The superoxide-dependent transfer of iron from ferritin to transferrin and lactoferrin

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By the use of gel filtration and [^{59}Fe]ferritin, apotransferrin and apolactoferrin were shown to take up iron released from ferritin by superoxide generated by hypoxanthine and xanthine oxidase. Apotransferrin also inhibited uptake of released iron by ferrozine. Ferritin and the xanthine oxidase system induced lipid peroxidation in phospholipid liposomes. This peroxidation was inhibited by apotransferrin or apolactoferrin. Thus, although superoxide and other free radicals can release iron from ferritin, either iron-binding protein, if present, should take up this iron and prevent its catalysing subsequent oxidative reactions.

INTRODUCTION

Excessive production of $\text{O}_2^{\cdot -}$ and other free-radical species is damaging to biological systems. There is considerable evidence that this damage involves the radicals reacting with H_2O_2 and iron to give $\text{OH}^{\cdot}$ or other highly oxidizing species (radical-driven Fenton reactions) or iron-catalysed peroxidation of polyunsaturated lipid (Aust *et al.*, 1985; Halliwell & Gutteridge, 1986). This requires the presence of iron in a catalytically active form. Very little of the iron in mammalian tissues is available as low- M_r complexes. Most stored iron (approx. 30% of the total body pool) is present as ferritin. It is contained within the central core of the protein as a ferric oxyhydroxide polymer, and as such is insulated from oxidative reactions occurring in the surroundings (Crichton, 1973; Theil, 1987). However, release of this iron could result in deleterious free-radical reactions.

Ferritin iron can be released either by a reductive process or, less efficiently, by direct chelation (Crichton *et al.*, 1980; Kontoghiorghes, 1987). Superoxide, either as potassium superoxide (Bolann & Ulvik, 1987) or produced by stimulated neutrophils (Biemond *et al.*, 1984) or xanthine oxidase (Thomas *et al.*, 1985), has been shown to release iron from ferritin. Radicals produced by reduction of paraquat, adriamycin and some quinones (Thomas & Aust, 1986a,b; H. P. Monteiro, G. F. Vile & C. C. Winterbourn, unpublished work) are also effective. Hence, ferritin could be a source of iron for catalysing subsequent reactions of these radicals. Consequently, it has been postulated that ferritin contributes to the tissue injury associated with $\text{O}_2^{\cdot -}$ production in inflammation (Biemond *et al.*, 1984) or ischaemia and reperfusion (Aust *et al.*, 1985; Biemond *et al.*, 1986) and the toxicity of redox cycling compounds.

To participate in lipid peroxidation or $\text{OH}^{\cdot}$ production, iron released from ferritin must be in a form that is catalytically active. Transferrin and lactoferrin both bind iron with high affinity, and this bound iron is a very poor catalyst of $\text{OH}^{\cdot}$ production or lipid peroxidation (Gutteridge *et al.*, 1983; Winterbourn, 1983;

Baldwin *et al.*, 1984). Transferrin normally exists in body fluids, and lactoferrin in neutrophils and various secretions (Van Snick *et al.*, 1974), in a partially saturated state. Hence, if iron released from ferritin by radicals like $\text{O}_2^{\cdot -}$ is accessible to either protein, complexation may prevent its catalysing further radical reactions. To examine this proposal, we have studied the mobilization of iron from ferritin induced by $\text{O}_2^{\cdot -}$ generated from hypoxanthine and xanthine oxidase. We show that $\text{O}_2^{\cdot -}$ causes rapid and efficient iron transfer from ferritin to apotransferrin and to apolactoferrin, and that both iron-binding proteins inhibit lipid peroxidation induced by ferritin and the $\text{O}_2^{\cdot -}$ -generating system.

