PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Metal Ions and Oxygen Radical Reactions in Human Inflammatory Joint Disease [and Discussion]

B. Halliwell, J. M. C. Gutteridge, D. Blake, Roberta J. Ward, P. M. May and H. Sies

Phil. Trans. R. Soc. Lond. B 1985 **311**, 659-671
doi: 10.1098/rstb.1985.0171

Phil. Trans. R. Soc. Lond. B **311**, 659–671 (1985)
Printed in Great Britain

Metal ions and oxygen radical reactions in human inflammatory joint disease

By B. Halliwell[1], J. M. C. Gutteridge[2] and D. Blake[3]

[1] *Department of Biochemistry, King's College London, Strand, London WC2R 2LS, U.K.*
[2] *National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, U.K.*
[3] *Rheumatology Research Wing, University Medical School, Birmingham B15 2TJ, U.K.*

Activated phagocytic cells produce superoxide (O_2^-) and hydrogen peroxide (H_2O_2); their production is important in bacterial killing by neutrophils and has been implicated in tissue damage by activated phagocytes. H_2O_2 and O_2^- are poorly reactive in aqueous solution and their damaging actions may be related to formation of more reactive species from them. One such species is hydroxyl radical ($OH^\cdot$), formed from H_2O_2 in the presence of iron- or copper-ion catalysts. A major determinant of the cytotoxicity of O_2^- and H_2O_2 is thus the availability and location of metal-ion catalysts of $OH^\cdot$ formation. Hydroxyl radical is an initiator of lipid peroxidation. Iron promoters of $OH^\cdot$ production present *in vivo* include ferritin, and loosely bound iron complexes detectable by the 'bleomycin assay'. The chelating agent Desferal (desferrioxamine B methanesulphonate) prevents iron-dependent formation of $OH^\cdot$ and protects against phagocyte-dependent tissue injury in several animal models of human disease. The use of Desferal for human treatment should be approached with caution, because preliminary results upon human rheumatoid patients have revealed side effects. It is proposed that $OH^\cdot$ radical is a major damaging agent in the inflamed rheumatoid joint and that its formation is facilitated by the release of iron from transferrin, which can be achieved at the low pH present in the micro-environment created by adherent activated phagocytic cells. It is further proposed that one function of lactoferrin is to protect against iron-dependent radical reactions rather than to act as a catalyst of $OH^\cdot$ production.

Introduction

There has been considerable interest in the role of oxygen-derived species such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) as agents of tissue damage by activated phagocytic cells in a number of disorders, including the adult respiratory distress syndrome, autoimmune disease, chronic joint inflammation, reperfusion injury after ischaemic cardiac damage, and cancer. In this paper recent developments of our knowledge of the role of oxygen-derived species in human rheumatoid arthritis will be discussed.

Production of oxygen-derived species by phagocytic cells

Reviewing the massive literature on the production of oxygen-derived species by phagocytes is not a task that can be attempted here, but the authors believe that the present state of the subject can be summarized as follows.

(i) It is well established that neutrophils, monocytes, macrophages and eosinophils produce superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) upon activation of the respiratory burst.

(ii) The products of the respiratory burst play a key role in bacterial killing by neutrophils, as shown by the greatly impaired killing seen in patients suffering from chronic granulomatous disease.

(iii) The myeloperoxidase system in neutrophils plays a lesser role in killing because inborn defects in myeloperoxidase activity rarely produce clinically observable defects in bacterial killing (see, for example, Nauseef *et al.* 1983).

(iv) There is no clear evidence that activated phagocytes produce singlet oxygen (see, for example, Kanofsky *et al.* 1984). Indeed, hypochlorous acid (HOCl) itself, and the products of its reaction with various amines, are toxic (Albrich *et al.* 1981); there is no need to invoke singlet O_2 to explain the toxicity of the myeloperoxidase –H_2O_2– halide system. HOCl and its reaction products may mediate some of the damage done by activated phagocytic cells to their environment (Test *et al.* 1984).

Studies of the chemistry of oxygen-derived products (for reviews see, for example, Halliwell & Gutteridge 1984, 1985; di Guiseppi & Fridovich 1984) also lead to the following conclusions. Pure O_2^- and pure H_2O_2 are poorly reactive in *aqueous* solution, yet biological or chemical O_2^--generating systems can damage many biomolecules. This damage is often prevented or decreased by addition of superoxide dismutase (SOD), or catalase, or both, to the reaction mixtures. Superoxide and H_2O_2 themselves do not peroxidize membrane lipids or degrade DNA. In addition, H_2O_2 can cross cell membranes whereas O_2^- cannot unless there is a specific channel for it (for example, the 'anion channel' in the erythrocyte membrane).

It therefore follows that the damage done by activated neutrophils that has been attributed to oxygen radicals, such as hyaluronic acid degradation (Greenwald & Moy 1980) or DNA fragmentation (Weitberg *et al.* 1983; Phillips *et al.* 1984), is unlikely to be a result of direct reactions of O_2^- or H_2O_2 themselves. A suggestion that O_2^- contributes to formation of a 'chemotactic factor' that attracts more neutrophils into a site of inflammation, has recently been challenged. Indeed, although copper–zinc superoxide dismutase has an anti-inflammatory effect, there is increasing doubt about whether this is entirely because of removal of O_2^- radical (Baret *et al.* 1984).

Perhaps, then, the damage done is due to some 'reactive factor' that O_2^- and H_2O_2 produce. Suggestions have included the following.

(i) Singlet oxygen. There is no clear evidence for the production of this by phagocytic cells.

(ii) $HO_2^{\cdot}$ radical. The protonated form of O_2^- is a more powerful oxidizing agent than is O_2^- itself and reacts with unsaturated fatty acids. Formation of $HO_2^{\cdot}$ is favoured at low pH values (its pK_a is *ca.* 4.8) but the pH beneath adherent activated macrophages has been reported to be 5 or less (Etherington *et al.* 1981) and so it may be biologically relevant.

(iii) Hydroxyl radical. Hydroxyl radical, $OH^{\cdot}$, is produced when water is exposed to high-energy ionizing radiation, and so its properties have been well documented by radiation chemists. Hydroxyl radical is highly reactive, and any produced *in vivo* will react at or close to its site of formation. The nature of the direct damage done by $OH^{\cdot}$ *in vivo* would therefore depend on what its site of formation was; so production of $OH^{\cdot}$ close to DNA could lead to strand breakage (Mello Filho & Meneghini 1984), whereas production close to an enzyme molecule that is present in excess in the cell, such as lactate dehydrogenase, might have no biological consequences. It should also be remembered that reaction of $OH^{\cdot}$ with a biomolecule will produce another radical, usually of lower reactivity (because of the extremely high reactivity of $OH^{\cdot}$). Such less-reactive radicals can cause their own problems, because they can

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

diffuse away from their site of formation and attack specific biomolecules. For example, uric acid reacts with $OH^{\cdot}$ radical; it protects lactate dehydrogenase against inactivation by $OH^{\cdot}$ but *accelerates* inactivation of alcohol dehydrogenase (Kittridge & Willson 1984). The urate-derived radicals are less reactive overall, so more of them survive to reach the sensitive sites on alcohol dehydrogenase that they can react with. Perhaps the best example of the importance of secondary radicals is the ability of $OH^{\cdot}$ to initiate lipid peroxidation by hydrogen-atom abstraction, with subsequent formation of peroxy radicals (for reviews see, for example, Halliwell & Gutteridge 1984, 1985).

