Protein oxidation at the air-lung interface

Review Article

F. J. Kelly and I. S. Mudway

Air Pollution and Health Research Group, School of Health & Life Sciences, King’s College London, London, United Kingdom

Received December 28, 2002
Accepted May 8, 2003
Published online July 31, 2003; © Springer-Verlag 2003

Summary. Whilst performing its normal functions the lung is required to deal with a range of toxic insults. Whether these are infectious agents, allergens or air pollutants they subject the lung to a range of direct and indirect oxidative stresses. In many instances these challenges lead to oxidative alterations of peptides and proteins within the lung. Measurement of protein oxidation products permits the degree of oxidative stress to be assessed and indicates that endogenous antioxidant defences are overwhelmed. The range of protein oxidation products observed is diverse and the nature and extent of specific oxidation products may inform us about the nature of the damaging ROS and NOS. Recently, there has been a significant shift away from the measurement of these oxidation products simply to establish the presence of oxidative stress, to a focus on identifying specific proteins sensitive to oxidation and establishing the functional consequences of these modifications. In addition the identification of specific enzyme systems to repair these oxidative modifications has lead to the belief that protein function may be regulated through these oxidation reactions. In this review we focus primarily on the soluble protein components of within the surface liquid layer in the lung and the consequence of their undue oxidation.

Keywords: Respiratory tract lining fluid – Lung – Protein carbonyl – Air pollution – α-tocopherol – Ascorbic acid – Glutathione

Abbreviations: ARDS, Acute Respiratory Distress System; BAL, Bronchoalveolar lavage; CF, Cystic Fibrosis; COPD, Chronic Obstructive Pulmonary Disease; DHA, Dehydropscorbate; EPO, Erythropoietin; ERK, Extracellular signal-regulated kinase; GSH, Reduced Glutathione; GSSG, Oxidised Glutathione; HIV, Human Immunodeficiency Virus; MPO, Myelo peroxidase; NOS, Nitric oxide synthase; NL, Nasal lavage; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; RTLF, Respiratory Tract Lining Fluid; SOD, Superoxide dismutase; SP-A, Surfactant Protein-A; 3-NT, 3-Nitrotyrosine; UA, Uric acid

Introduction

The lung, like the skin (Thiele, 2001) and the surface of the eye (Rose et al., 1998), presents the first physical interface between the outside environment and the body. As such it is susceptible to a range of environmental toxicants, both viable, such as bacteria, viruses, allergen and fungi, and non-viable, pollutant oxidant gases (ozone, nitrogen dioxide) and airborne particulate matter. Whilst these toxicants are diverse, in all cases their pathophysiological action on the lung seems to involve the imposition of an oxidative stress (the excess generation of reactive oxygen species (ROS) in relation to available antioxidant defences). This may occur in the case of oxidant gases through the direct oxidation of biomolecules at the surface of the lung (Pryor, 1994), or in the case of micro-organisms because they engender airway inflammation (Morcillo et al., 1999; Babior, 2000). All pulmonary conditions where inflammation has been implemented (asthma, COPD, cystic fibrosis) appear to involve a component of oxidative stress. In this review we examine the impact of these challenges on the lung, focusing specifically on protein oxidation events within the thin amphipathic layer overlaying the respiratory epithelium: the respiratory tract lining fluid (Bastacky et al., 1995; van der Vliet et al., 1999). In this context the endogenous antioxidant defences within this compartment will be outlined, as are the adaptive antioxidant pathways which function to mitigate against oxidative injury. Central to our review of the current literature will be the issue of whether protein oxidation is simply a biomarker of oxidative stress (allowing some discrimination of the radical species involved) or actually a mechanistically significant event, with major impacts on protein function and lung physiology. Whilst a considerable body of work has been performed on pulmonary tissues, examining protein oxidation in a variety of disease settings, comparatively little
work has focused on protein modifications within the RTLF. This, allied to the critical position of this compartment suggests that identification of oxidatively modified proteins within this compartment is likely to represent a fertile area of future research. Whilst much of the focus of this review will be on the soluble protein components of RTLF we will also deal with the oxidation of proteins within lung tissue itself.

The respiratory tract lining fluid

This aliphatic compartment consists of a two-layer structure, comprising a lower aqueous sol phase and an upper mucus, gel phase. The gel phase is largely composed of high molecular weight mucopolypeptide glycoproteins derived from sub-mucosal glands (Quinton, 1979). Whilst these compounds may act as sacrificial antioxidants in vivo due to their thiol content (van der Vliet and Cross, 2001) their main function is to trap microorganisms and large particles from the air-stream. These particles are then transported by mucociliary action to the posterior pharynx and swallowed; therefore acting as a mechanical clearance mechanism to limit the penetration of inhaled toxicants into the lung. As a consequence of this action the gel phase of the RTLF is continually turning over. The lower, sol phase which has been shown to have a wide range of antioxidant defences (van der Vliet et al., 1999; Cantin et al., 1987; Mudway et al., 2001) in contrast turns over relatively slowly, permitting secreted antioxidants to concentrate within this compartment (Cantin et al., 1987). In the distal airways, where mucus-secreting cells are absent, this layer is the first physical interface inhaled materials come into contact with. Furthermore, in airway inflammation the majority neutrophil, eosinophil, and macrophage actions occur within this compartment. Therefore much of the discussion in this review focusing on the antioxidant defences within the RTLF relates to events occurring with the sol compartment. Not all aspects of lung lining fluid are currently understood. For example, although it is appreciated that the depth of the fluid layer varies along the respiratory tree, its precise thickness is still under debate (Duneclift et al., 1997; Widdicombe et al., 1997). In the upper airways, lung-lining fluid may be 1–10 μm thick compared with 0.2–0.5 μm in the distal bronchoalveolar regions. In addition whether the RTLF comprises a continuous sheet covering the pulmonary epithelium throughout its length, as supported by high resolution EM-studies (Bastacky et al., 1995), or is patchy toward the more distal structures (Pryor et al., 1995) remains a point of contention. Both of these issues are critical in understanding the nature of oxidation reactions occurring at the lung surface as the RTLF may prevent or limit the extent of the reaction of inhaled toxicants with the lung epithelium.

RTLF can be obtained from human volunteers and patients using a variety of bronchoscopy based techniques, bronchial wash and bronchoalveolar lavage (BAL). More recently the use of less invasive techniques have also become widely employed: nasal lavage, (Noah et al., 1995; Housley et al., 1995), sputum induction with hypertonic saline (Nordenhall et al., 2001; Jones et al., 2001), and the condensation of exhaled breath (Antczak et al., 2002). There is currently little data to demonstrate the quantitative relationship between indirect proximal airway measures of pulmonary inflammation with the more traditional gold standard bronchoscopy based techniques. Despite this important proviso, these less invasive techniques have permitted more detailed time course studies in human investigations, and investigation of inflammatory responses in patients with severe airways disease such as asthma and COPD. These procedures, with the exception of the collection of breath condensate yield both cell pellets and dilute samples of the RTLF after separation of these components by centrifugation. In addition bronchoscopy based techniques often incorporate the collection of bronchial biopsies. These samples can therefore all be analysed for antioxidant concentrations and a range of oxidation markers. Collection and analysis of the RTLF in lavage fluid supernatants however presents a number of problems: First, the RTLF proteome may be contaminated with intra-cellular and plasma proteins if the lavage, or post aspiration processing is too robust. Second, prolonged saline, or PBS dwell times in the lung can result in erroneously high antioxidant concentrations, as these molecules move down a concentration gradient from cellular stores. Finally, unless lavage is performed rapidly, pH and contaminating transition metal sources are tightly controlled and samples are processed and stored appropriately, RTLF components may become oxidised due to the lavage procedure.

The RTLF proteome

The protein constituents of the RTLF have been reviewed in detail previously (Hatch, 1992), however recent developments in proteomics using 2D-gel maps of lavage fluid samples have produced a fuller picture (Noel-Georis et al., 2002; Lenz et al., 1993; Sabounchi-Schutt et al., 2001; Lindahl et al., 1995; Wattiez et al., 1999). These techniques have allowed the identification of protein profiles
characteristic in a range of pulmonary diseases (sarcoidosis, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis) and following acute airway insults (cigarette smoke) (Wattiez et al., 1999; Lindahl et al., 1996; Lenz et al., 1993; Lindahl et al., 1998; Lindahl et al., 1999a, b).

BAL protein components are diverse and derived from a variety of sources: local synthesis and export by epithelial and resident inflammatory cell populations and via transduction of plasma proteins across the blood-air barrier. Proteins constitute approximately 0.5–1% of the dry weight of bronchial secretions (Creeth, 1978), and total protein concentrations in the RTLF have been reported to be in the order of 3–5 mg/mL in healthy human subjects (Rennard et al., 1990; Thompson et al., 1990). These values are derived from BAL-fluid concentrations corrected for lavage dilution using the urea correction. Whilst there are acknowledged problems with this dilutional control (Haslam et al., 1999), this is the most widely used approach in the literature and does give a indication of overall dilution, as long as the lavage dwell time is short (van der Vliet et al., 1999).

The major protein constituents of RTLF are albumin (50–60% of the total protein pool), transferrin (5–6%), α1-antitrypsin (3–5%) and the immunoglobulins A and G (30%) (Hatch, 1992; Bell et al., 1981; Blomberg et al., 1999). The ratio of these major protein species varies depending on the compartment sampled, with albumin concentrations lower (12–20%), and lysozyme (15–35%) and lactoferrin (2–4%) concentrations higher in the nasal and bronchial RTLFs. These regional differences are also apparent in the expression of specific proteins, such as surfactant proteins in the distal lung, and mucus in the upper airways. Proteomic analysis by the group of Bernard has identified over 1200 silver stained spots, corresponding to approximately 78 protein species on 2D-gels of BAL-fluid proteins (Wattiez et al., 1999). Classification of this large number of proteins has been made on the basis of their origin: proteins derived from the plasma pool via transduction onto the surface of the lung, proteins synthesised in the lung, but also present in the plasma, and proteins unique to the surface of the lung. Using this approach it has been shown that the vast majority of the proteins found within the nasal RTLFs are derived from the plasma, whilst the distal lung has a significant complement of lung specific proteins (Noel-Georis et al., 2002). BAL proteins can also be classified according to their function: proteases/anti-proteases (α1-trypsin, α1-Antitrypsin, α1 antichymotrypsin, α1-antiplasmin, α1-antiprotease, α2-Macroglobulin, secretory leukoprotease inhibitor); antioxidant enzymes (AOEB166, superoxide dismutase, glutathione S transferase, thioredoxin, plasma glutathione peroxidase), metal-chelating proteins (transferrin, lactoferrin, ceruloplasmin); surfactant proteins (SP-A/B/C and D), proteins involved in tissue repair and cellular proliferation (fibronectin, calycyclin, calgizzarin, cathepsin D, zinc-α-2 glycoprotein, tranthryretin, clusterin, intestinal trefoil factor, α2-HS-glycoprotein); regulating airway immune responses (IgG, IgA, Ig-binding protein, complement fragments 3 and 4, lipocortin-1, Clara cell protein 16, lipocalin, α1-acid glycoprotein); anti-microbial proteins (defensins, cystatin S); lipid metabolism (acyl-CoA-binding protein, FABP-E, FABP-A, saposin D); and calcium binding (calvaskulin, calrectilin, calgranulin A). Detection of certain proteins by this method is problematic due to issues of hydrophobicity, low molecular mass of certain proteins and also low expression levels. In addition some of the proteins detected are clearly intra-cellular (γ- and β-actin, tubulin β2, tropomyosin, haemoglobin) suggesting cellular lysis. This list is far from exhaustive and does not include the wide range of cytokines/chemokines (IL-1/4/5/6/8/10/18, TNF-α, Gro-α, Rantes) and inflammatory cell activation markers (myeloperoxidase, eosinophil peroxidase, eosinophil cationic protein, and s-Selectin) that have been detected in BAL-fluid, both basally and during inflammatory episodes.

Antioxidant defences within the RTLF

Reactive oxygen (hydroxyl radicals, superoxide, and hydrogen peroxide) and nitrogen species (nitric oxide, nitrite, and peroxynitrite) are produced in the body as a part of normal metabolism: through leakage from electron transport chains, as by-products of a number of enzyme reactions (cytochrome-P450, lipooxygenase, cyclooxygenases, peroxi- dases), and through secretion from activated leukocytes. Whilst these species may play a modulating role in a range of physiological functions (Conner and Grisham, 1996), over production in a range of conditions has been shown to cause tissue injury (Repine et al., 1997). Endogenous antioxidant defences offset the potential damaging action of these reactive oxygen and nitrogen species. These may either act to directly detoxify these species or by converting them to less damaging species that can be removed by other components of the antioxidant network. However, if these defences are overwhelmed damage to cellular components will occur. By virtue of its large surface area and daily exposure to inhaled toxins the lung is particularly susceptible to oxidative and nitrosative injury. A number of other unique features of this compartment may also predispose it to oxidative injury. First, there is evidence, that the RTLF,
As oxalate is cytotoxic, most cells contain a variety of dehydratare oxidizing agents and scavengers. This includes the enzymatic antioxidants described in the previous section but also a range of small molecular weight antioxidants that act as direct free radical scavengers. It is important to point out that, in the context of expanding this discussion into the oxidation of proteins to state that the RTLF antioxidant network should not however be thought of as a static pool opposing any imposed oxidative insult. Instead the defenses within this compartment should be thought of as dynamic equilibrium with the antioxidant defenses within the epithelium and the more remote plasma pool. The most characteristic features of all oxidative challenges are the range of adaptive antioxidant responses observed; either through increased synthesis (Rahman and MacNee, 2000) or the enhanced movement of reducing equivalents to the RTLF (Freed et al., 1999). A fine balance therefore exists between basal antioxidant levels, oxidant fluxes and adaptive modifications to antioxidant reserves all of which modulate the capacity of ROS and RNS to oxidise protein amino acids.