MATERIALS AND METHODS

Reagents

Ferritin from horse spleen (46% iron-saturated), human transferrin, human lactoferrin (both substantially iron-free), catalase (thymol-free), cytochrome *c*, superoxide dismutase (SOD) (from bovine erythrocytes), buttermilk xanthine oxidase, hypoxanthine, ferrozine, 2-thiobarbituric acid (TBA) and butylated hydroxytoluene (BHT) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). $^{59}\text{FeCl}_3$ was purchased from New England Nuclear (North Ryde, N.S.W., Australia). Ultrogel AcA 34 was from LKB (Bromma, Sweden). Other chemicals were of analytical-reagent grade from BDH. Solutions were prepared in deionized water (Milli Q system), and buffers were stirred with Chelex 100 resin (Bio-Rad Laboratories) to free them of contaminating transition metals.

Xanthine oxidase was diluted in phosphate-buffered saline (PBS) containing 2.5 mM-EDTA and passed through a Sephadex G-25 column to remove the $(\text{NH}_4)_2\text{SO}_4$, EDTA and other contaminants present in the commercial preparation. Rates of $\text{O}_2^{\cdot -}$ production were assayed as described by McCord & Fridovich (1969).

Measurement of ferritin iron release

Ferritin (200 $\mu\text{g}/\text{ml}$) was incubated at 37 °C in PBS with ferrozine (200 μM), hypoxanthine (50 μM), xanthine

Abbreviations used: BHT, butylated hydroxytoluene; PBS, phosphate-buffered saline (10 mM-sodium phosphate/0.14 M-NaCl, pH 7.3); SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; $\text{O}_2^{\cdot -}$, superoxide radical.

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oxidase (approx. 0.011 unit/ml) and other additives as required. The appearance of the Fe^{2+} -ferrozine complex was continuously monitored, and rates of iron release (which were constant during the first few minutes of the reaction) were calculated by using an ϵ_{562} of $27900 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Stookey, 1970). All kinetic measurements were made on a Pye-Unicam PU spectrophotometer.

The total iron content of the ferritin was determined (Hoy *et al.*, 1974) by incubating 500 mM-sodium acetate, 500 mM-thiodiglycol and ferritin (0.1 mg/ml), overnight at room temperature with 1.0 mM-ferrozine and 2.0 mM-ascorbate. At the end of the incubation, the A_{562} of the solution was read and compared with a standard curve of $\text{Fe}(\text{NH}_4)_2\text{SO}_4$ and ferrozine.

^{59}Fe loading of ferritin

^{59}Fe -loaded ferritin was prepared by using the method of Hoy *et al.* (1974), giving an incorporation of 3.8×10^8 c.p.m./mg of protein without significant change in its iron saturation level. At the end of the incubation, [^{59}Fe]ferritin was dialysed for 24 h at 4°C against two changes of PBS, pH 7.3, the first containing $1 \mu\text{M}$ -EDTA. Chromatography on a column ($1.2 \times 50 \text{ cm}$) of Ultrogel AcA 34 with 0.15 M -NaCl/ 12 mM -phosphate, pH 7.4, as elution buffer showed that all the radioactivity was associated with the ferritin peak, which was eluted near the void volume. No radioactivity in low- M_r fractions was detected (see Fig. 5 below).

^{59}Fe transfer from ferritin to transferrin and lactoferrin

Transferrin (1 mg/ml) or lactoferrin (0.25 mg/ml) was incubated in PBS at 37°C with ferritin ($100 \mu\text{g/ml}$ containing $74 \mu\text{M}$ -Fe), hypoxanthine ($50 \mu\text{M}$) and xanthine oxidase at a concentration (approx. 0.01 unit/ml) that generated $6.9 \mu\text{M}$ - $\text{O}_2^{\cdot-}$ /min. Catalase ($60 \mu\text{g/ml}$) or SOD ($100 \mu\text{g/ml}$) was present where indicated. Samples (0.5 ml) were passed through the AcA 34 Ultrogel column, and the fractions were collected and counted for radioactivity. Ferritin and transferrin were well separated (Fig. 1). Lactoferrin had the same elution profile as transferrin.

The percentage of the ^{59}Fe transferred from ferritin to transferrin or lactoferrin was calculated from the pro-

Fig. 1. Elution profile after chromatography on Ultrogel ACA 34 of a mixture of 0.5 mg of apotransferrin (Tf) and 0.05 mg of ferritin (F)

portion of the total radioactivity in the fractions corresponding to the transferrin or lactoferrin peak, after subtracting the background radioactivity present in these fractions when ferritin alone was chromatographed.