Hydroxyl radical is produced when H_2O_2 comes into contact with iron ions. 'Free' iron ions cannot exist *in vivo*, but complexes of iron salts with phosphate esters (such as ATP and GTP), organic acids (such as citrate and lactate) and DNA are all effective in decomposing H_2O_2 (Floyd 1981, 1983; Flitter *et al.* 1983). Copper ions also promote the decomposition of H_2O_2 to $OH^{\cdot}$ (Samuni *et al.* 1981; Rowley & Halliwell 1983). The DNA damage done by H_2O_2 to human fibroblasts and mouse cells in culture can be minimized by metal chelating agents (Mello Filho & Meneghini 1984), suggesting that the H_2O_2 crosses the plasma membrane and combines with metal ions (probably iron) bound at or close to DNA.

Hence a major determinant of the actual toxicity of O_2^- and H_2O_2 to cells is the availability and location of metal-ion catalysts of $OH^{\cdot}$ radical formation. If, for example, iron salts are bound to DNA or membrane lipids, introduction of H_2O_2 and O_2^- can fragment the DNA and peroxidize the membrane lipids. The site of attack of the $OH^{\cdot}$ radicals will be determined by the site of the bound metal ion; such 'site-specific' damage is rarely prevented by 'scavengers' of $OH^{\cdot}$ (Czapski 1984) and the nature of the damage will probably not even resemble that done by $OH^{\cdot}$ radical generated in free solution and attacking the target randomly (Gutteridge & Halliwell 1982; Samuni *et al.* 1981; Gutteridge 1984*a*; Czapski 1984). The postulated 'crypto-$OH^{\cdot}$ radical' may represent formation and localized reactivity of 'real' $OH^{\cdot}$ radical (Youngman 1984; Moorhouse *et al.* 1985). For example, exposure of proteoglycan or mucus preparations to metal-dependent systems that generate $OH^{\cdot}$ seems to produce specific patterns of fragmentation rather than just random attack (Creeth *et al.* 1983; Cross *et al.* 1984), perhaps because the metal catalysts bind readily to specific sites.

There is much debate about the role played by the $OH^{\cdot}$ radical in initiating lipid peroxidation in systems containing iron salts (Aust & Svingen 1982). It is known that $OH^{\cdot}$ radicals, such as those produced radiolytically, can initiate peroxidation by abstracting hydrogen atoms, yet laboratory experiments in which peroxidation of liposomes or microsomal fractions is 'initiated' by iron-salt–ascorbate mixtures or NADPH–iron-salt–ADP mixtures often show little inhibition of peroxidation on addition of $OH^{\cdot}$ radical scavengers, even though formation of $OH^{\cdot}$ radicals can be demonstrated in the reaction mixtures (Aust & Svingen 1982; Gutteridge 1984*b*). There have been suggestions that perferryl, ferryl or Fe^{II}–Fe^{III}–O_2 complexes are the true initiators of peroxidation. This may be correct, but other possibilities exist. Those $OH^{\cdot}$ radicals important in initiation are formed by metal ions attached to the membranes; such site-specific production of $OH^{\cdot}$ could not be prevented by scavengers in bulk solution (Czapski 1984). Alternatively, once the peroxidation process has begun, initiation is continued by the alkoxy and peroxy radicals formed upon iron-dependent decomposition of lipid peroxides; $OH^{\cdot}$ radicals are not necessary under these conditions. Most lipid systems contain some pre-formed lipid hydroperoxide (Gutteridge 1984*b*, *c*), and it is merely necessary to fragment these peroxides to continue the chain reaction.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Chelating agents and oxidative tissue damage

If $OH^{\cdot}$ radicals are formed by low molecular mass metal complexes in free solution and then have to diffuse a short distance to attack a target, protection by added $OH^{\cdot}$ scavengers should be seen and has been reported in several *in vivo* systems (see, for example, Cohen 1978; Fox 1984). On the other hand, site-specific $OH^{\cdot}$ radical formation is much more difficult to protect against in this way (Czapski 1984; Moorhouse *et al.* 1985). Protection here can be achieved by (i) superoxide dismutase and H_2O_2-removing enzymes, to stop O_2^- and H_2O_2 ever reaching the bound metal complexes, and (ii) chelating agents that pull the metals away from sensitive sites and render them inactive. Complexes of iron salts with diethylenetriaminepentaacetic acid and bathophenanthroline show diminished reactivity in $OH^{\cdot}$ production (Halliwell 1978*a*, *b*), but the chelating agent most effective in preventing iron-dependent $OH^{\cdot}$ production is desferrioxamine (Gutteridge *et al.* 1979), which is also a powerful inhibitor of iron-dependent lipid peroxidation (Wills 1969). Desferrioxamine, available from Ciba-Geigy as its mesylate salt (Desferal) is widely used to prevent iron overload in thalassaemia and other conditions requiring repeated blood transfusion. Desferal cannot be given by mouth. Doses of 50–60 mg per kilogram of body mass (administered subcutaneously) appear safe in the treatment of iron overload, but high doses have been associated with ocular abnormalities and patients receiving Desferal for any purpose should be periodically checked for both ocular (Davies *et al.* 1983) and auditory changes, especially in diabetics (Arden *et al.* 1984).

EDTA renders copper ions less reactive in $OH^{\cdot}$ production (Samuni *et al.* 1981), but iron–EDTA chelates still react with O_2^- and H_2O_2 (Butler & Halliwell 1982). Hence EDTA has a number of potential effects in biological systems.

(i) It could minimize damage by pulling iron ions off sensitive sites and causing the $OH^{\cdot}$ formed by iron–EDTA chelates in free solution to react 'randomly' with less-important targets. Such an effect may account for its ability to inhibit DNA degradation by bleomycin–iron complexes (Gutteridge *et al.* 1981*a*).

(ii) It could make damage worse by pulling iron off 'safe sites'; i.e. the 'random attack' does more damage than the site-specific attack (see, for example, Wong *et al.* 1981). It thus 'decompartmentalizes' iron in a harmful way (Willson 1978).