**Ascorbic acid**

As a consequence of its high water solubility, L-ascorbate (vitamin C) is widely distributed throughout the body, including the RTLF. Ascorbate is an excellent reducing agent and scavenges a variety of free radicals and oxidants in vitro, including superoxide, peroxyl radicals, hydrogen peroxide, hypochlorous acid, singlet oxygen, ozone (Mudway and Kelly, 1998; Frei et al., 1989; Langford et al., 1995) and nitrogen dioxide (Kelly and Tetley, 1997; Postlethwait et al., 1995). During this scavenging activity, ascorbate loses one electron resulting in the formation of the semi-dehydroascorbate radical (Buettnner and Jurkiewicz, 1996), which rapidly decomposes to dehydroascorbate and a range of further oxidation products, terminating in oxalate. As oxalate is cytotoxic, most cells contain a variety of dehydroascorbic reductase enzymes that catalyse the regeneration of ascorbate from dehydroascorbate at the expense of GSH and NADPH (Wang et al., 1997; Washburn and Wells, 1999; May et al., 1997). Notably, whilst a range of enzymes has been reported to have DHA-reductase activity intracellularly (glutaredoxin, NADPH-dependent-3α-hydroxysteroid DHA reductase, erythrocyte glutathione peroxidase) only glutathione peroxidase has been reported in the RTLF. GSH can regenerate ascorbate from DHA non-enzymatically (Winkler et al., 1994) but again this is unlikely at RTLF concentrations. These two observations suggest that the ascorbate pool on the surface of the lung is likely to be highly liable, a contention further supported by the presence of non-transferrin bound iron in this compartment (Gutteridge et al., 1996), and basal hydrogen peroxide concentrations (Kharitonov and Barnes, 2002). Thus maintenance of adequate ascorbate concentrations at the lung surface require it to be rapidly transported from cellular sources or from the plasma pool. Which of these pathways occurs is currently not understood but absence of a clear association between plasma and lavage fluid ascorbate concentrations (over a wide range of plasma concentrations, 20–100 μM) suggests that simple diffusion from the plasma pool does not explain RTLF levels. Further, undiluted RTLF concentrations have been reported at 100–400 μM (Cantin et al., 1987; van der Vliet et al., 1999) above plasma saturation levels, suggesting that a simple diffusion gradient does not exist. In addition to the direct scavenging action of ascorbate it also acts indirectly to prevent lipid peroxidation through its reaction with membrane-bound tocopherol. In vitro studies, it has been demonstrated that ascorbate is able to reduce the tocopherol radical back to tocopherol, thereby restoring its scavenging activity (Halpner et al., 1998). This synergistic action of ascorbate though clearly demonstrated in vitro, has importantly, not yet been conclusively demonstrated in vivo. Whilst ascorbate has many antioxidant actions, it also has the capacity to act as a pro-oxidant in the presence of transition metals. The presence of transition metals absorbed onto their surface may be due to the presence of transition metals absorbed onto their surface (Ghio and Devlin, 2001). One might therefore speculate that these particles may actually subvert much of the protective character of the RTLF, with ascorbate acting as a pro-oxidant, resulting in tissue injury, instead of protecting against it.

**Glutathione and glutathione enzymes**

RTLF contains high concentrations of glutathione (L-γ-glutamyl-L-cysteinylglycine) at concentrations approximately
100 times greater than those in plasma, and in a predominately (>90% GSH) reduced form (Cantin et al., 1987). Initial estimates of the concentration of GSH in undiluted RTLF were approximately 400 μM (Cantin et al., 1987 #869), but concentrations are now considered to range between 100–200 μM (van der Vliet et al., 1999), with the concentration of GSSG being critically dependent on the magnitude of airway inflammation, but typically <90% of the total pool (Cantin et al., 1987). A number of cell types, alveolar macrophages, Clara cells and alveolar Type II cells have been reported to contain high levels of glutathione (4.8, 3.2 and 0.54 mM, respectively (Horton et al., 1987). Poor re-absorption of glutathione from the respiratory tract is also important. The cell surface enzyme responsible for the uptake of glutathione from the extra cellular space, γ-glutamyl transpeptidase is present in much lower concentrations in the lung compared with other organs but may be located in specific epithelial cells such as Clara and Type II cells (Dinsdale et al., 1992). RTLF is also replenished relatively slowly therefore any glutathione exported into this compartment is likely to remain there for prolonged periods. Glutathione is particularly good at defending against inflammatory cell derived oxidants such as superoxide, hypochlorous acid and hypobromous acid (Winterbourn, 1985). Though GSH concentrations are high in the RTLF, the glutathione redox cycle is not operative in this compartment due to the absence of glutathione reductase. Thus any GSSG formed either directly through the reaction of GSH with ROS/RNS, or through the detoxifying actions of glutathione peroxidase will tend to accumulate. Whilst glutathione reductase has been reported in lavage in some studies its presence has generally been taken as evidence of cell lysis. Therefore glutathione probably functions more in a sacrificial mode in the RTLF, reacting directly with ROS with the production of thiyl radicals (GS·) which can subsequently be converted to glutathione disulphide (GSSG) through a radical transfer process. Thiyl radicals should not however be considered wholly harmless as they have been shown to react with other targets propagation free radical induced injury (Pryor, 1994; Kalyanaraman, 1995).

In addition, the concentration and activity of plasma glutathione peroxidase is also influenced by oxidative stress. Its activity in the RTLF of smokers is increased (Melloni et al., 1996), while decreased activities are seen after ozone exposure (Avissar et al., 2000), and unaltered activities following exposure to nitrogen dioxide (Avissar et al., 2000). Recent studies have also provided evidence for GSH-dependent enzymes glutathione S transferase and thioredoxin within the RTLF (Noel-Georis et al., 2002). The role of these two enzymes within this compartment has not currently been investigated in human subjects. Glutathione S transferases represent a family of enzymes responsible for the detoxification of endobiotic and xenobiotic compounds by covalently linking them to glutathione. It is therefore possible that they may play a significant role in the detoxification of organic radicals inhaled in cigarette smoke (Squadrito et al., 2001) and introduced into the lung on the surface of inhaled particulates (Dellinger et al., 2001). This function if operative in the RTLF could explain the reported loss of GSH in certain acute lung insults where there is no evidence of an increase in GSSG. Thioredoxin catalyses thiol disulphide exchange reactions and is therefore critical in the regulation of protein function by thiol redox control. Given the propensity for numerous RTLF proteins to undergo thiol modification under oxidative stress, investigations into the regulation of this enzyme in a number of pulmonary conditions is clearly warranted.

**Uric acid**

Uric acid is also an important antioxidant in RTLF (Ames et al., 1981) especially in the upper airways where its concentration is high (Peden et al., 1990). Uric acid is an oxidised purine base which is an effective scavenger of hydroxyl radicals, ONOO−, and oxyhaem oxidants formed between the reactions of haemoglobin and peroxyl radicals, peroxyl radicals themselves and singlet oxygen (Becker, 1993). It acts in these reactions in a sacrificial mode, in that it is irreversibly damaged through the interaction producing a range of oxidation products including allantoin (Ames et al., 1981). It is also a highly effective scavenger of both inhaled ozone (Mudway et al., 1996; Mudway et al., 1999) and nitrogen dioxide (Kelly and Tetley, 1997). From our observations to date, we have noted that RTLF uric acid concentration seems to be associated closely with protein concentration. This seems to be the case at all levels of the respiratory tract. This finding infers that RTLF uric acid comes from either a direct flux from the plasma pool (Becker, 1993; Housley et al.,
or alternatively some UA may be stored and then actively secreted from sub-mucosal glands (Peden et al., 1993).

**α-Tocopherol**

α-Tocopherol (vitamin E) is present within RTLF, albeit at relatively low concentrations: 50–200 nM. It is thought that this α-tocopherol is secreted by type II cells in to RTLF along with surfactant (Rustow et al., 1993). α-Tocopherol is a powerful antioxidant, both in terms of its direct free radical scavenging activity, and through its ability to terminate lipid peroxidation (Burton et al., 1981; Burton et al., 1983). Vitamin E functions as a chain-breaking antioxidant in the lipid phase. Evidence of this function is however indirect, focusing almost entirely on observations made using in vitro systems (Burton et al., 1983). It is thought that reactivity with organic peroxyl radicals accounts for the majority of the biological activity of α-tocopherol (Burton et al., 1981). This reaction is of considerable importance because tocophersols reacting with lipid peroxyl radicals yield a relatively stable lipid hydroperoxide and a vitamin E radical, which effectively interrupts the lipid peroxidation chain reaction (McCay, 1985).

**Extra-cellular SOD**

Whilst 2D-Gel maps have reported the presence of Mn-SOD (Noel-Georis et al., 2002) in RTLF it is likely that this antioxidant enzyme is present as a contaminant due to cell lysis during lavage and lavage fluid processing. RTLF has also been shown to contain appreciable concentrations of extracellular SOD, both in nasal (Mudway et al., 1999) and BAL-fluid samples (Mudway et al., 2001) presumably closely associated with proteoglycan at the epithelial surface. The association of this antioxidant enzyme with epithelial and endothelial surfaces may be significant as it may function to limit the production of peroxynitrite in local environments in which superoxide and nitric oxide are produced.

**Metal chelating proteins**

The RTLF has also been shown to contain a range of metal binding proteins: caeruloplasmin, lactoferrin, transferrin, (Pacht and Davis, 1988; Noel-Georis et al., 2002). These chelators perform an important antioxidant function in vivo by regulating free iron concentrations and hence limiting the potential for Fenton-like chemistry, with the formation of the damaging hydroxyl radical. Despite detectable concentrations of these proteins in lavage fluid from healthy control subjects (Gutteridge et al., 1996), and evidence of their upregulation during a variety of pulmonary conditions and acute oxidant insults (Gutteridge et al., 1996; Nelson et al., 1996; Ghio et al., 1998), there is evidence that RTLF contains a significant ‘free’ non-transferrin bound iron pool (Gutteridge et al., 1996; Nelson et al., 1996), which is available for potential redox reactions.

**Distribution of antioxidants within the respiratory tract**

Although it is clear that there are differences in antioxidants between different regions of the respiratory tract, the extent of these differences has not yet been clarified. In the nasal cavity, uric acid is by far the most prevalent antioxidant (Peden et al., 1990; Housley et al., 1995). It should be noted however that there are gender differences with women having significantly lower levels of uric acid than men (Housley et al., 1996). A similar gender difference exists for plasma concentrations of uric acid, indicating that the major source of this antioxidant in NL is from plasma. However, Peden et al. demonstrated that in the nasal cavity, RTLF Sol phase uric acid concentration is increased following cholinergic stimulation of the airways (Peden et al., 1991). Furthermore, they found that the increase in uric acid correlated positively with lactoferrin concentration, but not with albumin. As lactoferrin is predominately derived from mucosal gland secretions (Gel phase) this supports the contention that RTLF uric acid in the upper airways is derived, in part, from glandular secretions. Control of uric acid secretion in this compartment may therefore be closely related to mucus secretion. Glutathione and ascorbic acid are also present in nasal cavity RTLF however at significantly lower concentrations. Furthermore, although not always the case, low concentrations of α-tocopherol are sometimes observed. In the peripheral airways (BL fluid) and bronchoalveolar region (BAL fluid) uric acid, ascorbic acid, GSH and α-tocopherol are usually present.

**ROS and RNS generation in the RTLF**

The respiratory tract is exposed to a variety of ROS and RNS, predominately through exposure to exogenous oxidants (ozone, nitrogen dioxide) and inflammation. During inflammatory processes a number of cell types can release ROS and RNS. Neutrophils, eosinophils, B-lymphocytes and monocytes (but not macrophages, except under special circumstance) all contain the NADPH oxidase enzyme system which catalyses the production of superoxide from
oxygen and NADPH (Babior, 2000). In phagocytic cell populations the superoxide, released into the extra-cellular environment by this enzyme, is involved in the destruction of microorganisms. Superoxide is relatively unstable, though unreactive (redox potential $-330 \text{ mV}$) radical, which will react to hydrogen peroxide, in SOD catalysed and un-catalysed reactions. Whilst superoxide is relatively unreactive it will participate in the liberation of $\text{Fe}^{2+}$ from ferritin (Harris et al., 1994), and aconitase (Gardner et al., 1995) with a loss of protein function and release potential dangerous ‘free’-iron. Ultimately therefore hydrogen peroxide is the predominant oxidant formed by phagocytes. Hydrogen peroxide is also a relatively weak oxidising species, which allied to cellular glutathione peroxidase and catalase defences means its toxicity \textit{in vivo} is not thought to be significant. Hydrogen peroxide can also be produced directly by a variety of oxidase enzymes including xanthine oxidase, monoamine, and amino acid oxidase. The damaging actions of hydrogen peroxide are therefore predominately related to its reduction to a variety of more damaging oxidant species. Hydrogen peroxide is a key substrate for the neutrophil derived myeloperoxidase, and the eosinophil derived, eosinophil peroxidase enzymes which catalyses the oxidation of halide anions (MPO, Cl$^-$, Br$^-$ and I$^-$; EPO, Br$^-$ and I$^-$ only) to form hypochlorous (HOCl) and hypobromous acid (HOBr) (Foote et al., 1983; Thomas et al., 1995). These highly reactive oxidants have been shown to react with thiols, and thiolethers (Visser and Winterbourn, 1995), halogenate nucleotides, and with amine groups to form halamines which may have even greater reactivity than the parent radical (Thomas et al., 1983).

