical scavengers. *Agents Actions*. 1992;37:134-9.
39. Graeve L, Baumann M, Heinrich PC. Interleukin-6 in autoimmune diseases. *Clin Invest*. 1993;71:664-71.
40. Hall GM, Desborough JP. Interleukin-6 and the metabolic response to surgery. *Br J Anaesth*. 1992;69:337-8.
41. Edwards MJ, Miller FN, Sims DE, Abney DL, Schuschke DA, Corey TS. Interleukin-2 acutely induces platelet and neutrophil-endothelial adherence and macromolecular leakage. *Cancer Res*. 1992;52:3425-31.
42. Kurokohchi K, Matsuo Y, Yoneyama H, Nishioka M, Ichikawa Y. Interleukin 2 induction of cytochrome P450-linked monooxygenase systems of rat liver microsomes. *Biochem Pharmacol*. 1993;45:585-92.
43. Kay AB. T cells, cytokines and asthma. *J R Coll Phys (Lond)*. 1994;28:325-31.
44. Rainsford KD. Diet, eicosanoids and chemical toxicity. In: Parke DV, Ioannides C, Walker R, eds. *Food, nutrition and chemical toxicity*. London: Smith-Gordon; 1993:171-80.
45. Gonzalez FJ, Nebert DW. Evolution of the P450 gene superfamily. *Trends Genet*. 1990;6:182-6.
46. Parke DV, Ioannides C, Lewis DFV. The role of cytochromes P450 in the detoxication and activation of drugs and other chemicals. *Can J Physiol Pharmacol*. 1991;69:537-49.
47. Ioannides C, Parke DV. The cytochrome P4501 gene family of microsomal haemoproteins and their role in the metabolic activation of chemicals. *Drug Metab Rev*. 1990;22:1-86.
48. Manns MP, Griffin KJ, Quattorchi LC et al. Identification of cytochrome P4501 A2 as a human autoantigen. *Arch Biochem Biophys*. 1990;280:229-32.
49. Kushimoto K, Nagasawa K, Udea A et al. Liver abnormalities and liver membrane autoantibodies in systemic lupus erythematosus. *Ann Rheum Dis*. 1989;48:946-52.
50. Neuberger J, Williams R. Immune mechanisms in tienilic acid associated hepatotoxicity. *Gut*. 1989;30:515-19.
51. De Leve LD, Kaplowitz N. Glutathione metabolism and its role in hepatotoxicity. *Pharmac Ther*. 1991;52:287-305.
52. Robinson MK, Rodrick ML, Jacobs DO et al. Glutathione depletion in rats impairs T-cell and macrophage immune function. *Arch Surg*. 1993;128:29-35.
53. Esposito F, Agosti V, Morrone G et al. Inhibition of the differentiation of human myeloid cell lines by redox changes through glutathione depletion. *Biochem J*. 1994;301:649-53.
54. Kilbourne EM, Rigau-Perez JG, Heath CWJ. Clinical epidemiology of toxic oil syndrome: manifestations of a new illness. *N Engl J Med*. 1983;309:1408-14.
55. Posada de la Paz M, Philen RM, Borda IA. Manufacturing processes at two French rapeseed oil companies: possible relationship to toxic oil syndrome in Spain. *Food Chem Toxicol*. 1991;29:797-803.
56. Kilbourne E, Bernet JJJ, Posada de la Paz M. Chemical correlates of pathogenicity of oils related to the toxic oil syndrome epidemic in Spain. *Am J Epidemiol*. 1988;127:1210-27.
57. Tabuenca JM. Toxic-allergic syndrome caused by the ingestion of rapeseed oil denatured with aniline. *Lancet*. 1981;2:567-8.
58. Pestana A, Munoz E. Anilides and the Spanish toxic oil syndrome. *Nature*. 1982;298:608.
59. Maneta-Peyret L, Picard J-P, Beziau J-H, Cassagne C. Fatty acids rendered immunogenic. *Immunol Lett*. 1992;31:227-31.