(iii) It will, in any case, change the nature of the damage and increase its susceptibility to inhibition by $OH^{\cdot}$ 'scavengers' (Gutteridge 1984*a*), because, in the presence of EDTA, the attack will be from $OH^{\cdot}$ generated close to the target 'in free solution' rather than from $OH^{\cdot}$ formed on the target.

EDTA is thus an interesting experimental tool, but for physiological purposes the authors use desferrioxamine, which tightly binds iron(III) *and* renders it inactive as a catalyst.

Hydroxyl radical formation by phagocytic cells

Direct measurement of $OH^{\cdot}$ radical formation *in vivo* is difficult because of its high reactivity, but recent studies in the authors' laboratories with the use of the technique of aromatic hydroxylation (Richmond *et al.* 1981; Grootveld & Halliwell 1985; Moorhouse *et al.* 1985) look promising. Conversion of dimethylsulphoxide (DMSO) into methane has been used as an assay method to show $OH^{\cdot}$ production in whole cells (Repine *et al.* 1979, 1981).

Hydroxyl radical formation by isolated phagocytic cells has been observed by using a wide

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

range of techniques, including e.s.r. spin trapping (Green *et al.* 1979), conversion of DMSO into methane (Repine *et al.* 1979), benzoate decarboxylation (Sagone *et al.* 1980) and ethene formation (Weiss *et al.* 1977). DMSO penetrates into cells and probably measures $OH^{\cdot}$ production both extra- and intracellularly, but the other techniques are probably detecting only external $OH^{\cdot}$ generation. This external $OH^{\cdot}$ generation is important in considering the damage done by activated phagocytes to their surroundings (see Halliwell 1982*a*; Fox 1984) and to themselves; an increased availability of catalytic iron complexes can decrease phagocyte activity by damaging the phagocytes (Sweder van Asbeck *et al.* 1984*a*, *b*). However, as far as bacterial killing is concerned, it is the formation of $OH^{\cdot}$ inside the phagocytic vacuole that matters. Again there are two possibilities, not mutually exclusive. First, that the phagocytes produce $OH^{\cdot}$ from O_2^- and H_2O_2 in the phagocytic vacuole and it attacks the bacteria from the outside. Some evidence consistent with this is available (Johnston *et al.* 1975; Repine *et al.* 1984). Secondly, that H_2O_2 generated in the phagocytic vacuole penetrates into the bacteria and produces $OH^{\cdot}$ inside them. Several bacteria contain iron complexes capable of catalysing radical reactions (Gutteridge & Wilkins 1984) and the killing of *S. aureus* cells by H_2O_2 becomes more effective if the internal iron content of the bacteria is increased (Repine *et al.* 1981).

Hydroxyl radical formation inside and outside phagocytic cells: what is the physiological metal catalyst?

Although both iron and copper salts mediate radical reactions, almost all the work concerning $OH^{\cdot}$ radical formation *in vivo* has been done with iron salts. Discussion will therefore be largely confined to these, although the development of an assay that measures available copper complexes (Gutteridge 1984*d*) should increase our knowledge of their importance. What iron catalysts are available *in vivo*?

Simple iron chelates

The first possibility is low molecular mass iron chelates such as iron–ATP, –GTP, or –citrate. Small 'transit' pools of these iron complexes are present within cells (Jacobs 1977; Halliwell 1982*b*), and the killing of fibroblasts by H_2O_2 requires intracellular metal ions, probably bound to DNA (Mello Filho & Meneghini 1984; Floyd 1981).

Gutteridge *et al.* (1981*a*, 1982*a*) have developed an assay for complexes of iron capable of accelerating radical reactions. This so-called 'bleomycin assay' shows that such complexes are not present in human serum or plasma, except in some cases of iron overload secondary to idiopathic haemochromatosis (Gutteridge *et al.* 1985*a*). They are present in sweat (Gutteridge *et al.* 1985*b*), cerebrospinal fluid (Gutteridge *et al.* 1982*b*) and in the knee-joint synovial fluid of many patients suffering from rheumatoid arthritis. Not all rheumatoid synovial fluids contain bleomycin-detectable iron, but many do and the concentrations present correlate positively with both clinical and laboratory parameters of disease activity (Rowley *et al.* 1984). The precise molecular form of bleomycin-detectable iron has not been established, but it is, at least partly, ultrafilterable and may represent iron chelated to organic acids, phosphate esters or urate, or perhaps iron *weakly* attached to such proteins as albumin, so that it can easily detach during ultrafiltration (Gutteridge *et al.* 1985*b*). The reasons for the presence of bleomycin-detectable iron in synovial fluid are considered later.

Evidence for the importance *in vivo* of iron-mediated radical reactions comes from studies with desferrioxamine. Desferal blocks the haemolytic action of several radical-generating drugs

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in mice (Clark & Hunt 1983) and decreases acute lung vascular injury in animals after complement activation due to infusion of cobra venom factor or to severe burn injury (Ward *et al.* 1983; Fligiel *et al.* 1984). It also has a beneficial effect on the course of allergic encephalomyelitis in rats (Bowern *et al.* 1984).

Ferritin

Ferritin is often regarded as a safe 'storage form' of iron, yet ferritin stimulates both lipid peroxidation (Gutteridge *et al.* 1983) and the formation of $OH^{\cdot}$ radicals from O_2^- and H_2O_2 (Bannister *et al.* 1984). The protein shell can be attacked by lipid peroxides, causing liberation of the iron, which is probably why ferritin stimulates lipid peroxidation (Gutteridge 1985). Superoxide seems to mobilize iron from ferritin, leading to subsequent $OH^{\cdot}$ production (Biemond *et al.* 1984). Haemosiderin also promotes hydroxyl radical formation but is less effective than ferritin on a unit-iron basis (O'Connell & Halliwell, in preparation).

Lactoferrin and transferrin

Ambruso & Johnston (1981) reported that iron-loaded lactoferrin (two moles of Fe^{III} per mole of protein) is an efficient catalyst of $OH^{\cdot}$ formation from O_2^- and H_2O_2. Bannister *et al.* (1982*a*) confirmed this with the use of a different assay system, although Winterbourn (1983) has pointed to some artefacts in the assays used and has concluded that iron-replete lactoferrin is, if effective at all, a poor catalyst compared with simple iron chelates; a view supported by the authors (Gutteridge *et al.* 1981*b*) and by Baldwin *et al.* (1984). Similarly, the activity of transferrin-bound iron in promoting $OH^{\cdot}$ production has been reported as good (McCord & Day 1978), moderate (Bannister *et al.* 1982*b*), poor (Motohashi & Mori 1983) and zero (Maguire *et al.* 1982; Baldwin *et al.* 1984).