Hydrogen peroxide can also undergo a transition metal catalysed reaction to the highly damaging hydroxyl radical. A range of biological reductants including ascorbate can then reduce the metal oxidised in this reaction. This reaction is critically dependent on the presence of ‘free’ $\text{Fe}^{2+}$ and Cu$^+$. \textit{In vivo}, these metals are typically sequestered into a range of proteins: ferritin, transferrin, lactoferrin, ceruloplasmin etc. Therefore whether this is a significant pathway \textit{in vivo} is questionable. Hydroxyl radicals can also be formed in the reaction between HOCl and superoxide but again the significance of this pathway \textit{in vivo} has not been fully established (Kettle and Winterbourn, 1994). Whilst the hydroxyl radical is highly reactive, not only does its formation require the presence of ‘free’ or unchelated iron, but such is its rate of reaction with a broad spectrum of organic molecules that its toxic action will tend to be diluted out by its very promiscuity. Therefore, whilst undoubtedly highly reactive, the hydroxyl radical is more likely to interact with non-vital cellular components than critical targets.

The hydrophobic gas nitric oxide (NO) is produced endogeneously \textit{in vivo} where it plays a role in a range of important functions in the maintenance of normal airway physiology and host defense: modulating the expression of inflammatory cytokines, adhesion molecules and enzymes of the eicosanoid biosynthetic, as well as having a modulating influence on a range of antioxidant systems (Halliwell et al., 1999; Brennan and Moncada, 2002). Nitric oxide is produced from L-arginine by a variety of cells within the airway, both epithelial (type II pneumocytes) and inflammatory (macrophages, neutrophils, mast cells) through the action of constitutive ($\text{Ca}^{2+}$ dependent, short term, low capacity) and inducible ($\text{Ca}^{2+}$ independent, long term, high capacity) forms of nitric oxide synthase. The expressions of these enzymes are increased during inflammation with concomitant increases in exhaled NO, as observed in asthmatics (Kharitonov et al., 1997), in patients with bronchiectasis, during airway infections and following inhalation of oxidant gases (Balint et al., 2001; Kharitonov and Barnes, 2002; Kharitonov and Barnes, 2000; Nightingale et al., 1999; Kharitonov and Barnes, 2001). Similarly the end products of NO metabolism, nitrite ($\text{NO}_2^-$) and nitrate ($\text{NO}_3^-$) are increased in RTLF in these conditions (Balint et al., 2001). Whilst most of the NO detected in exhaled breath is derived from the nasal airways and sinuses it is clear that NO can be produced throughout the length of the airway. Although a free radical itself, NO is not particularly reactive allowing it to diffuse readily \textit{in vivo} from its site of production to its site of action. It can however react with a range of other radical species (superoxide, thiol, lipid and protein radicals) to generate a variety of highly damaging radical intermediates of considerably greater toxicity than itself.

Nitric oxide can react with oxygen to form nitrogen oxides: the powerful oxidant nitrogen dioxide ($\text{NO}_2$), as well as nitrite ($\text{NO}_2^-$) and nitrate ($\text{NO}_3^-$) but these reactions are slow and not favoured \textit{vivo} (Beckman and Koppenhol, 1996). Instead much interest has focused on the rapid reaction between NO and superoxide (out competing its dismutation by SOD) with the formation of the highly damaging oxidant species: peroxynitrite (ONOO$^-$) (Beckman et al., 1994). It is also clear that both the inflammatory cells of the airways and the epithelium itself are capable of producing both NO and superoxide (Munoz-fernandez et al., 1992). It is now believed that much of the toxicity previously ascribed to the hydroxyl, superoxide and nitric oxide \textit{in vivo} is now often attributed to peroxynitrite and its derivatives (Tien et al., 1999). The ability
of ONOO\(^{-}\) to nitrite proteins as well as induce lipid peroxidation and DNA damage has been demonstrated \textit{in vitro}. In contrast to the hydroxyl radical the rate with which peroxynitrite will react with biomolecules is rather slow, favouring selective reactions with its major targets: sulphydryls (Stamler et al., 1992), iron/sulphur centres (Hausladen and Fridovich, 1994) and zinc-fingers. Peroxynitrate is surprisingly unreactive toward reduced glutathione and is therefore not an effective scavenger for this radical. Indeed unlike most oxidants, peroxynitrite will oxidize protein targets without having to first deplete endogenous antioxidant defences (van der Vliet et al., 1994). This potent species will participate in a wide range of oxidation reactions including the oxidation of protein and non-protein sulphydryls, deoxyribonucleic acid and lipids, as well as nitration of tyrosine residues by catalysed (low molecular weight transition metals and superoxide dismutase) and un-catalysed reactions.

Peroxynitrite will also undergo protonation in acid environments to produce the strongly oxidizing peroxynitrous acid radical, which decomposes to nitrate via intermediate formation of the hydroxyl radical and a nitrogen dioxide-like species (Beckman et al., 1990). This may represent a significant nitrating species on the surface of the lung of asthmatic subjects where RTLF has been demonstrated to be acidic (Hunt et al., 2000). Peroxynitrite will also react rapidly with CO\(_2\) to form nitrogen dioxide and the CO\(_2\) adduct nitrosperoxocarbonate (ONOOCO\(_2\)^\(-\)), which has been proposed to represent the major damaging peroxynitrate derived intermediate formed under physiological conditions (Tien et al., 1999). The ability of ONOO\(^{-}\) to nitrite proteins as well as induce lipid peroxidation and DNA damage has been demonstrated both \textit{in vitro} and \textit{in vivo}. It therefore appears that peroxynitrite, and its derivatives, is one of the major damaging oxidants formed \textit{in vivo}, and certainly accounts for much of the toxicity associated with the increased production of nitric oxide. Notably, in the absence of these physiological catalysts the rates of nitration seen in model systems are low and indeed diminish significantly as addition components are added to the models (Ischiropoulos et al., 1992). Protein nitration does not always imply the local production of ONOO\(^{-}\). Nitration by ONOO\(^{-}\) catalysed by MnSOD occurs through a mechanism thought to involve the cleavage of the peroxide bond to produce a nitryl cation like intermediate. Due to the short half-life of this species it does not exist free.

NO can also be converted to the highly reactive NO\(_2\) radical \textit{in vivo} through various biological pathways: The auto-oxidation of NO, via one electron reactions with antioxidants, thiols, aromatic amino acids, unsaturated lipids (Pryor and Squadrito, 1995) and via the one electron reduction of nitrite. In addition under acidic conditions (gastric compartment, phagosomes, tissue acidosis) nitrite can be protonated to nitrous acid (HNO\(_2\)), which can decompose to NO and NO\(_2\) (Ohshima et al., 1990). Nitrite also acts as a substrate for MPO and EPO in a reaction producing the highly reactive NO\(_2\) radical. High concentrations of these peroxidases can be found at sites of leukocyte activation, where they have been shown to be associated with evidence of protein nitration (Heinecke, 1997). This pathway is dependent on the concentrations of nitrite (10–140\(\mu\)M in nasal and 10–15\(\mu\)M in alveolar RTLF). Significantly nitrite does not accumulate \textit{in vivo} being further metabolised to nitrate (Parks et al., 1981) but levels do increase during inflammatory episodes.

**Inhaled ozone, nitrogen dioxide and PM**

The lung is also susceptible to oxidative damage from exogenous oxidant species. The secondary pollutant gas, ozone, represents an excellent example in that through its powerful oxidising ability it will react with numerous biomolecules. Despite the broad target specificity of ozone, the rate at which reactions occur with different molecules can vary over several orders of magnitude (Pryor, 1993). Indeed when ozone encounters an array of potential substrates it reacts initially with those with the highest intrinsic reactivities toward it. Uric acid, ascorbic acid and glutathione have all been shown (in defined settings) to display high intrinsic reactivates toward ozone (Pryor et al., 1995; Cross et al., 1992; Mudway and Kelly, 1998; Postlethwait et al., 1994). It has therefore been proposed that RTLF antioxidants function as sacrificial substrates for ozone (Pryor et al., 1985). By reacting with ozone they effectively remove it with only the production of ostensibly harmless products. It should however be noted that thyl and ascorbyl radicals are not without some biological significance. When these defences are overwhelmed oxidation to lipid and protein moieties can occur. As ozone is relatively insoluble it is unlikely that unreacted ozone can penetrate the RTLF to directly oxidise epithelial cell membranes. Instead its toxicity is transmitted to the underlying cells through the generation of a range of lipid oxidation products (LOPs) (Pryor et al., 1995), which have been reported to have pro-inflammatory properties (Kafoury et al., 1999).

The primary gaseous pollutant nitrogen dioxide is also highly oxidizing and its uptake into the RTLF is also coupled to its reaction with RTLF substrates, predominately reduced glutathione and ascorbate (Postlethwait et al., 1994). This reaction results in the production of nitrate (HNO\(_2\)) and NO\(_2\) which is then further metabolised to nitrate and nitrate (Parks et al., 1981).
et al., 1991; Connor et al., 2001). Because absorption is directly coupled to these reactions the products clearly must drive the events leading to tissue injury. The initial reaction products of these reactions are the thyl and ascorbyl radicals and nitrite. Thyl and ascorbyl radicals are able to generate ROS through a variety of mechanisms (Buettner and Jurkiewicz, 1993; Miller and Aust, 1989; Scarpa et al., 1983). Thyl radicals may react with thiolated (GS\textsuperscript{−}) to yield superoxide and GSSG, or molecular oxygen to produce GSOO\textsuperscript{−} and GSO2OO\textsuperscript{−} (Buettner and Jurkiewicz, 1993). The reaction between NO\textsubscript{2} and ascorbate can also result in the generation of oxidants: the ascorbyl radical and nitrite. Whilst the latter comprises a substrate for heme peroxidase catalysed formation of RNS, the ascorbyl radical can react with free iron, ultimately resulting in the formation of hydrogen peroxide (Miller and Aust, 1989; Scarpa et al., 1983). Therefore the reaction of both of these pollutant gases with the RTLF can give rise to a variety of radical species.

Cigarette smoke also contains an extensive variety of free radicals and oxidant species (including NO\textsubscript{2}), both in the gas and tar phase (Kim et al., 2002) and therefore will both introduce directly radicals to the lung surface, as well as generate them as a result of oxidation reactions at the RTLF-air interface. Much recent work in the air pollution field has focused on the damaging role of inhaled particulate matter of an aerodynamic diameter of <10 \mu m. These particles induce oxidative stress within the lung via the introduction of transition metal (Ghio et al., 1998) and stable organic radicals (Squadrito et al., 2001) into the lung. All of these exogenous agents are also capable of eliciting acute inflammatory responses in the human lung and hence a secondary source of ROS and RNS (Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society, 1996).

Protein oxidation

Reactive oxygen and nitrogen species can react directly or indirectly with proteins to cause a range of oxidative modifications. The key question when addressing the significance of these modifications is whether they are simply markers for the presence of oxidative stress, or have some substantive consequence on protein function that impacts on tissue injury or disease progression. To achieve this it is important to show that the amino acid modification is related to altered function, and not simply a parallel event. Here we will describe the major oxidative modifications, which have been reported, specifically focusing on those studies where oxidation has been related to altered function. By necessity this will require a consideration of findings derived from other organ systems, as to date only protein nitrosylation, methionine oxidation and protein carbonyl formation have been dealt with in any detail in the lung, and then mostly focusing on BAL cells or tissue biopsies. In the previous section considering the RTLF proteome it was clear that the major proteins could be classified into four groups: those related to (a) protease/anti-protease balance, (b) antioxidant defence and metal ion chelation, (c) regulation of inflammatory response, and (d) tissue repair and remodelling. It is therefore reasonable to assume that if oxidation of these proteins in other body compartment alters function (under an appropriate stress), these findings will be of relevance to the RTLF compartment. These types of detailed analysis in lavage fluid are still hampered by the low total protein concentrations in this compartment, though the use of proteomic technologies should help to resolve these issues.

Reactive oxygen and nitrogen species and their secondary lipid and sugar oxidation products may interact with proteins to cause oxidation of the polypeptide backbone of the protein, peptide bond cleavage, protein-protein cross-linking, and a range of amino-acid side chain modifications. All amino acids are potential targets for oxidation by reactive oxygen and nitrogen species but the derived products have only been fully characterised in a few cases. In the physiological context the major aromatic amino acids tyrosine, tryptophan, phenylalanine, the sulphur containing amino acids, cysteine, methionine, as well as the aliphatic amino acids arginine, lysine, proline, and histidine are the major targets of ROS/RNS attack. Cysteine and methionine, the two sulphur-containing amino acids appear especially sensitive to oxidation.