60. Kaufman LD, Seidman RJ. L-Tryptophan-associated eosinophilia-myalgia syndrome: perspective of a new illness. *Rheum Dis Clin N Am*. 1991;17:427-41.
61. Katz JD, Wakem CJ, Parke AL. L-Tryptophan associated eosinophilia myalgia syndrome. *J Rheumatol*. 1990;17:1559-61.

62. Mayeno AN, Lin F, Foote CS. Characterisation of 'peak E' a novel amino acid with the eosinophilia-myalgia syndrome. *Science*. 1990;250:1707-8.
63. Dröge W. Cysteine and glutathione deficiency in AIDS patients: a rationale for treatment with *N*-acetylcysteine. *Pharmacology*. 1983;46:61-5.
64. Haverkos HW, Kopstein AN, Wilson H, Drotman P. Nitrite inhalants: history, epidemiology, and possible links to AIDS. *Envir Health Persp*. 1994;102:858-61.
65. Newell GR, Adams SC, Mansell PWA, Hersh EM. Toxicity, immunosuppressive effects and carcinogenic potential of volatile nitrites: possible relationship to Kaposi's syndrome. *Pharmacotherapy*. 1984;4:284-91.
66. Meloche BA, O'Brien PJ. *S*-Nitrosylglutathione-mediated hepatocyte toxicity. *Xenobiotica*. 1993;23:863-71.
67. Buettner GR. The pecking order of free radicals and antioxidants: Lipid peroxidation,  $\alpha$ -tocopherol, and ascorbate. *Arch Biochem Biophys*. 1993;300:535-43.
68. Chow CK. Vitamin E and oxidative stress. *Free Rad Biol Med*. 1991;11:215-32.
69. Munday R, Winterbourn CC. Reduced glutathione in combination with superoxide dismutase as an important biological antioxidant defence mechanism. *Biochem Pharmacol*. 1989;38:4349-52.
70. Diplock AT. Low dietary selenium and its relationship to human disease. In: Parke DV, Ioannides C, Walker R, eds. *Food, nutrition and chemical toxicity*. London: Smith-Gordon; 1993:395-402.
71. Saba TM, Fortune JB, Wallace JR. Microaggregation hypothesis of multiple system organ failure. In: Fry DE, ed. *Multiple system organ failure*. St Louis: Mosby Year Book; 1992:25-41
72. Preece NE, Hall DE, Howarth JA, King LJ, Parke DV. Effects of acute and sub-chronic administration of iron nitrilotriacetate in the rat. *Toxicology*. 1989;59:37-58.
73. Liu PT, Symons AM, Parke DV. Autoxidative injury with loss of cytochrome P-450 following acute exposure of rats to fasting and ether anaesthesia. *Xenobiotica*. 1991;21:205-15.
74. Liu PT, Symons AM, Parke DV. The effects of fasting and ether anaesthesia on hepatic and renal function in surgical trauma. In: Parke DV, Ioannides C, Walker R, eds. *Food, nutrition and chemical toxicity*. London: Smith-Gordon; 1993:385-94.
75. Liu PT, Ioannides C, Shavilla J, Symons AM, Parke DV. Effects of ether anaesthesia and fasting on various cytochromes P450 of rat liver and kidney. *Biochem Pharmacol*. 1993;45:871-7.
76. Liu PT, Kentish PA, Symons AM, Parke DV. The effects of ether anaesthesia on oxidative stress in rats - dose response. *Toxicology*. 1993;80:37-49.
77. Babcock WW, ed. *Principles and practice of surgery*. Philadelphia: Lea and Febiger; 1994:5-55.
78. Bourne W. Anaesthetics and liver function. *Am J Surg*. 1936;34:486-92.
79. Lewis DFV, Ioannides C, Parke DV. Molecular orbital studies of oxygen activation and mechanisms of cytochrome P450-mediated oxidative metabolism of xenobiotics. *Chem Biol Interact*. 1989;70: 263-80.

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