At physiological pH, transferrin and lactoferrin seem to release iron much less readily than does ferritin; unlike ferritin-bound iron, iron bound to the former proteins is ineffective in stimulating lipid peroxidation (Gutteridge *et al.* 1981*b*). One possible explanation of the above discrepancies is that, when the fully iron-loaded proteins are studied, there is sometimes iron bound to non-specific sites on the protein molecule that becomes detached during the assay and is the true catalyst of $OH^{\cdot}$ production. It seems difficult to imagine that iron(III) embedded in the two specific binding sites of transferrin or lactoferrin can give rise to $OH^{\cdot}$ radical that escapes into free solution without reacting with the protein. The authors find it especially hard to understand the claims that iron on lactoferrin (Ambruso & Johnston 1981) or transferrin (McCord & Day 1978) equals iron–EDTA in its efficiency of forming $OH^{\cdot}$ radicals.

In normal human plasma, the transferrin present is only partly loaded with iron, i.e. few molecules have two Fe^{III} ions bound to them. Similarly, the lactoferrin released by phagocytic cells contains little iron and, indeed, its release has often been suggested to represent an antibacterial mechanism by binding the iron that bacteria require. These observations would make it even less likely that native transferrin and lactoferrin are significant catalysts of $OH^{\cdot}$ radical production *in vivo*. Even if lactoferrin is a catalyst of $OH^{\cdot}$ production by phagocytic cells, it is not the only one, because lactoferrin-deficient phagocytes still produce $OH^{\cdot}$ (Boxer *et al.* 1982; Newburger & Tauber 1982).

Lactoferrin and transferrin are similar in many respects, but a major difference in their properties is that iron is released from transferrin at pH values of 5.6 and below, whereas lactoferrin holds on to its iron down to pH values of 2 or less (Lonnerdal *et al.* 1981).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Iron and rheumatoid disease

Iron has long been known to play a part in the pathology of rheumatoid disease; in active human disease there is increased deposition of iron in the synovial membranes, a drop in blood haemoglobin and often the presence of iron complexes catalytic for radical reactions in synovial fluid (Blake *et al.* 1981*a*, 1984; Rowley *et al.* 1984). The extensive deposition of ferritin iron (Muirden 1970) within synovial membranes in the inflamed rheumatoid joint would increase its sensitivity to radical reactions; H_2O_2 generated by phagocytes could easily penetrate into the synovial cells and react with iron mobilized from ferritin to form $OH^{\cdot}$ radicals. The synovial fluid of rheumatoid knee joints also contains increased ferritin concentrations (Blake *et al.* 1980) but this ferritin contains little or no iron and appears abnormal in its properties. Idiopathic haemochromatosis is often associated with joint inflammation (Schumacher 1982), which resolves when the iron overload is controlled. Infusion of iron–dextran into rheumatoid patients can aggravate the synovial inflammation (Blake *et al.* 1985*a*). The bleomycin-detectable iron present in synovial fluid can accelerate lipid peroxidation and $OH^{\cdot}$ formation in *in vitro* experiments (Gutteridge *et al.* 1982*a*). There is as yet no direct evidence that $OH^{\cdot}$ is formed in the inflamed rheumatoid joint, although aromatic hydroxylation as an *in vivo* method is being used to study this in our laboratories. However, $OH^{\cdot}$ radical formation could account for some or all of the hyaluronic acid depolymerization (Gutteridge *et al.* 1979) and cartilage degradation (Dean *et al.* 1985) seen in the rheumatoid joint.

Treatment with Desferal

Blake *et al.* (1983) observed that low doses of Desferal aggravated acute rat models of inflammation, but larger doses were anti-inflammatory. In Glynn-Dumond synovitis in guinea pigs, Desferal (100 mg per kilogram body mass) aggravated the acute phase of the inflammation, but repeated administration depressed the chronic phase. A similar effect is seen in the rat allergic air-pouch model of acute to chronic inflammation (Yoshino *et al.* 1984). It is not clear why Desferal may make inflammation worse during the acute phase; by stopping $OH^{\cdot}$ production it may protect neutrophils against self-destruction (Sweder van Asbeck *et al.* 1984), or perhaps some $OH^{\cdot}$ production is necessary for effective regulation of inflammation, for example, by inactivation of arachidonic acid metabolites (Henderson & Klebanoff 1983).

The suppressive action of Desferal on chronic inflammation was sufficiently encouraging for preliminary trials with rheumatoid patients to be performed. Giordano *et al.* (1984) injected 1 g of Desferal intramuscularly and observed an abrupt rise in haemoglobin. No fall in the acute phase response was observed, but the speed of the change in haemoglobin suggests that this effect was mediated by suppressing inflammation. No ill effects were reported. Of seven rheumatoid patients given larger doses (up to 3 g d^{-1} for 5 days each week, for 1–3 weeks), four developed ocular abnormalities that reversed on Desferal withdrawal. Two patients who received prochlorperazine to combat nausea during Desferal therapy became unconscious for 48–72 h (Blake *et al.* 1985*b*), possibly because this combination of drugs mediates iron transfer across the blood–brain barrier and achieves removal of iron essential to the functioning of the nervous system (Gutteridge *et al.* 1982*b*). It is clear that doses of Desferal suitable for treatment of iron overload are not necessarily safe in other disease states, and the combination of Desferal with phenothiazine drugs should be strictly avoided. Although Desferal is of potential use in a number of human diseases (on the basis of animal experiments) caution should be employed

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in its use, and patients receiving it should be carefully checked for ocular and auditory abnormalities.

Why is bleomycin-detectable iron present in rheumatoid fluids?

As discussed earlier, samples of knee-joint synovial fluid from rheumatoid patients often contain bleomycin-detectable iron. Addition of excess commercial (Sigma) apotransferrin to the assay renders the iron in synovial fluid undetectable, i.e. it is present in a form that can bind to added transferrin under the conditions of the bleomycin assay.

Bleomycin-detectable iron does not seem to be an artefact of the collection or handling of body fluids. It is not present in serum, handled and stored in an identical manner, except in some cases of idiopathic haemochromatosis where the normal iron-binding capacity of transferrin is at or close to saturation (Gutteridge *et al.* 1985*b*). Inflammatory exudates taken from the chronic allergic air-pouch model of inflammation in rats (Yoshino *et al.* 1984) never show bleomycin iron. Synovial fluids taken from patients and assayed at once still often contain bleomycin-detectable iron; in those that do not, such iron does not appear on storage at 4 °C or on freeze–thawing.