Methionine oxidation

Methionine is easily oxidized to methionine sulfoxide derivatives in a mechanism mediated by a wide range of ROS (chloroamines, peroxynitrite, superoxide, hydrogen peroxide, hypochlorous acid) at physiological pHs. This oxidative modification can be reversed through the action of methionine sulfoxide reductase (Brot and Weissbach, 1983). Further oxidation products of methionine sulfoxide such as the sulphone cannot be reduced to methionine. The reversible oxidation-reduction of this residue has led to suggestions that this might act as a means of regulating enzyme activity, as well as protecting vital MET residue from irreversible oxidation with a corresponding loss of protein function (Levine et al., 2000). The oxidation of methionine residues has also been shown to result in an increase in protein surface hydrophobicity (Chao et al.,
This is related to the exposure of hydrophobic residues usually buried within the protein, as structural integrity is lost due to amino-acid oxidations. This increased hydrophobicity has been shown to increase the susceptibility of proteins to ligation with ubiquitin (Hershko and Ciechanover, 1986), targeting the proteins for proteolytic degradation by the proteosome.

A wide range of proteins has been shown to display altered function following methionine oxidation. Focusing on those proteins likely to be of significance on the surface of the lung, exposure of α-1-anti-trypsin to a variety of ROS in vitro has been shown to cause oxidation of surface exposed methionine residues at its reactive centre (met-351, met-358) associated with a loss of activity (Johnson and Travis, 1979; Taggart et al., 2000). Treatment with methionine reductase restored anti-elastase activity suggesting that the functional impairment was directly related to the methionine oxidation (Carp et al., 1983). Similarly, the proteinase inhibitor α-2 macroglobulin has been shown to possess oxidisable methionine residues (Reddy et al., 1994) though their oxidation in this case did not appear to result in a loss of antiprotease function. Instead their oxidation appeared to spare the oxidation of a critical tryptophan residue related to the protein function suggesting that in certain proteins methionine may have protective antioxidant functions. Other antiproteases have also been reported to have their function modified through oxidation of their methionine residues: α-2-antiplasmin (Geary and Gonia, 1989), mucus proteinase inhibitor (Boudier and Bieth, 1994) and secretory leukocyte proteinase inhibitor (Tomova, 1994). Methionine oxidation has also been implemented in altered function in a variety of proteins regulating the inflammatory response, present within the RTLF: Interferon γ (Keck, 1996), IL-6 (Nishimura et al., 1991), and lysozyme (Jori et al., 1968). In these studies the involvement of methionine oxidation in the modulation of protein function was generally examined in simple in-vitro models; whether these specific oxidations occur in vivo remains an open question. There is however evidence for methionine oxidation of RTLF proteins in vivo. Elevated concentrations of oxidised methionine residues have been detected in BAL proteins obtained from lung transplantation patients suffering from bronchiolitis obliterans syndrome (Behr et al., 2000; Hirsch et al., 1999). Increased concentrations of methionine sulphoxide have also been detected in BAL fluid obtained from patients with acute and chronic bronchitis (Maier et al., 1992), chronic obstructive pulmonary disease (Maier et al., 1992), and lung cancer patients under-going radio chemotherapy (Beinert et al., 2000). In each of these cases, the protein species that has been modified remains unknown. The measurement of methionine sulfoxide concentrations is simply used as an index of oxidative stress. Whilst it might be possible to relate a loss of a candidate proteins function, one which is known to have altered function related to specific methionine oxidation, with generic total protein levels of methionine sulphoxide, there is still a requirement to identified the modified protein if a direct causation is to be made.

Cysteine oxidation, S-thiolation and S-nitrosation

Cysteine residues are easily oxidised resulting in enzyme inactivation, alterations in protein tertiary structure and protein cross-linking (Thomas et al., 1995). Like methionine, cysteine residues can be oxidised to their corresponding sulfoxides: sulfenic (RSOH), sulfinic (RO2H), and sulfonic acid (RSO3H) by a range of reactive oxygen and nitrogen species (Claiborne et al., 1999). Of these, only the formation of sulfenic acid is reversible under physiological conditions, though this species readily oxidises to irreversible oxidation products referred to above (Pruzt, 1992), or reacts with GSH to form a disulphide bond (Claiborne et al., 1993). The conversion of critical cysteine residues to sulfenic acids by S-nitrosogluthathione, and other reactive nitrogen species has been implemented in the inactivation of glutathione reductase (Becker et al., 1998), and Cathepsin K (Percival et al., 1999) in in vitro models.

The most studied oxidative modifications of cysteine are the formation of disulphide bonds both within and between proteins and the formation of mixed disulfides with small molecular weight thiols: cysteine, GSH, homocysteine (Klatt and Lamas, 2000). Under conditions of oxidative stress these thiolation reactions occur as a result of either partially oxidised protein sulphydryl (thiyl radicals and sulfenic acid intermediates) with thiols such as cysteine and glutathione, or by thiol/disulphide exchange reactions with GSSG (Thomas et al., 1995). Further the formation of these mixed disulphides has been shown to alter the function of a diverse range of proteins under conditions of oxidative stress: ion translocators (Shattoc and Matsuura, 1993), protein kinases (Ward et al., 1998), phosphatases (Mahadev et al., 2001), antioxidant enzymes (Schinina et al., 1996), G-proteins (Mallis et al., 2001), metabolic (Cappiello et al., 1996) proteosome complexes (Demasi et al., 2001) and structural proteins (Eaton et al., 2002; Hanson et al., 1999), as well as DNA isomerases (Wang et al., 2001). These modifications are easily reversed, either through changes in intra-cellular redox, or via enzymatic reduction by thioredoxin, glutaredoxin, or disulphide isomerase at the expense of NADPH (Grant, 2001). Many studies have
shown that S-thiolated proteins formed during oxidative stress are readily de-thiolated once the stress is removed (Seres et al., 1996) associated with complete restoration of function (Ravichandran et al., 1994). It has therefore been proposed that thiolation/de-thiolation may regulate protein function in much the same way as other reversible posttranslational modifications, such as phosphorylation and adenylation. Regulatory cysteine residues are generally located in protein regions characterised by high pKa’s, as these areas favour the formation of reactive thiolated ions which are sensitive to oxidative modification by a number of oxidants: GSSG, nitric oxide, nitrosothiols, peroxynitrite, hypochlorous acid, and reactive lipids (Padgett and Whorton, 1998; Mohr et al., 1999; Pullar et al., 2001; Stamler et al., 2001).

The formation of mixed disulfides has been observed in a wide range of cells and tissues exposed to oxidative stress, both in vitro and in vivo. Mixed disulfides have been reported in rat lung tissue after exposure to cigarette smoke (Park et al., 1998) and hyperoxia (Awasthi et al., 1998), in the human lens during cataract formation (Lou et al., 1999), in models cadmium-induced oxidative stress (Figueiredo-Pereira et al., 1998), and during ischemia/reperfusion in isolated rat hearts (Eaton et al., 2002). Further, stimulation of respiratory burst in isolated human neutrophils (Seres et al., 1996; Chai et al., 1994) and monocytes (Ravichandran et al., 1994) has been shown to result in the rapid formation of disulfide bonds between intra-cellular low molecular weight thiols (GSH, γ-glutamylcysteine, homocysteine and cysteine) and a range of proteins (glyceraldehyde-3-phosphate dehydrogenase, actin) in a hydrogen peroxide dependent mechanism. These disulfides were subsequently reduced back to sulphydryls in a thioredoxin reductase dependent manner as the respiratory burst attenuated (Seres et al., 1996). As well as a potential regulatory role on protein function, S-thiolation has been proposed to play a protective role by preventing the oxidation of protein cysteine residues to irreversible oxidation products. Supportive evidence for this has been observed in the glutathiolation of the γ-glutamyl transpeptidase which appears to protect this membrane-bound enzyme from the hydrogen peroxide produced during its catabolism of GSH in the presence of metal cations (Dominici et al., 1999).

Whilst nitric oxide and many of its reactive derivatives have been implemented in the formation of mixed disulphide, probably through the oxidation of free thiols and subsequent disulphide exchange reactions, NO can also react directly with free and protein bound thiols with the formation of S-nitrosothiols (SNO). The products of these S-nitrosoation reactions have been proposed to simultaneously facilitate the transport of NO in vivo (Kharitonov et al., 1995), conferring NO properties on the carrier, whilst mitigating against its toxic effects (Stamler et al., 1992). A number of proteins have been identified as physiological NO reservoirs permitting the transport of this bioactive gas to effector sites in vivo: haemoglobin (Bonaventura et al., 2002), albumin (Tsikas et al., 2001) and calbindin D28K (Tao et al., 2002). In addition to this beneficial role nitrosylation of cysteine residues has also been shown to inactivate, or indeed activate, a broad spectrum of proteins: caspase B (Stamler et al., 1992), ion channels (Bolotina et al., 1994; Sun et al., 2001), receptors (Lipton et al., 1993), G-proteins (Lander et al., 1995), nuclear proteins and transcription factors (Marshall and Stamler, 2002; Laval and Wink, 2004), metabolic proteins (Mohr et al., 1996). S-nitroso compounds such as S-nitroso-L-glutathione (GSNO) may inhibit enzymes responsible for the modulation of the response to oxidative stress: glutathione peroxidase (Becker et al., 1995), glutathione reductase (Becker et al., 1995) and glutathione S transferase (Clark and Sinclair, 1988). NO can be liberated from these S-nitrosoative cysteine residues through a variety of mechanisms including metal catalysed homolytic cleavage, non-enzymatic and enzymatically catalysed transnitrosation reactions and metabolism of S-nitrosothiols by various enzymes (Hogg, 2002).

Increased concentrations of S-nitrosothiols have been reported in BAL fluid recovered from patients following lung transplantation (Gaston et al., 1993) and associated with infective pneumonia (Gaston et al., 1994). Increased concentrations have also been reported in exhaled breath condensate of patients with severe asthma, COPD, CF and in current smokers (Corradi et al., 2001). The formation of nitrosothiols depends on the availability of GSH within the RTL, and there is some evidence that concentrations of this antioxidant are in a range of chronic pulmonary conditions (Smith et al., 1993; Cross et al., 1994), and following acute airway insults (Blomberg et al., 1999; Cantin et al., 1987). At question is whether the formation of these nitrosothiols represents an adaptive response of the airway to nitrosative stress and related to this, what the functional consequences of these elevated concentrations are.

Oxidation of tyrosine residues by ROS and RNS

During the oxidation of the aromatic amino acid tyrosine, tyrosine radicals are formed, which in the absence of repair by cellular reductants may combine to form di-tyrosines and 3,4-dihydroxyphenylalanine, generally referred to as DOPA (Davies et al., 1999). As di-tyrosine derivatives are fluorescent this property has been made use of to assess oxidative
damage to proteins in a range of model systems. Under physiological conditions the formation of these species are relatively minor but their detection has proven a reliable method of assessing protein oxidation (Huggins et al., 1993; Giulivi and Davies, 1994). Hypochlorous acid released from activated neutrophils also reacts with tyrosine residues in protein to produce 3-chlorotyrosine and 3,5-dichloro tyrosine (Kettle, 1996). Although this is a relatively minor reaction, it is the only known physiological source of chlorotyrosine. As such, chlorotyrosine may be a specific marker for oxidant activity of cells that contain MPO such as neutrophils and monocytes. Elevated chlorotyrosine concentrations have been reported in atherosclerotic lesions (Podrez et al., 2000) and lungs of patients with adult respiratory distress syndrome (ARDS), cystic fibrosis and following lung transplantation (Lamb et al., 1999; De Andrade et al., 2000; van der Vliet et al., 2000). Similarly, hypobromous acid released from eosinophils can lead to the bromination of tyrosine residues. The generation of bromotyrosine is thought to be particularly important as this product has been shown to increase 10-fold in BAL fluid obtained from asthmatic subjects following allergen challenge (Wu et al., 2000) and 100-fold in severe asthmatics admitted to emergency departments (MacPherson et al., 2001).