Lipid peroxidation

Phospholipids were extracted from fresh lamb brains (Gutteridge, 1977). Multilamellar liposomes were prepared daily by adding phospholipids (10 mg/ml) to 0.15 M -NaCl, pH 7.0, and shaking with glass beads. Liposomes (1 mg of phospholipid in a total volume of 1 ml of PBS) were incubated at 37°C in air for 30 min with hypoxanthine, xanthine oxidase, catalase, ferritin and transferrin or lactoferrin at concentrations given in the legend to Table 2 (below). Peroxidation was determined by measuring TBA-reactive products (Buege & Aust, 1978). BHT [0.03 vol. in 2% (v/v) ethanol] was added to the TBA reagent to prevent further lipid peroxidation during the assay procedure.

RESULTS

Xanthine oxidase-dependent iron release from ferritin

Iron was released from horse spleen ferritin incubated with hypoxanthine and xanthine oxidase and was detectable as a Fe^{2+} -ferrozine complex. Fig. 2 shows that increasing the $\text{O}_2^{\cdot-}$ flux, by increasing the xanthine oxidase concentration, increased the amount of iron mobilization. Although chelating agents can release iron from ferritin (Crichton *et al.*, 1980), the process is slow, and no significant release was observed in the absence of xanthine oxidase. The release of comparable amounts of iron in the presence of ferrozine or transferrin (see below) implies that ferrozine does not facilitate $\text{O}_2^{\cdot-}$ -dependent release. Increasing the ferritin concentration, with a constant activity of xanthine oxidase, increased the amount of iron released (Fig. 3). Thus the efficiency of iron release (mol/mol of $\text{O}_2^{\cdot-}$) was greatest at high ferritin concentrations and low $\text{O}_2^{\cdot-}$ -generation rates. The $0.24 \mu\text{M}$ -iron release/min from ferritin (1 mg/ml) by an

Fig. 2. Rates of iron release from ferritin as a function of increasing activity of xanthine oxidase

Reaction conditions: PBS buffer, pH 7.3; hypoxanthine, $50 \mu\text{M}$; ferrozine, $200 \mu\text{M}$; ferritin, $200 \mu\text{g/ml}$; and catalase, $60 \mu\text{g/ml}$. Reactions were carried out at 37°C with various concentrations of xanthine oxidase. Rates of $\text{O}_2^{\cdot-}$ production were measured independently as cytochrome *c* reduction.

Fig. 3. Rate of iron release as a function of increasing concentration of ferritin

Reaction conditions were the same as those described in the legend to Fig. 2. Xanthine oxidase was added to give 4.1 μM-O₂⁻/min. A ferritin concentration of 100 μg/ml corresponds to 74 μM ferritin iron.

Fig. 4. Effect of apotransferrin on Fe²⁺-ferrozine formation during incubation of ferritin with the xanthine oxidase system

Reaction conditions were the same as those described in the legend to Fig. 2, with xanthine oxidase added to give 3.8 μM-O₂⁻/min. Percentage inhibition was calculated from initial rates of iron release. Transferrin at 1 μM is capable of binding 2 μM-iron and corresponds to a 0.08 mg/ml solution.

O₂⁻ flux of 4.1 μM/min (Fig. 3) gives an efficiency of 0.058. In agreement with previous findings (Biemond *et al.*, 1984; Thomas *et al.*, 1985; Bolann & Ulvik, 1987), the addition of catalase (60 μg/ml) increased the rate of accumulation of the Fe²⁺ complex approx. 1.5-fold, probably by preventing H₂O₂-dependent Fe²⁺ oxidation. SOD (10 μg/ml) inhibited iron release by 94%.

Addition of increasing concentrations of transferrin to the system containing ferritin, ferrozine and the xanthine

oxidase led to progressively less iron detectable as Fe²⁺-ferrozine (Fig. 4). Adding apotransferrin to a preformed Fe²⁺-ferrozine complex, however, did not cause disappearance of the complex. This implies that transferrin is able to compete with ferrozine for the Fe²⁺ released from the transferrin and suggests that O₂⁻ can transfer iron from ferritin to transferrin.