Is bleomycin-detectable iron present because the transferrin in the fluid is fully iron loaded? This may be so in cerebrospinal fluid (Gutteridge *et al.* 1982*b*), but it is certainly not in synovial fluid. Our (unpublished) data show a low percentage degree of saturation of transferrin in rheumatoid synovial fluids, calculated by comparing the total iron content of the fluids with the amount of immunologically determined transferrin protein. Addition of iron–NTA chelates to the fluids directly demonstrates this unsaturation; there is substantial iron-binding capacity. Why then is bleomycin-detectable iron present in the synovial fluid? A possible explanation could be that as macrophages and other phagocytes 'slide over' cell or cartilage surfaces, a micro-environment sealed off from the bulk synovial fluid is created (Wright & Silverstein 1984). The pH in this micro-environment can fall to 5 or less (Etherington *et al.* 1981). Oxygen radicals, proteolytic enzymes, myeloperoxidase (from neutrophils) and other products are released into this micro-environment. The low pH facilitates damage to collagen, cartilage and cell membranes by first favouring formation of $HO_2^{\cdot}$ from O_2^{-}, and secondly, by causing release of iron from transferrin, assisted by the presence of ascorbic acid in synovial fluid. The released iron is kept in the iron(II) state by $HO_2^{\cdot}$, O_2^{-}, or ascorbate or both and thus cannot re-bind to transferrin or bind to lactoferrin (from neutrophils). Hydroxyl radicals are formed and attack the surface to which the phagocytes are attached (as well as the phagocytes themselves) and also inactivate antiproteases in the micro-environment, causing further damage.

When the phagocytic cells detach or move away, the micro-environment is 'opened' to the bulk synovial fluid. All that can then be seen in this fluid is a slightly lower pH than normal, an oxidation of ascorbic acid (Blake *et al.* 1981*b*), partial inactivation of 'bulk' antiprotease activity (Lewis *et al.* 1984) and the presence of micromolar concentrations of iron available to bleomycin. This iron will not re-bind easily to transferrin or lactoferrin because ascorbate keeps it in the reduced state, and because transferrin does not bind iron easily from some physiological iron complexes (Bates & Schlabach 1973).

We further suggest that one function of lactoferrin released from neutrophils, far from acting as a catalyst of $OH^{\cdot}$ formation, is to minimize damage by binding some iron, because it can hold iron(III) at much lower pH values than can be achieved even in the postulated micro-environment. The fact that macrophages do not synthesize lactoferrin may facilitate

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

damage by iron-dependent $OH^{\cdot}$ radical formation from the extracellular H_2O_2 that they produce.

We are grateful to the Arthritis and Rheumatism Council, Wellcome Trust, British Technology Group, West Midlands Health Authority and Ciba-Geigy Pharmaceuticals for research support. BH is a Lister Institute Research Fellow. BH is grateful to Professor Roger Dean for helpful discussions.

References

Albrich, J. M., McCarthy, C. A. & Hurst, T. K. 1981 Biological reactivity of hypochlorous acid: implications for microbicidal mechanism of leukocyte myeloperoxidase. *Proc. natn. Acad. Sci. U.S.A.* **78**, 210–214.

Ambruso, D. R. & Johnston, R. B. 1981 Lactoferrin enhances hydroxyl radical production by human neutrophils, neutrophil particulate fractions and an enzymatic generating system. *J. clin. Invest.* **67**, 352–360.

Arden, G. B., Wonke, B., Kennedy, C. & Huehns, E. R. 1984 Ocular changes in patients undergoing long-term desferrioxamine treatment. *Br. J. Ophthal.* **68**, 873–877.

Aust, S. D. & Svingen, B. A. 1982 The role of iron in enzymatic lipid peroxidation. In *Free radicals in biology*, vol. 5 (ed. W. A. Pryor), pp. 1–28. New York: Academic Press.

Baldwin, A., Jenny, E. R. & Aisen, P. 1984 The effect of human serum transferrin and milk lactoferrin on hydroxyl radical formation from superoxide and hydrogen peroxide. *J. biol. Chem.* **259**, 13391–13394.

Bannister, J. V., Bannister, W. H., Hill, H. A. O. & Thornalley, P. J. 1982*a* Enhanced production of hydroxyl radicals by the xanthine–xanthine oxidase system in the presence of lactoferrin. *Biochim. biophys. Acta* **715**, 116–120.

Bannister, J. V., Bannister, W. H. & Thornalley, P. J. 1984 The effect of ferritin iron loading on hydroxyl radical production. *Life Chem. Rep.* suppl. 2, pp. 64–72.

Bannister, J. V., Bellavite, P., Davoli, A., Thornalley, P. J. & Rossi, F. 1982*b* The generation of hydroxyl radicals following superoxide production by neutrophil NADPH oxidase. *FEBS Lett.* **150**, 300–302.

Baret, A., Jadot, G. & Michelson, A. M. 1984 Anti-inflammatory properties in the rat of CuZnSOD from various species. *Life Chem. Rep.* suppl. 2, pp. 417–421.

Bates, G. W. & Schlabach, M. R. 1973 The reaction of ferric salts with transferrin. *J. biol. Chem.* **248**, 3228–3232.

Biemond, P., van Eijk, H. G., Swaak, A. J. G. & Koster, J. F. 1984 Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leucocytes. *J. clin. Invest.* **73**, 1576–1579.

Blake, D. R., Bacon, P. A., Eastham, E. J. & Brigham, K. 1980 Synovial fluid ferritin in rheumatoid arthritis. *Br. med. J.* **281**, 715–716.

Blake, D. R., Gallagher, P. J., Potter, A. R., Bell, M. J. & Bacon, P. A. 1984 The effect of synovial iron on the progression of rheumatoid disease. *Arthritis Rheum.* **27**, 495–501.

Blake, D. R., Hall, N. D., Bacon, P. A., Dieppe, P. A., Halliwell, B. & Gutteridge, J. M. C. 1981*a* The importance of iron in rheumatoid disease. *Lancet ii*, 1141–1144.

Blake, D. R., Hall, N. D., Bacon, P. A., Dieppe, P. A., Halliwell, B. & Gutteridge, J. M. C. 1983 Effect of a specific iron-chelating agent on animal models of inflammation. *Ann. Rheum. Dis.* **42**, 89–93.

Blake, D. R., Hall, N. D., Treby, D. A., Halliwell, B. & Gutteridge, J. M. C. 1981*b* Protection against superoxide and hydrogen peroxide in synovial fluid from rheumatoid patients. *Clin. Sci.* **61**, 483–486.

Blake, D. R., Lunec, J., Ahern, M., Ring, E. F. J., Bradfield, J. & Gutteridge, J. M. C. 1985*a* The effect of intravenous iron-dextran on rheumatoid synovitis. *Ann. Rheum. Dis.* **44**, 183–188.

Blake, D. R., Winyard, P., Lunec, J., Williams, A., Good, P. A., Gutteridge, J. M. C., Rowley, D. & Halliwell, B. 1985*b* Cerebral and ocular toxicity induced by desferrioxamine. *Q. Jl Med.* (In the press.)