The most studied oxidative modification of tyrosine residues is their nitration to 3-nitrotyrosine (3-NT). This may occur through a variety of mechanisms: via the reaction of superoxide with nitric oxide to form peroxynitrite (Beckman et al., 1994) and its derivative species, by peroxidase dependent nitrite oxidations, catalysed by MPO and EPO (Eiserich et al., 1998), and through nitric oxide interactions with tyrosyl radicals (Gunther et al., 1997), and nitrous acid (Knowles et al., 1974). These mechanisms have all been demonstrated in simple in vitro models, however questions remain about which mechanisms operate in vivo, where competing reactions (including those with antioxidants) will limit the degree of protein nitration. For example the toxic action of peroxynitrite can be seriously impaired in the presence of urate (Reiter et al., 2000). Despite this proviso, there is clear evidence that nitration of proteins does occur in vivo (MacMillan-Crow et al., 1996; Gole et al., 2000; Viner et al., 1996; Strong et al., 1998). Elevated concentrations have been detected in animal models of hyperoxia (Haddad et al., 1994), endotoxemia (Wizemann et al., 1994), lung ischaemia/reperfusion (Ishii et al., 1995), influenza induced pneumonia (Akaike et al., 1996) and asbestosis (Tanaka et al., 1998). Nitrotyrosine concentrations have been shown to be increased in a range of pulmonary diseases: asthma (Saleh et al., 1998; Kaminsky et al., 1999) idiopathic pulmonary fibrosis (Walker et al., 2001); Cystic fibrosis (van der Vliet et al., 1997); lung transplantation (McDermott et al., 1997); as well as in acute lung injury (Kooy et al., 1995; Haddad et al., 1994). A global increase in nitrotyrosine has been demonstrated in lung autopsy tissues from paediatric patients who had died of acute pulmonary conditions (Kooy et al., 1995), as well as in the RTLF of intensive care patients at risk of developing pneumonia and ARDS (Lamb et al., 1999; Mathy-Hartert et al., 2000). Concentrations of nitrite, nitrate and 3-NT have been shown to be elevated in BAL-fluid, cells and lung biopsy samples obtained from patients with ARDS (Gole et al., 2000; Lamb et al., 1999). Increased concentrations of 3-NT have been reported in asthma, with evidence that these levels were reduced following inhaled corticosteroid treatment (Saleh et al., 1998). Elevated concentrations of 3-chlorotyrosine, 3,3'-di tyrosines, nitrite, nitrate and 3-NT have been reported in induced sputum samples obtained from CF patients (van der Vliet et al., 2000).

Whilst the presence of 3-NT residues in protein serves as a useful marker of nitrosative stress, whether these modifications have any impact on protein function, and allied to this whether they may play an active role in tissue pathology is a critical issue. In simple model systems in which target proteins are exposed to peroxynitrite there is considerable evidence that this results in a loss of function in range of proteins: nuclear proteins (Chazotte-Aubert et al., 2000; Haqqani et al., 2002); metabolic enzymes (Souza and Radi, 1998); calcium binding proteins (Rowan et al., 2002); surfactant proteins (Zhu et al., 1996; Haddad et al., 1996; Haddad et al., 1993); tyrosine kinases (Knapp et al., 2001; MacMillan-Crow et al., 1996); phosphatases (Takakura et al., 1999), ion channels (Viner et al., 1996; Hu et al., 1994), inflammatory proteins (Sato et al., 1999; Zou et al., 1997) and antioxidant enzymes (MacMillan-Crow et al., 1996). Further, recent studies have been able to show that this loss of function is due to nitrosylation of critical tyrosine residues and not simply parallel oxidations within the protein. Of specific interest to the lung, nitration of tyrosine residues 161, 164 and 166 in SP-A have been shown to be related to its loss lipid aggregation capacity (Zhu et al., 2000). Nitration of Tyr67 on cytochrome C has been shown to have profound effects on its redox-regulating properties (Cassina et al., 2000). In studies with purified enzymes peroxynitrite has been shown to be a potent inhibitor of prostaglandin I2 synthase, related to the nitration of a critical tyrosine residue within the active site (De Andrade et al., 2000). Cytoskeletal proteins are also thought to be significant targets for nitrosylation due to their intra-cellular abundance and high tyrosine content (Eiserich et al., 1999). Further nitration of Tyr34 allied to the formation of
3,3'-dityrosine accounts for much of the inactivation of MnSOD seen in vivo (MacMillan-Crow et al., 1996; Yamakura et al., 1998). This list of specific modifications is increasing, but there is still a requirement for their identification in vivo. Circulating levels of free 3-NT has been reported in a range of pathological conditions (Fukuyama et al., 1997 #1060) and has been shown to be taken up into A549 cells where it is irreversibly incorporated into α-tubulin through the action of tyrosine ligase, disrupting binding of microtubule-associated proteins with potential impacts on cell growth, differentiation and motility (Eiserich et al., 1999; Chang et al., 2002). Whilst these studies can demonstrate altered protein function in specific proteins in simple cell and cell free models exposed to considerable nitrosative stresses, only a limited number of studies have identified the specific proteins nitrated in vivo (Gole et al., 2000; MacMillan-Crow et al., 1996). In an important study by Gole et al plasma ceruloplasmin, transferrin, α1-protease inhibitor, α1-antichymotrypsin and fibrinogen were all shown to be nitrated with an associative loss of function in ARDs patients (Gole et al., 2000). Whilst this approach partially bridges the conceptual gap between the formation of 3-NT and altered protein function, there still remains the possibility that the parallel oxidation of other amino acids within the protein drives the effect. Therefore at present it is probably best to view these identified protein as having altered function due to general oxidation as illustrated by the presence of 3-NT. Significantly, the recent identification of a de-nitrase activity isolated from rat spleen and lung (Kamisaki et al., 1998) has raised the possibility that many of these tyrosine nitrations reactions may be reversible and hence provide a mechanism for regulating protein activity.

As mentioned previously, recent studies have demonstrated that the heme peroxidases, myeloperoxidase and eosinophil peroxidase can also catalyse tyrosine nitration from nitrite in the presence of hydrogen peroxide, providing a significant alternative pathway for their formation in vivo during inflammation (Eiserich et al., 1998). Increased 3-NT staining in tissue sections are often co-localised with neutrophils, and associated with elevated concentrations of MPO. Clearly the operation of this pathway is critically dependent on the concentrations of nitrite within the local environment and notably significant concentrations have been reported in the RTLF: 10–140 μM in nasal and 10–15 μM in alveolar compartment, increasing markedly during inflammation. The involvement of MPO in the formation of 3-NT in vivo has been illustrated by the observation of diminished 3-NT concentrations in the livers of MPO deficient mice compared with wild type (Baldus et al., 2001). Recent studies have revealed that MPO released from inflammatory cells at the endothelium may undergo cellular transcytosis to the basolateral cell surface where they associate with endothelial cell glycosoglycans, especially fibronectin (Baldus et al., 2001). This mechanism is significant as it implies that the site of action of MPO may both be remote from its site of production and extended.

There is now good evidence that eosinophils can use nitrite as a substrate for the nitration of tyrosine residues (Wu et al., 1999). Pulmonary inflammation in asthma is characterized by the differential recruitment of eosinophils to the airway mucosa and lumen. Activated eosinophils release large amounts of cationic proteins from morphologically distinct cytoplasmic granules (Erjefalt and Persson, 2000). The most abundant of these granule proteins is a cationic heme-containing protein, eosinophil peroxidase (EPO). EPO appears to be specific for the eosinophil cell type and represents nearly 25% of the total protein mass of the secondary granule (Carlson et al., 1985). The release of EPO, together with the generation of superoxide and hydrogen peroxide from the respiratory burst (Elsner et al., 1996), leads to the generation of hypobromous acid (Slungaard and Mahoney, 1991). This potent defense mechanism can kill invading pathogens such as bacteria (Jong et al., 1980). Indeed in model systems EPO is a more potent catalyst of protein nitration than MPO under physiological conditions (Wu et al., 1999) raising the possibility that much of the nitration seen in asthma in vivo is related to its action. A recent study has shown a significantly higher concentration of 3-bromotyrosine in asthmatics compared with control subjects (Aldridge et al., 2002). Notably, levels of 3-chlorotyrosine were not elevated in this group emphasising the importance of EPO derived oxidants in asthma.

**Protein carbonyls**

There is a considerable body of evidence that the reaction of reactive oxygen and nitrogen species with lys, arg, cys, his, pro, glu, asp and thr residues in proteins leads to an increase in carbonyl groups (Stadtman and Levine, 2000). The introduction of these groups into proteins can arise through five distinct oxidative mechanisms: (1) through oxidative cleavage of polypeptide bonds by α-amidation or oxidation of glu and asp amino acid side chains; (2) by direct oxidation of lys, arg, pro and thr residues; (3) Michael addition of unsaturated aldehydes such as 4-hydroxy-2-nonenal to lys amino groups, cys sulphhydryl groups, and histidine imidazole groups; (4) by the reaction of malondialdehyde with lys to
form Schiff base derivatives; and finally (5) through the reaction of reducing sugars and their oxidation products with lys in glycation reactions. These mechanisms have been reviewed in detail by a number of authors previously (Stadtman and Levine, 2000). In contrast to many of the specific amino acid modifications already considered, where the absolute numbers of modifications are low, carbonyl modification may occur in numerous residues and may therefore have profound structural changes to a broad spectrum of proteins. A variety of highly sensitive HPLC and antibody-based techniques are available to measure protein carbonyls and thus their determination has been widely used to assess oxidative stress in a number in vitro and in vivo models. Increased concentrations of protein carbonyls have been measured following exposure of model protein solutions to ozone (Mudway et al., 1998; Berlett et al., 1996), transitional metal based free radical generating systems (Requena and Stadtman, 1999), cigarette smoke (Reznick et al., 1992) and activated neutrophils (Oliver, 1987). Increased concentrations have also been detected in the lungs of rats exposed to hyperoxia (Winter and Liehr, 1991) and paraquat (Winter and Liehr, 1991). Carbonyls concentrations are also increased in a murine model of emphysema (Yoshida et al., 2001) associated with increased NF-κB expression and ROS formation by alveolar macrophages. Elevated protein carbonyl concentrations have also been reported in plasma from ARDS patients (Quinlan et al., 1994) and in plasma BAL fluid obtained from in critically ill patients following major trauma or sepsis. Here the increased concentration of carbonyls correlated well with lipid oxidation product measurements and indices of neutrophilia and neutrophil activation (Winterbourn et al., 2000). Increased levels of protein oxidation have also been observed in tracheal aspirates from premature infants undergoing ventilation therapy, similarly related to pulmonary inflammation (Buss et al., 2000). Similar results have been shown by other groups where the increase in protein carbonyl concentrations has been shown to be positively associated with increased concentrations of matrix-metalloproteinase-9, and its inhibitor, TIMP-1 (Schock et al., 2001) suggesting that the processing of epithelial remodelling may be regulated through oxidative stress. There appears to be little evidence at present that protein carbonyl levels are elevated in asthma (Aldridge et al., 2002), although some evidence of a small increase has been reported in subjects following allergen challenge (Foreman et al., 1999) related to eosinophilia migration. Notably, in the later study, as with many of the protein oxidation markers described previously, the major protein oxidised was α1-antitrypsin.

**Further amino acid oxidative modifications**

Histidine residues are converted to 2-oxohistidine (Uchida and Kawakishi, 1993) or to asparagine or aspartic acid (Berlett et al., 1996) by a variety of reactive oxygen species and oxidants. The rate of these reactions and the ultimate product formed is a function both of the oxidising species and the local amino acid environment. Whilst ozone and hydroxyl radicals randomly attack histidine residues, metal catalysed oxidation is relatively site-specific targeting residue near the metal binding site (Stadtman and Oliver, 1991). Phenylalanine can also be oxidised to a range of products: ortho- and meta-tyrosine (Huggins et al., 1996), 2-, 3-, 4-hydroxy and 2,3-dihydroxy derivatives (Gieseg et al., 1993; Dean et al., 1993). Protein cross-linking in response to oxidation results in the formation of larger molecular weight species. These aggregates are formed through a variety of mechanisms: the formation of disulphide bridges, through covalent C–C bond formation arising from the interaction between two carbon-centred radicals, by interactions between lysine residues on separate proteins with lipid peroxidation products, or finally, via formation of a Schiff base cross link between a carbonyl group on one protein and a N-amino group of a lysine residue on a second. None of these modifications have been addressed in detail in models of pulmonary injury to date.

Recently considerable attention has focused on the formation of protein lipid oxidation product adducts, specifically with the lipid peroxide degradation products 4-hydroxynonenal and malondialdehyde in a number of diseases characterised by oxidative stress (Eaton et al., 2001; Walker et al., 2001; Houglum et al., 1990). The focus on this protein oxidative modification has been driven by the availability of commercially available antibodies against 4-HNE-protein adducts, permitting identification and quantification of modified proteins by Western blotting and immunohistochemistry (Eaton et al., 2001). In addition, elevated concentrations of these lipid oxidation species have been identified in almost all of the major pulmonary diseases characterised by acute and chronic inflammation making the formation of these adducts highly feasible in vivo. Increased concentrations of these adducts have been reported in the lungs of smokers with and without COPD (Rahman et al., 2002). Notably, 4-HNE concentrations in the pulmonary epithelium, airway endothelium and particularly in neutrophils of the COPD patients were found to be inversely associated with lung function. Significantly, the formation of these protein-lipid adducts have been shown to have profound effects on protein function. For example the binding of lipid...
oxidation products to the epidermal growth factors receptor has been shown to result in the phosphorylation of the receptors, with subsequent stimulation of the ERK pathway (Liu et al., 1999).

Summary

The lung is assaulted on a daily basis by a range of toxic insults. Whether these are infectious agents, allergens, or air pollutants they act upon the lung by eliciting oxidation reactions, either directly, or through inflammatory processes. Measurement of protein oxidation products therefore permits the degree of oxidative stress to be assessed and indicates that endogenous antioxidant defences are overwhelmed. The range of protein oxidation products observed is diverse and the nature and extent of specific oxidation products may inform us about the nature of the damaging ROS and NOS. Recently, there has been a significant shift away from the measurement of these oxidation products simply to establish the presence of oxidative stress, to a focus on identifying specific proteins sensitive to oxidation and establishing the functional consequences of these modifications. In addition the identification of specific enzyme systems to repair these oxidative modifications has led to the belief that protein function may be regulated through these oxidation reactions.