Transfer of iron from ferritin to transferrin or lactoferrin

To investigate directly the transfer of iron to the iron-binding proteins, ferritin was loaded with ⁵⁹Fe and incubated with the xanthine oxidase system and transferrin or lactoferrin. The available iron-binding capacity (24 μM for transferrin and 6 μM for lactoferrin) was well in excess of the iron released by the xanthine oxidase system (up to 3 μM in the transferrin experiments and 1.5 μM in the lactoferrin experiments). The distribution of ⁵⁹Fe between the two proteins was determined after separation by gel filtration.

When [⁵⁹Fe]ferritin was incubated with transferrin in the absence of xanthine oxidase or any reducing agent, there was a small amount of ⁵⁹Fe transferred to the transferrin peak (2.0% of the total ferritin iron over 30 min) Fig. 5 shows that addition of the xanthine oxidase O₂⁻-generating system increased the amount of ⁵⁹Fe transferred to 7.0%. The central role for O₂⁻ in the iron transfer is evidenced by its total inhibition by SOD at 100 μg/ml (Fig. 5). The 7% iron release from ferritin in 30 min (Fig. 5) compares with 6.8% measured in a separate experiment with ferrozine present instead of apotransferrin, but under otherwise similar conditions.

Fig. 5. ⁵⁹Fe elution profiles after incubation of [⁵⁹Fe]ferritin with transferrin

Chromatography on Ultrogel AcA 34 was carried out 30 min after incubation of both proteins at 37 °C in the following situations: A, only ferritin (●); B, A plus transferrin (□); C, B plus hypoxanthine (50 μM/xanthine oxidase (6.9 μM-O₂⁻/min) (■); D, C plus SOD (100 μg/ml) (▲). 'Relative radioactivity/min' means the proportion of total c.p.m. in each of the eluted fractions.

Fig. 6. Time-dependence for iron transfer from [^{59}Fe]ferritin to transferrin

The percentage of iron transferred from [^{59}Fe]ferritin to transferrin was calculated from the ^{59}Fe radioactivity eluted with the ferritin and transferrin peaks after incubation under the same conditions as for Fig. 5 in the absence (●) or presence (▲) of xanthine oxidase and hypoxanthine.

As shown in Fig. 6, the amount of ^{59}Fe transferred to transferrin increased with increasing the time of exposure to the $\text{O}_2^{\cdot-}$ flux.

In analogous experiments, ^{59}Fe released from ferritin by the hypoxanthine/xanthine oxidase system was taken up by lactoferrin present in the medium in a time-dependent manner (Table 1). Table 1 also shows that, with no lactoferrin present, a similar amount of ^{59}Fe was released by the xanthine oxidase system, but it was recovered in the low- M_r fraction on gel filtration.

Inhibition by transferrin and lactoferrin of lipid peroxidation mediated by $\text{O}_2^{\cdot-}$ and ferritin

Thomas *et al.* (1985) showed that ferritin, in combination with xanthine and xanthine oxidase, causes lipid peroxidation. Utilizing a similar $\text{O}_2^{\cdot-}$ -generating system and phospholipid liposomes, our results confirm these findings. Table 2 shows that adding ferritin to liposomes gave a low yield of TBA-reactive products in the absence, and a much higher yield in the presence, of the $\text{O}_2^{\cdot-}$ -generating system. The TBA reactivity in the absence of $\text{O}_2^{\cdot-}$, which was not affected by apotransferrin or apolactoferrin, was the same whether the ferritin was incubated with the liposomes or added immediately before heating with the TBA reagent. It could be further decreased to blank levels by adding desferrioxamine just before heating. It represents reactions occurring during heating, therefore, and not catalysis of peroxidation by ferritin alone. The enhanced TBA reactivity seen with ferritin in the presence of the xanthine oxidase system was substantially inhibited by both transferrin and lactoferrin (Table 2). So also was the basal peroxidation induced by the xanthine oxidase system alone and catalysed by trace-metal contaminants.

DISCUSSION

The present results show that iron is mobilized from ferritin by $\text{O}_2^{\cdot-}$ produced from xanthine oxidase and can be recovered as an Fe^{2+} -ferrozine complex. Xanthine

Table 1. Iron transfer from ferritin to lactoferrin mediated by xanthine oxidase system: dependence on incubation time

All reaction mixtures contained: [^{59}Fe]ferritin (100 $\mu\text{g}/\text{ml}$), lactoferrin (250 $\mu\text{g}/\text{ml}$), hypoxanthine (200 μM), catalase (60 $\mu\text{g}/\text{ml}$), xanthine oxidase (to give 1.8 $\mu\text{M}-\text{O}_2^{\cdot-}/\text{min}$) and phosphate buffer, pH 7.4. Samples (0.5 ml) were separated by gel filtration as described in the legend to Fig. 1.

Additions	Incubation time (min)	Percentage of total radioactivity in lactoferrin fractions
Ferritin alone	40	2.5
Ferritin + lactoferrin	0	4.1
+ xanthine oxidase system	20	9.2
	40	12.5
Ferritin + xanthine oxidase system	40	12.6*

* Radioactivity present in low- M_r fractions.

Table 2. Lipid peroxidation in liposomes mediated by ferritin and xanthine oxidase: inhibitory effects of lactoferrin and transferrin

Reaction mixtures containing liposomes (1 mg/ml), ferritin (50 $\mu\text{g}/\text{ml}$), transferrin (1 mg/ml) or lactoferrin (250 $\mu\text{g}/\text{ml}$), catalase (60 $\mu\text{g}/\text{ml}$), hypoxanthine (50 μM), xanthine oxidase (to give 3.5 $\mu\text{M}-\text{O}_2^{\cdot-}/\text{min}$) and were incubated for 30 min at 37 °C. The TBA-reactive products were determined as described in the Materials and methods section. Results are the means of duplicates that agreed within 13 %.

Additions to liposomes	A_{535}		Inhibition (%)*
	Xanthine oxidase absent	Xanthine oxidase present	
Transferrin	0.073	0.108	—
Lactoferrin	0.057	0.069	70
Ferritin	0.059	0.059	100
Ferritin	0.145	0.373	—
Ferritin + transferrin	0.142	0.175	86
Ferritin + lactoferrin	0.128	0.197	70

* Inhibition by transferrin or lactoferrin, measured in the presence of the xanthine oxidase system, after subtracting blanks measured in the absence of xanthine oxidase.

oxidase was shown originally by Mazur *et al.* (1958) to promote iron release from ferritin. Recent studies have shown that, aerobically, this is mediated primarily by $\text{O}_2^{\cdot-}$ (Thomas *et al.*, 1985), although some mobilization independent of $\text{O}_2^{\cdot-}$ can occur (Biemond *et al.*, 1986). Our observation of inhibition by SOD agrees with these findings.

We found that adding apotransferrin inhibited the

appearance of the Fe²⁺-ferrozine complex. This suggests that iron released from ferritin by O₂^{•-} can be taken up by transferrin. The gel-filtration experiments with ⁵⁹Fe directly demonstrated this transfer to both transferrin and lactoferrin. Transfer was mediated by O₂^{•-}, since it was inhibited by SOD. The extent of iron mobilization was similar to that measured with ferrozine, indicating that the binding proteins take up iron once it is released, rather than influencing the O₂^{•-}-mediated release process. This presumably involves initial reduction of iron within the ferritin core, as observed for electrochemical reduction (Watt *et al.*, 1985).

There was low level of iron transfer from ferritin to transferrin in the absence of the xanthine oxidase system, which became more significant at longer incubation times. This is in agreement with Jin & Crichton (1987), who saw some transfer after 20 h incubation. They observed slight acceleration by bicarbonate, citrate, lactate and ascorbate. However, according to Boyer & McCleary (1987), ascorbate-dependent iron release from ferritin (to ferrozine) is mediated by O₂^{•-}. Iron transfer from ferritin to transferrin is an important physiological process (Crichton, 1973). Macrophages convert iron released from ingested red cells into ferritin, from which it is mobilized and transported by transferrin to other cells where it is required (Crichton, 1973; Theil, 1987). Iron is released from macrophages degrading red cells *in vitro* in a ferritin-like form that can be taken up by transferrin (Saito *et al.*, 1986), but in general the physiological mechanism of transfer is not well understood (Theil, 1987). On the basis of our results we can speculate that O₂^{•-} may play a role in this process.

In support of our proposal that transferrin or lactoferrin could inhibit reactions of iron released from ferritin by O₂^{•-}, both apoproteins inhibited lipid peroxidation induced by ferritin and the xanthine oxidase system. Previous authors have described lipid peroxidation caused by ferritin and either O₂^{•-} generated by xanthine oxidase (Thomas *et al.*, 1985; Koster & Slee, 1986) or ascorbate (Wills, 1966; Gutteridge *et al.*, 1983; O'Connell *et al.*, 1985). In agreement with most of these studies, our results imply that ferritin-dependent peroxidation requires reduction and release of ferritin iron. They also show that this iron can be complexed and rendered catalytically inactive by apotransferrin or apolactoferrin before interaction with the lipid occurs. Both apoproteins can suppress lipid peroxidation and OH[•] production catalysed by iron salts (Gutteridge *et al.*, 1981; Aruoma & Halliwell, 1987). Thus it has been proposed (Halliwell & Gutteridge, 1986) that by binding iron and preventing its participation in free-radical reactions, transferrin and lactoferrin play a major role in extracellular antioxidant defence. Our results support this concept and extend it to cover ferritin-dependent reactions.

Ferritin is predominantly an intracellular storage protein. It is present in plasma, however, and is deposited in the synovium of rheumatoid joints (Muirden, 1966; Muirden & Senator, 1968). Since transferrin is restricted to extracellular locations, its major influence would be on free-radical reactions involving extracellular ferritin. Lactoferrin is a major constituent of the specific granules of neutrophils and is released when the cells ingest particles or respond to inflammatory stimuli. Stimulation of neutrophils produces a large amount of O₂^{•-}. For this O₂^{•-} to undergo Fenton reactions or cause lipid peroxidation, the cells must acquire an exogenous catalyst,

since they contain no endogenous catalyst of either reaction (Britigan *et al.*, 1986; Thomas *et al.*, 1986; Winterbourn, 1986). Such a role has been suggested for ferritin iron mobilized by O₂^{•-} generated by the cells (Biemond *et al.*, 1984). This ferritin could be extracellular or present within phagocytosed cells or organisms. However, since neutrophils exist in a medium containing partially saturated transferrin, and they release apolactoferrin on stimulation, we would predict from our results that mobilized ferritin iron could be taken up by either binding protein and, therefore, should not be able to catalyse subsequent O₂^{•-}-dependent reactions. We have recently shown that when bacteria are killed by neutrophils, bacterial iron is released and taken up by lactoferrin (A. L. Molloy & C. C. Winterbourn, unpublished work). This, taken together with the present findings, supports an antioxidant role for lactoferrin within the neutrophil, taking up iron released by oxidative or degradative processes and preventing its participation in further oxidative reactions.

Reactions involving ferritin iron released as a result of intracellular free-radical generation would not readily be inhibited by transferrin or lactoferrin, because of their limited access. However, if damage to cell integrity and iron decompartmentalization occurred (Willson, 1977), transferrin or neutrophil lactoferrin may gain access to this iron and prevent it from catalysing more widespread oxidative damage.

This work was supported by a grant from the Medical Research Council of New Zealand. H.P.M. is a Fellow of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Brazil.

REFERENCES

- Aruoma, O. I. & Halliwell, B. (1987) *Biochem. J.* **241**, 273–278
- Aust, S. D., Morehouse, L. A. & Thomas, C. E. (1985) *J. Free Radicals Biol. Med.* **1**, 3–25
- Baldwin, D. A., Jenny, E. R. & Aisen, P. (1984) *J. Biol. Chem.* **259**, 13391–13394
- Biemond, P., van Eijk, H. G., Swaak, A. J. G. & Koster, J. F. (1984) *J. Clin. Invest.* **73**, 1576–1579
- Biemond, P., Swaak, A. J. G., Beindorf, C. M. & Koster, J. F. (1986) *Biochem. J.* **239**, 169–173
- Bolann, B. J. & Ulvik, J. (1987) *Biochem. J.* **243**, 55–59
- Boyer, R. F. & McCleary, C. (1987) *Free Radical Biol. Med.* **3**, 389–395
- Britigan, B. E., Rosen, G. M., Chai, Y. & Cohen, M. S. (1986) *J. Biol. Chem.* **261**, 4426–4431
- Buege, J. A. & Aust, S. D. (1978) *Methods Enzymol.* **52**, 302–310
- Crichton, R. R. (1973) *Struct. Bonding* **17**, 67–134
- Crichton, R. R., Roman, F. & Roland, F. (1980) *FEBS Lett.* **110**, 271–274
- Gutteridge, J. M. C. (1977) *Anal. Biochem.* **82**, 76–82
- Gutteridge, J. M. C., Paterson, S. K., Segal, A. W. & Halliwell, B. (1981) *Biochem. J.* **199**, 259–261
- Gutteridge, J. M. C., Halliwell, B., Treffry, A., Harrison, P. M. & Blake, D. (1983) *Biochem. J.* **209**, 557–560
- Halliwell, B. & Gutteridge, J. M. C. (1986) *Arch. Biochem. Biophys.* **246**, 501–514
- Hoy, T. G., Harrison, P. M. & Shabbir, M. (1974) *Biochem. J.* **139**, 603–607
- Jin, Y. & Crichton, R. R. (1987) *FEBS Lett.* **1**, 41–46

- Kontoghiorghe, G. J. (1987) *Biochim. Biophys. Acta* **924**, 13–18
- Koster, J. F. & Slee, R. G. (1986) *FEBS Lett.* **199**, 85–88
- Mazur, A., Green, S., Saha, A. & Carleton, A. (1958) *J. Clin. Invest.* **37**, 1809–1817
- McCord, J. M. & Fridovich, I. (1969) *J. Biol. Chem.* **244**, 6049–6055
- Muirden, K. D. (1966) *Ann. Rheum. Dis.* **25**, 387–401
- Muirden, K. D. & Senator, G. B. (1968) *Ann. Rheum. Dis.* **27**, 38–53
- O'Connell, M. J., Ward, R. J., Baum, H. & Peters, T. J. (1985) *Biochem. J.* **229**, 135–139
- Saito, K., Nishisato, T., Grasso, J. A. & Aisen, P. (1986) *Br. J. Haematol.* **62**, 275–286
- Stookey, L. L. (1970) *Anal. Chem.* **42**, 779–781
- Theil, E. C. (1987) *Annu. Rev. Biochem.* **56**, 289–315
- Thomas, C. E. & Aust, S. D. (1986a) *Arch. Biochem. Biophys.* **248**, 684–689
- Thomas, C. E. & Aust, S. D. (1986b) *J. Biol. Chem.* **261**, 13064–13070
- Thomas, C. E., Morehouse, L. A. & Aust, S. D. (1985) *J. Biol. Chem.* **260**, 3275–3280
- Thomas, M. J., Shirley, P. S., Hedrick, C. C. & DeChatelet, L. R. (1986) *Biochemistry* **25**, 8042–8048
- Van Snick, J. L., Masson, P. L. & Heremans, J. F. (1974) *J. Exp. Med.* **140**, 1069–1083
- Watt, G. D., Frankel, R. B. & Papaefthymiou, G. C. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3640–3643
- Wills, E. D. (1966) *Biochem. J.* **99**, 667–676
- Willson, R. L. (1977) *Ciba Found. Symp.* **51**, 331–354
- Winterbourn, C. C. (1983) *Biochem. J.* **210**, 15–19
- Winterbourn, C. C. (1986) *J. Clin. Invest.* **78**, 545–550

Received 21 March 1988/21 June 1988; accepted 15 July 1988