Bowern, N., Ramshaw, I. A., Clark, I. A. & Doherty, P. C. 1984 Inhibition of autoimmune neuropathological process by treatment with an iron-chelating agent. *J. exp. Med.* **160**, 1532–1543.

Boxer, L. A., Coates, T. D., Haak, R. A., Wolach, J. B., Hoffstein, S. & Baehner, R. L. 1982 Lactoferrin deficiency associated with altered granulocyte function. *New Engl. J. Med.* **307**, 404–410.

Butler, J. & Halliwell, B. 1982 Reaction of iron–EDTA chelates with the superoxide radical. *Archs Biochem. Biophys.* **218**, 174–178.

Clark, I. A. & Hunt, N. H. 1983 Evidence for reactive oxygen intermediates causing haemolysis and parasite death in malaria. *Infect. Immun.* **39**, 1–6.

Cohen, G. 1978 The generation of hydroxyl radicals in biologic systems: toxicological aspects. *Photochem. Photobiol.* **28**, 669–675.

Creeth, J. M., Cooper, B., Donald, A. S. R. & Clamp, J. R. 1983 Studies on the limited degradation of mucus glycoproteins. *Biochem. J.* **211**, 323–332.

Cross, C. E., Halliwell, B. & Allen, A. 1984 Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus. *Lancet*, i, pp. 1328–1330.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Czapski, G. 1984 On the use of $OH^{\cdot}$ scavengers in biological systems. *Israel J. Chem.* **24**, 29–32.
Davies, S. C., Marcus, R. E., Hungerford, J. L., Miller, M. H., Arden, G. B. & Huehns, E. R. 1983 Ocular toxicity of high-dose intravenous desferrioxamine. *Lancet* i, pp. 181–184.
Dean, R. T., Roberts, C. R. & Forni, L. 1985 Oxygen-centred free radicals efficiently degrade the polypeptide of proteoglycans in cartilage. *Biosci. Rep.* (In the press.)
Etherington, D. J., Pugh, G. & Silver, I. A. 1981 Collagen degradation in an experimental inflammatory lesion: studies on the role of the macrophage. *Acta biol. med. germ.* **40**, 1625–1631.
Fligiel, S. E. G., Ward, P. A., Johnson, K. J. & Till, G. O. 1984 Evidence for a role of hydroxyl radical in immune-complex-induced vasculitis. *Am. J. Path.* **115**, 375–382.
Flitter, W., Rowley, D. A. & Halliwell, B. 1983 Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. *FEBS Lett.* **158**, 310–312.
Floyd, R. A. 1981 DNA–ferrous iron catalysed hydroxyl free radical formation from hydrogen peroxide. *Biochem. biophys. Res. Commun.* **99**, 1209–1215.
Floyd, R. A. 1983 Direct demonstration that ferrous ion complexes of di- and triphosphate nucleotides catalyse hydroxyl free radical formation from hydrogen peroxide. *Archs Biochem. Biophys.* **225**, 263–270.
Fox, R. B. 1984 Prevention of granulocyte-mediated oxidant lung injury in rats by a hydroxyl radical scavenger, dimethylthiourea. *J. clin. Invest.* **74**, 1456–1465.
Giordano, N., Fioravanti, A., Sancasciani, S., Marcolongo, R. & Borghi, C. 1984 Increased storage of iron and anaemia in rheumatoid arthritis: usefulness of desferrioxamine. *Br. med. J.* **289**, 961–962.
Green, M. R., Hill, H. A. O., Okolow-Zubkowska, M. J. & Segal, A. W. 1979 The production of hydroxyl and superoxide radicals by stimulated human neutrophils – measurements by e.p.r. spectroscopy. *FEBS Lett.* **100**, 23–26.
Greenwald, R. A. & Moy, W. W. 1980 Effect of oxygen-derived free radicals on hyaluronic acid. *Arthritis Rheum.* **23**, 455–463.
Grootveld, M. & Halliwell, B. 1985 An aromatic hydroxylation assay for hydroxyl radicals utilizing high-performance liquid chromatography (HPLC). Use to investigate the effect of EDTA on the Fenton reaction. *Free radical research communications.* (In the press.)
di Guiseppi, J. & Fridovich, I. 1984 The toxicology of molecular oxygen. *CRC Crit. Rev. Toxicol.* **12**, 315–342.
Gutteridge, J. M. C. 1984*a* Reactivity of hydroxyl and hydroxyl-like radical discriminated by release of thiobarbituric-acid-reactive material from deoxyribose, nucleosides and benzoate. *Biochem. J.* **224**, 761–767.
Gutteridge, J. M. C. 1984*b* Lipid peroxidation initiated by superoxide-dependent hydroxyl radicals using complexed iron and hydrogen peroxide. *FEBS Lett.* **172**, 245–250.
Gutteridge, J. M. C. 1984*c* Ferrous iron–EDTA-stimulated phospholipid peroxidation. A reaction changing from alkoxyl radical to hydroxyl-radical-dependent initiation. *Biochem. J.* **224**, 697–701.
Gutteridge, J. M. C. 1984*d* Copper–phenanthroline-induced site-specific oxygen radical damage to DNA. *Biochem. J.* **218**, 983–985.
Gutteridge, J. M. C. 1985 Age pigments and free radicals: fluorescent lipid complexes formed by iron- and copper-containing proteins. *Biochim. biophys. Acta.* **834**, 144–148.
Gutteridge, J. M. C. & Halliwell, B. 1982 The role of superoxide and hydroxyl radicals in the degradation of DNA and deoxyribose induced by a copper-phenanthroline complex. *Biochem. Pharmacol.* **31**, 2801–2805.
Gutteridge, J. M. C., Halliwell, B., Treffry, A., Harrison, P. M. & Blake, D. 1983 Effect of ferritin-containing fractions with different iron loading on lipid peroxidation. *Biochem. J.* **209**, 557–560.
Gutteridge, J. M. C., Paterson, S. K., Segal, A. W. & Halliwell, B. 1981*b* Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. *Biochem. J.* **199**, 259–261.
Gutteridge, J. M. C., Richmond, R. & Halliwell, B. 1979 Inhibition of the iron-catalysed formation of hydroxyl radicals from superoxide and of lipid peroxidation by desferrioxamine. *Biochem. J.* **184**, 469–472.
Gutteridge, J. M. C., Rowley, D. A. & Halliwell, B. 1981*a* Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. Detection of 'free' iron in biological systems by using bleomycin-dependent degradation of DNA. *Biochem. J.* **199**, 263–265.
Gutteridge, J. M. C., Rowley, D. A., Griffiths, E. & Halliwell, B. 1985*b* Low molecular mass iron complexes and oxygen radical reactions in idiopathic haemochromatosis. *Clin. Sci.* **68**, 463–467.
Gutteridge, J. M. C., Rowley, D. A. & Halliwell, B. 1982*a* Superoxide-dependent formation of hydroxyl radicals and lipid peroxidation in the presence of iron salts. *Biochem. J.* **206**, 605–609.
Gutteridge, J. M. C., Rowley, D. A., Halliwell, B., Cooper, D. F. & Heeley, D. M. 1985*a* Copper and iron complexes catalytic for oxygen radical reactions in sweat from human athletes. *Clinica chim. Acta.* **145**, 267–273.
Gutteridge, J. M. C., Rowley, D. A., Halliwell, B. & Westermarck, T. 1982*b* Increased non-protein-bound iron and decreased protection against superoxide radical damage in cerebrospinal fluid from patients with neuronal ceroid lipofuscinoses. *Lancet* ii, pp. 459–461.
Gutteridge, J. M. C. & Wilkins, S. 1984 Non-protein-bound iron within bacterial cells and the action of bleomycin. *Biochem. Int.* **8**, 89–93.
Halliwell, B. 1978*a* Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. *FEBS Lett.* **92**, 321–326.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Halliwell, B. 1978*b* Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. *FEBS Lett.* **96**, 238–242.
Halliwell, B. 1982*a* Production of superoxide, hydrogen peroxide and hydroxyl radicals by phagocytic cells: a cause of chronic inflammatory disease? *Cell Biol. Int. Rep.* **6**, 529–541.
Halliwell, B. 1982*b* Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts is a feasible source of hydroxyl radicals *in vivo*. *Biochem. J.* **205**, 461–462.
Halliwell, B. & Gutteridge, J. M. C. 1984 Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **219**, 1–14.