Future work

A major challenge for researchers working in this area will be to try and establish which specific amino acid oxidations are related to altered protein function. Oxidised proteins are likely to carry a range of oxidative modifications and thus determining which are the crucial changes will require careful work. In this respect it is clear that some protein modifications occur at a low frequency, i.e., try and met oxidations, while others have a much more global effect, e.g. carbonyl formation. Determining which, if any, of these modifications are most important in vivo is a clear challenge. Much more work is required to clarify specific oxidation products that arise due to the wide range of ROS and RNS that are present under defined conditions. Likewise, the interaction between endogenous antioxidant defences and protein oxidation requires more study. It is likely that certain amino acids are oxidised on proteins to spare other critical residues from oxidation, i.e. they are performing an antioxidant role. Much of this work will most easily be approached with in vitro studies. However, clearly it will be very important to test the relevance of these data within the in vivo situation.

References

Akaike T, Noguchi Y, Ijiri S, Setoguchi K, Saga M, Zheng YM, Dietzschold B, Maeda H (1996) Pathogenesis of influenza virus-induced pneumonia: involvement of both nitric oxide and oxygen radicals. Proc Natl Acad Sci USA 93: 2448–2453

Aldridge R, Chan T, van Dalen C, Senthilmohan R, Winn M, Venge P, Town G, Kettle A (2002) Eosinophil peroxidase produces hypobromous acid in the airways of stable asthmatics. Free Radic Biol Med 33: 847–856

Ames BN, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci USA 78: 6858–6862

Antczak A, Montuschi P, Kharitonov S, Gorski P, Barnes PJ (2002) Increased exhaled cysteiny1-leukotrienes and 8-isoprostane in aspirin-induced asthma. Am J Respir Crit Care Med 166: 301–306

Avissar N, Finkelstein JN, Horowitz S, Willey JC, Coy E, Frampton MW, Watkins RH, Khullar P, Xu YL, Cohen HJ (1996) Extracellular glutathione peroxidase in human lung epithelial lining fluid and in lung cells. Am J Physiol 270: L173–182

Avissar NE, Reed CK, Cox C, Frampton MW, Finkelstein JN (2000) Oxygen, but not nitrogen dioxide, exposure decreases glutathione peroxides in epithelial lining fluid of human lung. Am J Respir Crit Care Med 162: 1342–1347

Awasthi S, Gyurasics A, Knight SA, Welty SE, Smith CV (1998) Protein oxidation biomarkers in hyperoxic lung injury in rats: effects of U-74389. Toxicol Lett 95: 47–61

Babior BM (2000) Phagocytes and oxidative stress. Am J Med 109: 33–44

Baldus S, Eiserich JP, Mani A, Castro L, Mani A, Frampton MW, Ma W, Tousson A, White CR, Bullard DC, Brennan ML, Lusis AJ, Moore KP, Freeman BA (2001) Endothelial transcytosis of myeloperoxidase confers specificity to vascular ECM proteins as targets of tyrosine nitration. J Clin Invest 108: 1759–1770

Balint B, Donnelly LE, Hanazawa T, Kharitonov SA, Barnes PJ (2001) Increased nitric oxide metabolites in exhaled breath condensate after exposure to tobacco smoke. Thorax 56: 456–461

Bastacky J, Lee CY, Goerke J, Koushafar H, Yager D, Kenaga L, Speed et al., 1999).

References
Biennial T, Binder D, Oehm C, Ziems M, Priem F, Stuschke M, Schweigert B, Oehm C, Ziemer S, Priem F, Stuschke M, Schweigert Burton GW, Cheeseman KH, Doba T, Ingold KU, Slater TF (1983) An improved procedure for Bunnell E, Pacht ER (1993) Oxidized glutathione is increased in the Buettner GR, Jurkiewicz BA (1996) Catalytic metals, ascorbate and free radicals: combinations to avoid. Radiat Res 145: 532–541 Buettner GR, Jurkiewicz BA (1993) Ascorbate free radical as a marker of oxidative stress: an EPR study. Free Radic Biol Med 14: 49–55 Boudier C, Bieth JG (1994) Oxidized mucus proteinase inhibitor: a fairly potent neutrophil elastase inhibitor. Biochem J 303 (Pt 1): 61–68 Brennan PA, Moncada S (2002) From pollutant gas to biological messenger: the diverse actions of nitric oxide in cancer. Ann R Coll Surg Engl 84: 75–78 Brot N, Weissbach H (1983) Biochemistry and physiological role of methionine sulfone in residues in proteins. Arch Biochem Biophys 223: 271–281 Brown DM, Beswick PH, Bell KS, Donaldson K (2000) Depletion of glutathione and ascorbate in lung lining fluid by respirable fibres. Ann Occup Hyg 44: 101–108 Buettner GR, Jurkiewicz BA (1993) Ascorbate free radical as a marker of oxidative stress: an EPR study. Free Radic Biol Med 14: 49–55 Buettner GR, Jurkiewicz BA (1996) Catalytic metals, ascorbate and free radicals: combinations to avoid. Radiat Res 145: 532–541 Bunnett N, Pacht ER (1993) Oxidized glutathione is increased in the alveolar fluid of patients with the adult respiratory distress syndrome. Am Rev Respir Dis 148: 1174–1178 Burton GW, Ingold KU, Thompson KE (1981) An improved procedure for the isolation of ghost membranes from human red blood cells. Lipids 16: 946–947 Burton GW, Cheeseman KH, Doba T, Ingold KU, Slater TF (1983) Vitamin E as an antioxidant in vitro and in vivo. Ciba Found Symp 101: 4–18 Buss IH, Darlow BA, Winterbourn CC (2000) Elevated protein carbonyls and lipid peroxidation products correlating with myeloperoxidase in tracheal aspirates from premature infants. Pediatr Res 47: 640–645 Cantin AM, North SL, Hubbard RC, Crystal RG (1987) Normal alveolar epithelial lining fluid contains high levels of glutathione. J Appl Physiol 63: 152–157 Cantin AM, Hubbard RC, Crystal RG (1989) Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis. Am Rev Respir Dis 139: 370–372 Cappiello M, Voltarelli M, Cecconi I, Vilardo PG, Dal Monte M, Marini I, Del Corso A, Wilson DK, Quiocho FA, Petrasch JM, Mura U (1996) Specifically targeted modification of human aldose reductase by physiologic disulfides. J Biol Chem 271: 33539–33544 Carlson MG, Peterson CG, Venge P (1985) Human eosinophil peroxidase: purification and characterization. J Immunol 134: 1875–1879 Carp H, Janoff A, Abrams W, Weinbaum G, Drew RT, Weissbach H, Brot N (1983) Human methionine sulfoxide-peptide reductase, an enzyme capable of reactivating oxidized alpha-1-proteinase inhibitor in vitro. Am Rev Respir Dis 127: 301–305 Cassina AM, Hodara R, Souza JM, Thomson L, Castro L, Ischiropoulos H, Freeman BA, Radi R (2000) Cytochrome c nitration by peroxynitrite. J Biol Chem 275: 21409–21415 Chai YC, Hendrich S, Thomas JA (1994) Protein S-thiolation in hepatoctyes stimulated by t-butyl hydroperoxide, menadione, and neutrophils. Arch Biochem Biophys 310: 264–272 Chang W, Webster DR, Salam AA, Gruber D, Prasad A, Eisicher JP, Bulins Jc (2002) Alteration of the C-terminal amino acid of tubulin specifically inhibits myogenic differentiation. J Biol Chem 277: 30690–30698 Chao CC, Ma YS, Stadtman ER (1997) Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. Proc Natl Acad Sci USA 94: 2969–2974 Chazotte-Aubert L, Hainaut P, Ohshima H (2000) Nitric oxide nitrates tyrosine residues of tumour-suppressor p53 protein in MCF-7 cells. Biochem Biophys Res Commun 267: 609–613 Claiborne A, Miller H, Parsonage D, Ross RP (1993) Protein-sulfenic acid stabilization and function in enzyme catalysis and gene regulation. Faseb J 7: 1483–1490 Claiborne A, Yeh JI, Mallet C, Luba J, Crane EJ, 3rd, Charrier V, Parsonage D (1999) Protein-sulfenic acids: diverse roles for an unlikely player in enzyme catalysis and redox regulation. Biochemistry 38: 15407–15416 Clark AG, Sinclair M (1988) The Meisenheimer complex of glutathione and trinitrobenzene. A potent inhibitor of the glutathione S-transferase from Galleria mellonella. Biochem Pharmacol 37: 259–263 Comhair SA, Lewis MJ, Bhathena PR, Hammel JP, Erzurum SC (1999) Increased glutathione and glutathione peroxidase in lungs of individuals with chronic beryllium disease. Am J Respir Crit Care Med 159: 1824–1829 Comhair SA, Bhathena PR, Dweik RA, Kavuru M, Erzurum SC (2000) Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response. Lancet 355: 624–625 Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society (1996) Health effects of outdoor air pollution. Am J Respir Crit Care Med 153: 3–50 Conner EM, Grisham MB (1996) Inflammation, free radicals, and antioxidants. Nutrition 12: 274–277 Connor LM, Biddi A, Goerke J, Clements JA, Postlethwait EM (2001) NO2 interfacial transfer is reduced by phospholipid monolayers. J Appl Physiol 91: 2024–2034 Corradi M, Montuschi P, Donnelly LE, Peschi A, Kharitonov SA, Barnes PJ (2001) Increased nitrosothiols in exhaled breath condensate in inflammatory airway diseases. Am J Respir Crit Care Med 163: 854–858 Creeth JM (1978) Constituents of mucus and their separation. Br Med Bull 34: 17–24 Cross CE, Motchnik PA, Bruener BA, Jones DA, Kaur H, Arnes BN, Halliwell B (1992) Oxidative damage to plasma constituents by ozone. FEBS Lett 298: 269–272 Cross CE, van der Vliet A, O’Neill CA, Louie S, Halliwell B (1994) Oxidants, antioxidants, and respiratory tract lining fluids. Environ Health Perspect 102 Suppl 10: 185–191 Davies MJ, Fu S, Wang H, Dean RT (1999) Stable markers of oxidant damage to proteins and their application in the study of human disease. Free Radic Biol Med 27 1151–1163 De Andrade JA, Crow JP, Viera L, Bruce Alexander C, Randall Young K, McGiffin DC, Zorn GL, Zhu S, Matalon S, Jackson RM (2000) Protein nitration, metabolites of reactive nitrogen species, and inflammation in lung allografts. Am J Respir Crit Care Med 161: 2035–2042 Dean RT, Gieseg S, Davies MJ (1993) Reactive species and their accumulation on radical-damaged proteins. Trends Biochem Sci 18: 437–441
Kelly FJ, Blomberg A, Frew A, Holgate ST, Sandstrom T (1996) Anti-
Keck RG (1996) The use of t-butyl hydroperoxide as a probe for
Kamisaki Y, Wada K, Bian K, Balabanli B, Davis K, Martin E, Behbod F, Lee
Kaminsky DA, Mitchell J, Carroll N, James A, Soultanakis R, Janssen Y
392 F. J. Kelly and I. S. Mudway
Kalyanaraman B (1995) Thiyl radicals in biological systems: significant
Kafoury RM, Pryor WA, Squadrito GL, Salgo MG, Zou X, Friedman M
Jori G, Galiazzo G, Marzotto A, Scoffone E (1968) Selective and
Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, Gaston
Huggins TG, Wells-Knecht MC, Detorie R, Richards RJ (1995) Deple-
Houglum K, Filip M, Witztum JL, Chojkier M (1990) Malondialdehyde
Horton JK, Meredith MJ, Bend JR (1987) Glutathione biosynthesis from
Hogg N (2002) The biochemistry and physiology of S-nitrosothiols. Annu
Housley DG, Eccles R, Richards RJ (1995) Gender difference in the
concentration of the antioxidant uric acid in human nasal lavage. Acta
Otolaryngol 116: 751–754
Hu P, Ischiropoulos H, Beckman JS, Matalon S (1994) Peroxynitrite
inhibition of oxygen consumption and sodium transport in alveolar type
II cells. Am J Physiol 266: L628–L634
Huggins TG, Wells-Knecht MC, Detorie NA, Baynes JW, Thorpe SR
(1993) Formation of o-tyrosole and dityrosine in proteins during radi-
oytic and metal-catalyzed oxidation. J Biol Chem 268: 12341–12347
Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, Gaston
B (2000) Endogenous airway acidification. Implications for asthma
pathophysiology. Am J Respir Crit Care Med 161: 694–699
Ischiropoulos H, Zhu L, Beckman JS (1992) Peroxynitrite formation from
macrophage-derived nitric oxide. Arch Biochem Biophys 298: 446–451
Ischiropoulos H, al-Mehdi AB, Fisher AB (1995) Reactive species in
ischemic rat lung injury: contribution of peroxynitrite. Am J Physiol
269: L159–L164
Johnson D, Travis J (1979) The oxidative inactivation of human alpha-1
proteinase inhibitor. Further evidence for methionine at the reactive
center. J Biol Chem 254: 4022–4026
Jones PD, Hankin R, Simpson J, Gibson PG, Henry RL (2001) The tolera-
bility, safety, and success of p6mation induction and combined hyperoxic
treatment in children. Am J Respir Crit Care Med 164: 1146–1150
Jong EC, Henderson WR, Klebanoff SJ (1980) Bactericidal activity of
eicosinoph peroxidase. J Immunol 124: 1378–1382
Jori G, Galiazzo G, Marzotto A, Scoffone E (1968) Selective and
reversible photo-oxidation of the methionyl residues in lysozyme. J
Biol Chem 243: 4272–4278
Kafoury RM, Pryor WA, Squadrito GL, Salgo MG, Zou X, Friedman M
(1999) Induction of inflammatory mediators in human airway epithelial
cells by lipid ozonation products. Am J Respir Crit Care Med 160:
1934–1942
Kalyanaraman B (1995) Thyl radicals in biological systems: significant or
trivial? Biochem Soc Symp 61: 55–63
Kaminsky DA, Mitchell J, Carroll N, James A, Soultanakis R, Janssen Y
(1999) Nitrosylation formation in the airways and lung parenchyma of
patients with asthma. J Allergy Clin Immunol 104: 747–754
Kaminsky DA, Mitchell J, Carroll N, James A, Soultanakis R, Janssen Y
(1999) Newly identified proteins in human nasal and clinical applications. Electrophoresis 20: 3670–3676
Kelly FJ, Tetley TD (1997) Nitrogen dioxide depletes uric acid and
ascorbic acid but not glutathione from lung lining fluid. Biochem J
325 (Pt 1): 95–99
Kettle AJ (1996) Neutrophils convert tyrosyl residues in albumin to
chlorotyrosine. FEBS Lett 379: 103–106
Kettle AJ, Winterbourn CC (1994) Superoxide-dependent hydroxylation by
myeloperoxidase. J Biol Chem 269: 17146–17151
Khairitoun SA, Barnes PJ (2000) Clinical aspects of exhaled nitric oxide.
Eur Respir J 16: 781–792
Khairitoun SA, Barnes PJ (2001) Exhaled markers of pulmonary disease.
Am J Respir Crit Care Med 163: 1693–1722
Khairitoun SA, Barnes PJ (2002) Biomarkers of some pulmonary dis-
eases in exhaled breath. Biomarkers 7: 1–32
Khairitoun VG, Sundquist AR, Sharma VS (1995) Kinetics of nitrosation of
thiols by nitric oxide in the presence of oxygen. J Biol Chem 270:
28158–28164
Khairitoun SA, Rajakulasingam K, O’Connor B, Durham SR, Barnes PJ
(1997) Nasal nitric oxide is increased in patients with asthma and
allergic rinitis and may be modulated by nasal glucocorticoids. J
Allergy Immunol 99: 58–64
Kim HJ, Liu X, Wang H, Kohyama T, Kobayashi T, Wen FQ, Romberger
DJ, Abe S, MacNee W, Rahman I, Rennard SI (2002) Glutathione
prevents inhibition of fibroblast-mediated collagen gel contraction by
cigarette smoke. Am J Physiol Lung Cell Mol Physiol 283: L409–L417
Klatt P, Lamas S (2000) Regulation of protein function by S-glutathio-
lation in response to oxidative and nitrosative stress. Eur J Biochem 267:
4928–4944
Knapp LT, Kanterewicz BJ, Hayes EL, Klann E (2001) Peroxynitrite-
induced tyrosine nitration and inhibition of protein kinase C. Biochem
Biophys Res Commun 268: 764–770
Knolles ME, McWeeny DJ, Couchman L, Thorogood M (1974) Inter-
action of nitrite with proteins at gastric pH. Nature 247: 288–289
Koo YW, Royall JA, Ye YZ, Kelly DR, Beckman JS (1995) Evidence for
in vivo peroxynitrite production in human acute lung injury. Am J
Respir Crit Care Med 151: 1250–1254
Lamb NJ, Gutierrez JM, Baker C, Evans TW, Quinlan GJ (1999) Oxidative
damage to proteins of bronchoalveolar lavage fluid in patients with acute
respiratory distress syndrome: evidence for neutrophil-mediated hydro-
xylation, nitration, and chlorination. Crit Care Med 27: 1738–1744
Lander HM, Ogiste JS, Teng KK, Novogrodsky A (1995) p21ras as a
common signaling target of reactive free radicals and cellular redox
stress. J Biol Chem 270: 21195–21198
Langford SD, Bidani A, Postlethwait EM (1995) Ozone-reactive absorp-
tion by pulmonary epithelial lining fluid constituents. Toxicol Appl
Pharmacol 132: 122–130
Laval F, Wink DA (1994) Inhibition by nitric oxide of the repair protein, O6-
methylguanine-DNA-methyltransferase. Carcinogenesis 15: 443–447
Lenz AG, Meyer B, Costabel U, Maier K (1993) Bronchoalveolar lavage
fluid proteins in human lung disease: analysis by two-dimensional
electrophoresis. Electrophoresis 14: 242–244
Levine RL, Moskowitz J, stadman ER (2000) Oxidation of methionine in
proteins: roles in antioxidant defense and cellular regulation. IUBMB
Life 50: 301–307
Lindahl M, Stahlbom B, Tagesson C (1995) Two-dimensional gel electo-
rophoresis of nasal and bronchoalveolar lavage fluids after occupa-
tional exposure. Electrophoresis 16: 1199–1204
Lindahl M, Ekstrom T, Sorensen J, Tagesson C (1996) Two dimensional
protein patterns of bronchoalveolar lavage fluid from non-smokers,
smokers, and subjects exposed to asbestos. Thorax 51: 1028–1235
Lindahl M, Stahlbom B, Svartz J, Tagesson C (1998) Protein patterns of
human nasal and bronchoalveolar lavage fluids analyzed with two-
dimensional gel electrophoresis. Electrophoresis 19: 3222–3229
Lindahl M, Stahlbom B, Tagesson C (1999a) Newly identified proteins in
human nasal and bronchoalveolar lavage fluids: potential biomedical
and clinical applications. Electrophoresis 20: 3670–3676
Kelly FJ, Blomberg A, Frew A, Holgate ST, Sandstrom T (1996) Anti-
oxidant kinetics in lung lavage fluid following exposure of humans to
tungsten dioxide. Am J Respir Crit Care Med 154: 1700–1705
Lindahl M, Svartz J, Tagesson C (1999b) Demonstration of different forms of the anti-inflammatory proteins lipocortin-1 and Clara cell protein-16 in human nasal and bronchoalveolar lavage fluids. Electro- phoresis 20: 881–890

Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HS, Sucher NJ, Losalzo J, Singel DJ, Stamler JS (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364: 626–632

Liu W, Akhand AA, Kato M, Yokoyama I, Miyata T, Kurokawa K, Uchida K, Nakashima I (1999) 4-hydroxynonenal triggers an epidermal growth factor receptor-linked signal pathway for growth inhibition. J Cell Sci 112 (Pt 14): 2409–2417

Lou MF, Dickerson JE Jr, Tung WH, Wolfe JK, Chylack LT Jr (1999) Correlation of nuclear color and opalescence with protein S-thiolation in human lenses. Exp Eye Res 68: 547–552

MacMillan-Crow LA, Crow JP, Kerby JD, Thompson JA (1996) Studies of ascorbate-dependent, iron-catalyzed dethiolation. Arch Biochem Biophys 358:

Mallis RJ, Buss JE, Thomas JA (2001) Oxidative modification of H-ras: S-thiolation and S-nitrosylation of reactive cysteines. Biochem J 355: 145–153

Marshall HE, Stamler JS (2002) Nitrosative Stress-induced Apoptosis through Inhibition of NF-kappa B. J Biol Chem 277: 34223–34228

Mathy-Hartert M, Damas P, Nys M, Deby-Dupont G, Canivet JL, Ledoux D, Lamy M (2000) Nitroptated proteins in bronchoalveolar lavage fluid of patients at risk of ventilator-associated bronchopneumonia. Eur Respir J 16: 299–301

May JM, Mendiratta S, Hill KE, Burk RF (1997) Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. J Biol Chem 272: 22007–22010

McCay PB (1985) Vitamin E: interactions with free radicals and ascrobate. Annu Rev Nutr 5: 323–340

McDermott CD, Gavita SM, Shennib H, Giaid A (1997) Immunohistochemical localization of nitric oxide synthase and the oxidant peroxynitrite in lung transplant recipients with obliterative bronchiolitis. Transplantation 64: 270–274

Melloni B, Lefebvre MA, Bonnafon F, Vergnenegre A, Grossin L, Rigaud M, Cantin A (1996) Antioxidant activity in bronchoalveolar lavage fluid from patients with lung cancer. Am J Respir Crit Care Med 154: 1706–1711

Miller DM, Aust SD (1989) Studies of ascorbate-dependent, iron-catalyzed lipid peroxidation. Arch Biochem Biophys 271: 113–119

Molho S, Stamler JS, Brune B (1996) Posttranslational modification of glyceraldehyde-3-phosphate dehydrogenase by S-nitrosylation and subsequent NADH attachment. J Biol Chem 271: 4209–4214

Molho S, Hallak H, de Boite A, Lapetina EG, Brune B (1999) Nitric oxide-induced S-glutathionylation and inactivation of glyceraldehyde-3-phosphate dehydrogenase. J Biol Chem 274: 9427–9430

Morcillo EJ, Estrella J, Cortijo J (1999) Oxidative stress and pulmonary inflammation: pharmacological intervention with antioxidants. Pharmacol Res 40: 393–404

Mudway IS, Blomberg A, Frew AJ, Holgate ST, Sandstrom T, Kelly FJ (1999) Antioxidant consumption and repletion kinetics in nasal lavage fluid following exposure of healthy human volunteers to ozone. Eur Respir J 13: 1429–1438

Mudway IS, Kelly FJ (1998) Modeling the interactions of ozone with pulmonary epithelial lining fluid antioxidants. Toxicol Appl Pharmacol 148: 91–100

Mudway IS, Housley D, Eccles R, Richards RJ, Dahs AK, Tetley TD, Kelly FJ (1996) Differential depletion of human respiratory tract antioxidants in response to ozone challenge. Free Radic Res 25: 499–513

Mudway IS, Stenfors N, Blomberg A, Helleday R, Dunster C, Marklund SL, Frew AJ, Sandstrom T, Kelly FJ (2001) Differences in basal airway antioxidant concentrations are not predictive of individual responsiveness to ozone: a comparison of healthy and mild asthmatic subjects. Free Radic Biol Med 31: 962–974

Munoz-Fernandez MA, Fernandez MA, Fresno M (1992) Activation of human macrophages for the killing of intracellular Trypanosoma cruzi by TNF-alpha and IFN-gamma through a nitric oxide-dependent mechanism. Immunol Lett 33: 35–40

Nelson ME, O’Brien-Ladner AR, Wessellius LJ (1996) Regional variation in iron and iron-binding proteins within the lungs of smokers. Am J Respir Crit Care Med 153: 1535–1538

Nightingale JA, Rogers DF, Barnes PJ (1999) Effect of inhaled ozone on exhaled nitric oxide, pulmonary function, and induced sputum in normal and asthmatic subjects. Thorax 54: 1061–1069

Nishimura C, Ikeda T, Masuda S, Futatsumi K, Itoh S, Yasukawa K, Kishimoto T, Arata Y (1991) Chemical modification and 1H-NMR studies on the receptor-binding region of human interleukin 6. Eur J Biochem 196: 377–384

Noah TL, Henderson FW, Henry MM, Peden DB, Devlin RB (1995) Nasal lavage cytokines in normal, allergic, and asthmatic school-age children. Am J Respir Crit Care Med 152: 1290–1296

Noel-Georis I, Bernard A, Falmagne P, Wattiez R (2002) Database of bronchoalveolar lavage fluid proteins. J Chromatogr B Analyt Technol Biomed Life Sci 771: 221–236

Nordenhall C, Pourazar J, Ledin MC, Levin JO, Sandstrom T, Adeloth E (2001) Diesel exhaust enhances airway responsiveness in asthmatic subjects. Eur Respir J 17: 909–915

Ohshima H, Friesen M, Brouet I, Bartsch H (1990) Nitrosoyamine as a new marker for endogenous nitrosation and nitration of proteins. Food Chem Toxicol 28: 647–652

Oliveier CN (1987) Inactivation of enzymes and oxidative modification of proteins by stimulated neutrophils. Arch Biochem Biophys 253: 62–72

Pacht ER, Davis WB (1988) Role of transferrin and ceruloplasmin in antioxidant activity of lung epithelial lining fluid. J Appl Physiol 64: 2092–2099

Pacht ER, Diaz P, Clanton T, Hart J, Gadek JE (1997) Alveolar fluid glutathione decreases in asymptomatic HIV-seropositive subjects over time. Chest 112: 785–788

Padgett CM, Whorton AR (1998) Cellular responses to nitric oxide: role of protein S-thiolation/dethiolation. Arch Biochem Biophys 358: 232–242

Park EM, Park YM, Gwak YS (1998) Oxidative damage in tissues of rats exposed to cigarette smoke. Free Radic Biol Med 25: 79–86

Parks NJ, Krohn KJ, Mathis CA, Chasko JH, Geiger KR, Gregor ME, Peak NF (1981) Nitrogen-13-labeled nitrite and nitrate: distribution and metabolism after intratracheal administration. Science 212: 58–60

Peden DB, Holman R, Brown ME, Mason RT, Berkbeile C, Fales HM, Kaliner MA (1990) Uric acid is a major antioxidant in human nasal airway secretions. Proc Natl Acad Sci USA 87: 7638–7642

Peden DB, Brown ME, Wade Y, Raphael GD, Berkbeile C, Kaliner MA (1991) Human nasal glandular secretion of novel antioxidant activity: cholinergeric control. Am Rev Respir Dis 143: 545–552

Peden DB, Swierz M, Ohkubo K, Hahn B, Emery B, Kaliner MA (1993) Nasal secretion of the ozone scavenger uric acid. Am Rev Respir Dis 148: 455–461
Percival MD, Ouellet M, Campagnolo C, Claveau D, Li C (1999) Inhibition of cathepsin K by nitrile oxide donors: evidence for the formation of mixed disulfides and a sulfenic acid. Biochemistry 38: 13574–13583

Podrez EA, Abu-Soud HM, Hazen SL (2000) Myeloperoxidase-generated oxidants and atherosclerosis. Free Radic Biol Med 28: 1717–1725

Postlethwait EM, Langford SD, Bidani A (1991) Interaface transfer kinetics of NO2 into pulmonary epithelial lining fluid. J Appl Physiol 71: L1502–1510

Postlethwait EM, Langford SD, Bidani A (1994) Determinants of inhaled ozone absorption in isolated rat lungs. Toxicol Appl Pharmacol 125: 77–89

Postlethwait EM, Langford SD, Jacobson LM, Bidani A (1995) NO2 reactive absorption substrates in rat pulmonary surface lining fluids. Free Radic Biol Med 19: 553–563

Prutz WA (1992) Catalytic reduction of Fe(III)-cytochrome-c involving stable radiolysis products derived from disulphides, proteins and thiols. Int J Radiat Biol 61: 593–602

Pryor WA (1994) Mechanisms of radical formation from reactions of ozone with target molecules in the lung. Free Radic Biol Med 17: 451–465

Pryor WA, Squadrito GL (1995) The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol 268: L699–L722

Pryor WA, Squadrito GL, Friedman M (1995a) The cascade mechanism to explain ozone toxicity: the role of lipid ozonation products. Free Radic Biol Med 19: 935–941

Pryor WA, Squadrito GL, Friedman M (1995b) A new mechanism for the toxicity of ozone. Toxicol Lett 82/83: 287–293

Pullar JM, Vissers MC, Winterbourn CC (2001) Glutathione oxidation by hypochlorous acid in endothelial cells causes glutathione sulfoximation as a major product but not glutathione sulfide. J Biol Chem 276: 22120–22125

Quinlan GJ, Evans TW, Gutteridge JM (1994) Oxidative damage to plasma proteins in adult respiratory distress syndrome. Free Radic Res 20: 289–298

Quinton PM (1979) Composition and control of secretions from tracheal bronchial submucosal glands. Nature 279: 551–552

Rahman I, van Schadewijk AA, Crowther AJ, Hiemstra PS, Stolk J, MacNee W (1992) Catalytic reduction of Fe(III)-cytochrome-c involving stable radiolysis products derived from disulphides, proteins and thiols. Int J Radiat Biol 61: 593–602

Rostow B, Haapt R, Stevens PA, Kunze D (1996) Protein S-thiolation and dethiolation during the respiratory burst in human monocytes. A reversible post-translational modification with potential for buffering the effects of oxidant stress. J Immunol 156: 1973–1980

Ravichandran V, Murrayci U, Rokutan K, Thomas JA, Johnston RB Jr (1996) Protein S-thiolation and dethiolation during the respiratory burst in human monocytes. A reversible post-translational modification with potential for buffering the effects of oxidant stress. J Immunol 156: 1973–1980

Shattuck MJ, Matsuur H (1993) Measurement of Na+ + K+-pump current in isolated rabbit ventricular myocytes using the whole-cell voltage-clamp technique. Inhibition of the pump by oxidant stress. Circ Res 72: 91–101

Slungaard A, Mahoney JR Jr (1991) Bromide-dependent toxicity of eosinophil peroxidase for endothelium and isolated working rat hearts: a model for eosinophilic endocarditis. J Exp Med 173: 117–126

Smith LJ, Houston M, Anderson J (1993) Increased levels of glutathione residues. Eur J Biochem 237: 433–439

Schock BC, Sweet DG, Halliday HL, Young IS, Ennis M (2001) Oxidative stress in lavage fluid of preterm infants at risk of chronic lung disease. Am J Physiol Lung Cell Mol Physiol 281: L1386–1391

Seres T, Ravichandran V, Moriguchi T, Rokutan K, Thomas JA, Johnston RB Jr (1996) Protein S-thiolation and dethiolation during the respiratory burst in human monocytes. A reversible post-translational modification with potential for buffering the effects of oxidant stress. J Immunol 156: 1973–1980

Souza JM, Radi R (1998) Glyceraldehyde-3-phosphate dehydrogenase inactivation by peroxynitrite. Arch Biochem Biophys 360: 187–194

Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, Singel DJ, Loscalzo J (1992) S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. Proc Natl Acad Sci USA 89: 444–448
Stamler JS, Lamas S, Fang FC (2001) Nitrosylation. the prototypic redox-based signaling mechanism. Cell 106: 675–683

Strong MJ, Sopper MM, Crow JP, Strong WL, Beckman JS (1998) Nitration of the low molecular weight neurofilament is equivalent in sporadic amyotrophic lateral sclerosis and control cervical spinal cord. Biochem Biophys Res Commun 248: 157–164

Sun J, Xin C, Eu JP, Stamler JS, Meissner G (2001) Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. Proc Natl Acad Sci USA 98: 11158–11162

Taggart C, Cervantes-Lauren D, Kim G, McElvaney NG, Wehr N, Moss J, Levine RL (2000) Oxidation of either methionine 351 or methionine 358 in alpha 1-antitrypsin causes loss of anti-neutrophil elastase activity. J Biol Chem 275: 27258–27265

Takakura K, Beckman JS, MacMillan-Crow LA, Crow JP (1999) Rapid and irreversible inactivation of protein tyrosine phosphatases PTP1B, CD45, and LAR by peroxynitrite. Arch Biochem Biophys 369: 197–207

Tanaka S, Choe N, Hemenway DR, Zhu S, Matalon S, Kagan E (1998) The respiratory tract lactoferrin and lysozyme arise primarily in the airways and not in the lungs and pleura of the rat. J Clin Invest 102: 445–454

Tao L, Murphy ME, English AM (2002) S-nitrosation of Ca(2+)-loaded and Ca(2+)-free recombinant calbindin D(28K) from human brain. Biochemistry 41: 6185–6192

Thiele JJ (2001) Oxidative targets in the stratum corneum. A new basis for antioxidative strategies. Skin Pharmacol Appl Skin Physiol 14 Suppl 1: 87–91

Thomas EL, Grisham MB, Jefferson MM (1983) Myeloperoxidase-dependent effect of amines on functions of isolated neutrophils. J Clin Invest 72: 441–454

Thomas EL, Bozeman PM, Jefferson MM, King CC (1995a) Oxidation of bromide by the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. Formation of bromamines. J Biol Chem 270: 2906–2913

Thomas JA, Poland B, Honzatko R (1995b) Protein sulfhydryls and their role in the antioxidative function of protein S-thiolation. Arch Biochem Biophys 319: 1–9

Thompson AB, Bohling T, Payvandi F, Rennard SI (1990) Lower respiratory tract lactoferrin and lysozyme arise primarily in the airways and are elevated in association with chronic bronchitis. J Lab Clin Med 115: 148–158

Tien M, Berlett BS, Levine RL, Chock PB, Stadtman ER (1999) Selective oxidation of methionyl residues in the human recombinant secretory proteinase inhibitor. Effect on the inhibitor binding properties. J Mol Recognit 7: 31–37

Tsiakas D, Sandmann J, Luessen P, Savva A, Rossa S, Stichtenoth DO, Frolich JC (2001) S-Transnitrosylation of albumin in human plasma and blood in vitro and in vivo in the rat. Biochim Biophys Acta 1546: 422–434

Uchida K, Kawakishi S (1993) 2-Oxo-histidine as a novel biological marker for oxidatively modified proteins. FEBS Lett 332: 208–210

van der Vlieet A, Cross CE (2000) Myeloperoxidase and protein oxidation in cystic fibrosis. Am J Physiol Lung Cell Mol Physiol 279: L537–L546

van der Vlieet A, O’Neill CA, Cross CE, Koostoa JM, Volz WG, Halliwell B, Louie S (1999) Determination of low-molecular-mass antioxidant concentrations in human respiratory tract lining fluids. Am J Physiol 276: L289–296

Viner RI, Hahnert AF, Bigelow DJ, Schoneich C (1996) The oxidative inactivation of sarcoplasmic reticulum Ca(2+)-ATPase by peroxynitrite. Free Radic Res 24: 223–259

Vissers MC, Winterbourn CC (1995) Oxidation of intracellular glutathione after exposure of human red blood cells to hypochlorous acid. Biochem J 307 ( Pt 1): 57–62

Walker LM, York JL, Imam SZ, Ali SF, Muldev KL, Mayeux PR (2001) Oxidative stress and reactive nitrogen species generation during renal ischemia. Toxicol Sci 63: 143–148

Wang H, Mao Y, Chen AY, Zhou N, LaVoie EL, Liu LF (2001) Stimulation of topoisomerase II-mediated DNA damage via a mechanism involving protein thiolation. Biochemistry 40: 3316–3323

Wang Y, Russo TA, Kwon O, Chanock S, Rumsey CE, Levine M (1997) Ascorbate recycling in human neutrophils: induction by bacteria. Proc Natl Acad Sci USA 94: 13816–13819

Ward NE, Pierce DS, Chung SE, Gravitt KR, O’Brian CA (1998) Irreversible inactivation of protein kinase C by glutathione. J Biol Chem 273: 12558–12566

Washburn MP, Wells WW (1999) Identification of the dehydrouracil-based signaling mechanism. Cell 106: 675–683

Wattiez R, Hermans C, Bernard A, Lesur O, Falmagne P (1999) Human bronchoalveolar lavage fluid: two-dimensional gel electrophoresis, amino acid microsequencing and identification of major proteins. Electrophoresis 20: 1634–1645

Widdicombe JH, Bastacky SJ, Wu DX, Lee CY (1997) Regulation of depth and composition of airway surface liquid. Eur Respir J 10: 2892–2897

Winkler BS, Orselli SM, Rex TS (1994) The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. Free Radic Biol Med 17: 333–349

Winter ML, Liehr JG (1991) Free radical-induced carbonyl content in protein of estrogen-treated hamsters assayed by sodium borohydride reduction. J Biol Chem 266: 14446–14450

Winterbourn CC (1985) Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite. Biochim Biophys Acta 840: 204–210

Winterbourn CC, Buss IH, Chan TP, Plank LD, Clark MA, Windsor JA (2000) Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients. Crit Care Med 28: 143–149

Wizemann TM, Laskin DL (1994) Effects of acute endotoxemia on production of cytokines and nitric oxide by pulmonary alveolar and interstitial macrophages. Ann NY Acad Sci 730: 336–337

Wu W, Chen Y, Hazen SL (1999) Eosinophil peroxidase nitrates protein tyrosyl residues. Implications for oxidative damage by nitrating intermediates in eosinophil inflammatory disorders. J Biol Chem 274: 25933–25944

Wu W, Samszuk MK, Comhair SA, Thomassen MJ, Farver CF, Dweik RA, Kavuru MS, Erzurum SC, Hazen SL (2000) Eosinophils generate brominating oxidants in allergen-induced asthma. J Clin Invest 105: 1455–1463

Yamakura F, Taka H, Fujimura T, Murayama K (1998) Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. J Biol Chem 273: 14085–14089

Protein oxidation in the lung 395
Yoshida M, Korfhagen TR, Whitsett JA (2001) Surfactant protein D regulates NF-kappa B and matrix metalloproteinase production in alveolar macrophages via oxidant-sensitive pathways. J Immunol 166: 7514–7519

Zhu S, Haddad IY, Matalon S (1996) Nitration of surfactant protein A (SP-A) tyrosine residues results in decreased mannose binding ability. Arch Biochem Biophys 333: 282–290

Zhu S, Basiouny KF, Crow JP, Matalon S (2000) Carbon dioxide enhances nitration of surfactant protein A by activated alveolar macrophages. Am J Physiol Lung Cell Mol Physiol 278: L1025–L1031

Zou M, Martin C, Ullrich V (1997) Tyrosine nitration as a mechanism of selective inactivation of prostacyclin synthase by peroxynitrite. Biol Chem 378: 707–713

Authors’ address: Prof. Frank J. Kelly, Environmental Research Group, School of Health & Life Sciences, Franklin-Wilkins Building, King’s College London, 150 Stamford Street, London, SE1 9NN, UK, Fax: (44) 20 7848 3891, E-mail: frank.kelly@kcl.ac.uk