Halliwell, B. & Gutteridge, J. M. C. 1985 *Free radicals in biology and medicine*. Oxford University Press.
Henderson, W. R. & Klebanoff, S. J. 1983 Leukotriene B_4, C_4, D_4 and E_4 inactivation by hydroxyl radicals. *Biochem. biophys. Res. Commun.* **110**, 266–272.
Jacobs, A. 1977 Iron overload – clinical and pathological aspects. *Semin. Hematol.* **14**, 89–113.
Johnston, R. B., Keele, B. B., Misra, H. P., Lehmeyer, J. E., Webb, L. S., Baehner, R. L. & Rajagopalan, K. V. 1975 The role of superoxide anion generation in phagocytic bactericidal activity. *J. clin. Invest.* **55**, 1357–1372.
Kanofsky, J. R., Wright, J., Miles-Richardson, G. E. & Tauber, A. I. 1984 Biochemical requirements for singlet oxygen production by purified human myeloperoxidase. *J. clin. Invest.* **74**, 1489–1495.
Kittridge, K. J. & Willson, R. L. 1984 Uric acid substantially enhances the free radical-induced inactivation of alcohol dehydrogenase. *FEBS Lett.* **170**, 162–164.
Lewis, D. A., Parrott, D. P., Bird, J., Cosh, J. A. & Ring, F. 1984 Inactive forms of plasma antiproteases in arthritic synovial fluids. *Int. Res. Commun. Syst. Med. Sci.* **12**, 304–305.
Lonnerdal, B., Keen, C. L. & Hurley, L. S. 1981 Iron, copper, zinc and manganese in milk. *Ann. Rev. Nutr.* **1**, 149–174.
McCord, J. M. & Day, E. D. 1978 Superoxide-dependent production of hydroxyl radical catalysed by iron–EDTA complex. *FEBS Lett.* **86**, 139–142.
Maguire, J. T., Kellogg, E. W. & Packer, L. 1982 Protection against free radical formation by protein-bound iron. *Toxic. Lett.* **14**, 27–34.
Mello Filho, A. C. & Meneghini, R. 1984 *In vivo* formation of single-strand breaks in DNA by hydrogen peroxide is mediated by the Haber–Weiss reaction. *Biochim. biophys. Acta* **781**, 56–63.
Modell, B., Letsky, E. A., Flynn, D. M., Peto, R. & Weatherall, D. J. 1982 Survival and desferrioxamine in thalassaemia major. *Br. med. J.* **284**, 1081–1084.
Moorhouse, C. P., Halliwell, B., Grootveld, M. & Gutteridge, J. M. C. 1985 Cobalt (II) ion as a catalyst of hydroxyl radical and possible 'crypto hydroxyl' radical formation. Differential effects of hydroxyl radical scavengers. *Biochim biophys. Acta* (In the press.)
Motohashi, N. & Mori, I. 1983 Superoxide-dependent formation of hydroxyl radical catalysed by transferrin. *FEBS Lett.* **157**, 197–199.
Muirden, K. D. 1970 The anaemia of rheumatoid arthritis. The significance of iron deposits in the synovial membrane. *Aust. Ann. Med.* **2**, 97–104.
Nauseef, W. M., Root, R. K. & Malech, H. L. 1983 Biochemical and immunological analysis of hereditary myeloperoxidase deficiency. *J. clin. Invest.* **71**, 1297–1307.
Newburger, P. E. & Tauber, A. I. 1982 Heterogeneous pathways of oxidizing radical production in human neutrophils and the HL-69 cell line. *Pediat. Res.* **16**, 856–860.
Phillips, B. J., James, T. E. B. & Anderson, D. 1984 Genetic damage in CHO cells exposed to enzymically generated active oxygen species. *Mutat. Res.* **126**, 265–271.
Repine, J. E., Eaton, J. W., Anders, M. W., Hoidal, J. R. & Fox, R. B. 1979 Generation of hydroxyl radical by enzymes, chemicals and human phagocytes *in vitro*. *J. clin. Invest.* **64**, 1642–1651.
Repine, J. E., Fox, R. B. & Berger, E. M. 1981 Hydrogen peroxide kills *Staphylococcus aureus* by reacting with staphylococcal iron to form hydroxyl radical. *J. biol. Chem.* **256**, 7094–7096.
Repine, J. E., Johansen, K. S. & Berger, E. M. 1984 Hydroxyl radical scavengers produce similar decreases in the chemiluminescence responses and bactericidal activities of neutrophils. *Infect. Immun.* **43**, 435–438.
Richmond, R., Halliwell, B., Chauhan, J. & Darbre, A. 1981 Superoxide-dependent formation of hydroxyl radicals; detection of hydroxyl radicals by the hydroxylation of aromatic compounds. *Analyt. Biochem.* **118**, 328–335.
Rosen, H. & Klebanoff, S. J. 1981 Role of iron and EDTA in the bactericidal activity of a superoxide anion-generating system. *Archs Biochem. Biophys.* **208**, 512–519.
Rowley, D. A., Gutteridge, J. M. C., Blake, D., Farr, M. & Halliwell, B. 1984 Lipid peroxidation in rheumatoid arthritis: thiobarbituric-acid-reactive material and catalytic iron salts in synovial fluid from rheumatoid patients. *Clin. Sci.* **66**, 691–695.
Rowley, D. A. & Halliwell, B. 1983 Superoxide-dependent and ascorbate-dependent formation of hydroxyl radicals in the presence of copper salts: a physiologically-significant reaction? *Archs Biochem. Biophys.* **225**, 279–284.
Sagone, A. L., Decker, M. A., Wells, R. M. & Democko, C. 1980 A new method for the detection of hydroxyl radical production by phagocytic cells. *Biochim. biophys. Acta* **628**, 90–102.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Samuni, A., Chevion, M. & Czapski, G. 1981 Unusual copper-induced sensitization of the biological damage due to superoxide radical. *J. biol. Chem.* **256**, 12632–12635.
Schumacher, H. R. 1982 Articular cartilage in the degenerative arthropathy of haemochromatosis. *Arthrit. Rheum.* **25**, 1460–1467.
Sweder van Asbeck, B., Marx, J. J. M., Struyvenberg, A., van Kats, J. H. & Verhoef, J. 1984*a* Effect of iron(III) in the presence of various ligands on the phagocytic and metabolic activity of human polymorphonuclear leukocytes. *J. Immun.* **132**, 851–856.
Sweder van Asbeck, B., Marx, J. J. M., Struyvenberg, A., van Kats, J. H. & Verhoef, J. 1984*b* Deferoxamine enhances phagocytic function of human polymorphonuclear leukocytes. *Blood* **63**, 714–718.
Test, S. T., Lampert, M. B., Ossanna, P. J., Thoene, J. G. & Weiss, S. J. 1984 Generation of nitrogen-chlorine oxidants by human phagocytes. *J. clin. Invest.* **74**, 1341–1349.
Ward, P. A., Till, G. O., Kunkel, R. & Beauchamp, C. 1983 Evidence for role of hydroxyl radical in complement and neutrophil-dependent tissue injury. *J. clin. Invest.* **72**, 789–801.
Weiss, S. J., King, G. W. & Lo Buglio, A. F. 1977 Evidence for hydroxyl radical generation by human monocytes. *J. clin. Invest.* **60**, 370–376.
Weitberg, A. B., Weitzman, S. A., Destrempes, M., Latt, S. A. & Stossel, T. P. 1983 Stimulated human phagocytes produce cytogenetic changes in cultured mammalian cells. *New Engl. J. Med.* **308**, 26–30.
Wills, E. D. 1969 Lipid peroxide formation of microsomes. The role of non-haem iron. *Biochem. J.* **113**, 325–332.
Willson, R. L. 1978 Iron, zinc, free radicals and oxygen in tissue disorders and cancer control. In *Iron metabolism*: *CIBA Foundation Symposium* **65**, pp. 331–354. Amsterdam: Elsevier.
Winterbourn, C. C. 1983 Lactoferrin-catalysed hydroxyl radical production. *Biochem. J.* **210**, 15–19.
Wong, S. F., Halliwell, B., Richmond, R. & Skowroneck, W. R. 1981 The role of superoxide and hydroxyl radicals in the degradation of hyaluronic acid induced by metal ions and by ascorbic acid. *J. inorg. Biochem.* **14**, 127–135.
Wright, S. D. & Silverstein, S. C. 1984 Phagocytosing macrophages exclude proteins from the zones of contact with opsonised targets. *Nature, Lond.* **309**, 359–360.
Yoshino, S., Blake, D. R. & Bacon, P. A. 1984 The effect of desferrioxamine on antigen-induced inflammation in the rat air pouch. *J. Pharm. Pharmacol.* **36**, 543–545.
Youngman, R. J. 1984 Oxygen activation: is the hydroxyl radical always biologically relevant? *Trends biochem. Sci.* **9**, 280–284.

Discussion

ROBERTA J. WARD (*Division of Clinical Cell Biology, M.R.C., Clinical Research Centre, Harrow*). What percentage of the total synovial fluid iron is non-protein bound iron? In our studies at Northwick Park Hospital, we have shown a significantly lower mean total iron concentration (7.5 ± 3 μmol l^{-1}, $n = 10$) in the synovial fluid of rheumatoid arthritis patients with severe disease activity compared with rheumatoid arthritis patients with mild disease activity (16.0 ± 6 μmol l^{-1}, $n = 10$); $P > 0.02$. Therefore if the non-protein bound iron was a consistent percentage of the total iron, Dr Halliwell's results of a linear relation between non-protein iron and TBA-reacting material might indicate that there should be higher concentrations of TBA-reacting material in the synovial fluid of patients with mild disease activity. (TBA is thiobarbituric acid.)

B. HALLIWELL. Bleomycin-detectable iron is only a small percentage of the total iron present in synovial fluid and shows no consistent relation to the total iron present, either in synovial fluid or in plasma from patients suffering idiopathic haemochromatosis. Our results are thus not necessarily inconsistent with those of Dr Ward. Iron bound to lactoferrin or transferrin will not accelerate lipid peroxidation or register in the bleomycin assay, yet will show up in 'total iron' determinations.

P. M. MAY (*Department of Applied Chemistry, UWIST, Cardiff*). Dr Halliwell suggests that chelating agents might be used to sequester iron from the low molecular mass pool in cells. However, it is important to remember the competitive effects of other metal ions in the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

biological medium. For example, has he actually observed the reactions of EDTA that he discusses? I would expect them to be entirely inhibited by the binding of endogenous Ca^{II} or Mg^{II} to the chelating agent.

B. Halliwell. Because EDTA promotes iron-dependent formation of hydroxyl radicals we do not recommend it for use *in vivo*. Desferrioxamine has a binding constant for Fe^{III} several orders of magnitudes greater than that for any other metal ion.

EDTA administered to animals in other experiments is often given as its calcium chelate.

H. Sies (*Institut für Physiologische Chemie I, Universität Düsseldorf, F.R.G.*). I might comment on some clinical observations in West Germany. Superoxide dismutase has been introduced as a drug in 1982 under the name of Peroxinorm, largely in the field of orthopedic surgery. As far as I know, about 600000 patients have been treated for disorders such as gonarthritis, coxarthritis and other inflammations, and the results have been positive.

B. Halliwell. These observations are encouraging in showing the role of oxygen radicals in inflammation *in vivo*, but are the beneficial effects of SOD any better than those of steroids, non-steroidal anti-inflammatory drug therapy or other drugs? It must not be assumed that the ability of SOD to scavenge O_2^- necessarily accounts for its anti-inflammatory effect (Baret *et al.* 1984).

Reference

Baret, A., Jadot, G. & Michelson, A. M. 1984 Anti-inflammatory properties in the rat of CuZnSOD from various species. *Life Chem. Rep.* suppl. 2, pp. 417–421.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES