Generation of Superoxide via the Interaction of Nitrofurantoin with Oxyhemoglobin*

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Nitrofurantoin was found to interact with HbO₂ to cause the concomitant formation of methemoglobin and superoxide. The rate of formation of methemoglobin and superoxide was linearly dependent upon the concentration of nitrofurantoin and could be inhibited by superoxide dismutase, catalase, or the prior conversion of O₂ to ethylisocyanoferrrohemoglobin. The ability of nitrofurantoin to interact with HbO₂ and cause superoxide formation may represent one mechanism by which it produces red cell toxicity and suggests that generation of superoxide in erythrocytes may occur via a different mechanism than that which occurs in microsomes.

Nitrofurantoin has been shown to cause the generation of activated oxygen species in several tissue microsomal preparations including rat liver (1) and rat lung (2) as well as in human erythrocytes (3). Mason and Holtzman have directly observed the one-electron reduction of NF to a nitroaromatic anion free radical in rat liver microsomes via EPR spectroscopy and proposed that this free radical reduces molecular oxygen to superoxide (1). Sasame and Boyd observed the generation of elevated levels of both superoxide and hydrogen peroxide in rat lung microsomes incubated aerobically in the presence of NF (2), whereas under anaerobic conditions, NF was bound covalently to microsomal macromolecules (4). These authors have proposed that NF accepts a single electron from a flavoprotein reductase thus forming a nitroaromatic anion free radical. Under aerobic conditions this nitroanion free radical transfers an electron to molecular oxygen, resulting in superoxide and elevated levels of hydrogen peroxide, while in the absence of oxygen the free radical, or a subsequent reduction product, binds covalently to macromolecules. In addition Harada and Omura have recently shown that the initial one-electron reduction in the conversion of nitrobenzene to aniline by rat liver microsomes is exclusively catalyzed by NADPH-cytochrome P-450 reductase and that subsequent reductions require the participation of both the reductase and cytochrome P-450 (5).

Persons genetically deficient in the enzyme glucose-6-phosphate dehydrogenase are susceptible to red cell hemolysis when administered NF (6). Since this enzyme is required to maintain an adequate level of NADPH in the red cell and because the primary function of NADPH in erythrocytes is to serve as a source of reducing equivalents for the reduction of GSSG to GSH, it would thus seem plausible that the mechanism for NF-mediated red cell toxicity would require (a) an increase in NADPH utilization and (b) simultaneous production of an intermediate dependent on GSH for its detoxification. For example, conversion of NF to a free radical in the presence of molecular oxygen would result in an increase in superoxide and hydrogen peroxide levels in the red cell. Since GSH can act as a scavenger of free radicals as well as serve as a source of reducing equivalents for the metabolism of hydrogen peroxide to water via glutathione peroxidase, GSH would be converted to GSSG at an increased rate, and the rate of NADPH oxidation would increase to convert the GSSG to GSH via glutathione reductase. These effects of NF on the generation of activated oxygen species, coupled with the fact that NF inhibits glutathione reductase (7), might well provide the basis for NF-mediated red cell toxicity.

Recent experiments in our laboratory on the mechanism of NF-induced red cell toxicity have revealed that NF causes a marked depletion of red cell GSH levels (8) with a concomitant increase in hydrogen peroxide and MetHb formation (3). Depletion of red cell GSH, however, could be prevented by ethyl isocyanide (9), a high affinity ligand capable of binding to ferrohemoglobin and displacing oxygen (10). This observation suggests that Hb may participate in NF-induced GSH depletion, perhaps via the reduction of NF to a free radical or subsequent reduction products, or by the NF-mediated release of superoxide from HbO₂.

Since the formation of nitroaromatic anion free radicals in microsomes appears to be achieved solely by the participation of a flavoprotein, the mechanism of NF-induced red cell toxicity may differ from those tissues which contain microsomal enzymes. We have thus undertaken a study of the interaction between NF and purified human HbO₂ to investigate directly NF-induced formation of superoxide from HbO₂.

EXPERIMENTAL PROCEDURES

Materials

A stock solution of HbO₂ was prepared by dissolving Hb (human, type IV, twice recrystallized, Sigma) in 20 mM KP, pH 7.5 at 4 °C, bubbling with O₂ for 10 min, and then adding a small excess of sodium dithionite (Fisher). The solution was then passed over two Sephadex G-25 columns at 4 °C to remove dithionite and other small molecules. The HbO₂ thus prepared was characterized via UV-visible spectrosopy, and the observed maxima agreed with previously published values (11).

Acetylated cytochrome c was prepared as described by Azzi et al. (12). Bovine liver catalase (specific activity 10,000 units/mg), bovine blood superoxide dismutase (specific activity 2,900 units/mg), and
Superoxide Generation from Nitrofurantoin-HbO₂ Interaction

Methods

Autoxidation of HbO₂—The rate of autoxidation of HbO₂ was determined using a method previously described by Mieyal and Blumer (13). Solutions of 1.9 μM HbO₂ and of 0-840 μM NF, both 20 mM in potassium phosphate buffer, pH 7.5, were added to opposite chambers of Yankeloff mixing cuvettes, allowed to reach thermal equilibrium, and a flat baseline was recorded with the Cary 219 spectrophotometer operating in the autoslit mode at 37 °C. The sample cuvette was then inverted, and the difference spectrum was recorded repetitively from 400-600 nm. In experiments using superoxide dismutase or catalase, the enzyme was placed in the cuvette chamber containing the HbO₂. A plot of the change in the absorbance of the Soret peak versus time described the autoxidation of HbO₂, containing NF permits the measurement of superoxide anion generated. A solution of 40 μM HbO₂ (final concentration) which was 100 mM in potassium phosphate buffer, pH 7.5, and contained 100 mM Na₂EDTA, was placed in 1-ml cuvettes to which was added acetylated cytochrome c to achieve a final concentration of 17.5 μM. The sample and reference cuvettes were allowed to equilibrate at 37 °C for several minutes. A fixed volume of 5 μl of a solution of dimethylformamide, which contained varying concentrations of NF (0-336 mM), was added to the sample cuvette. The reduction of acetylated cytochrome c was monitored at 550 nm, and ε₅₅₀ = 19.6 mm⁻¹ cm⁻¹ was used as the value for the reduced minus oxidized extinction coefficients (14). When 5 μl of dimethylformamide was added alone to the sample cuvette it produced a finite, but small, rate of change in A₅₅₀ as compared to that measured in the presence of NF. This rate, however, was in all cases subtracted from the rate measured in the presence of NF. Furthermore, dimethylformamide was found to be 15-fold less potent than NF in facilitating autoxidation of HbO₂. In experiments using superoxide dismutase or ethyl isocyanide the enzyme or ligand was added prior to the addition of NF in dimethylformamide and in such a manner so as to achieve the final concentration required.

RESULTS

A typical difference spectrum obtained when solutions of HbO₂ and NF are mixed is presented in Fig. 1. The Soret absorbance of HbO₂ in the absence of NF occurs at 415 nm with α and β bands at 577 and 542 nm, respectively. The addition of NF to HbO₂ produces a hypochromic shift in the Soret absorbance and decreases in the absorbances of the α and β bands of HbO₂, consistent with the difference spectrum obtained in the conversion of HbO₂ to MetHb. These alterations in the Soret and the α and β band absorbance wavelengths result in the initial difference spectrum recorded in Fig. 1 which is indicative of MetHb formation. The magnitude of the absorbance changes in the difference spectrum is also time and NF concentration dependent, thus providing spectral evidence for the interaction of NF with HbO₂ which results in an accelerated rate of autoxidation of HbO₂ to MetHb. The concentration dependence of this NF-mediated oxidation process is shown graphically in Fig. 2. In the absence of NF, HbO₂ is autoxidized to MetHb at a finite rate, resulting in a nonzero intercept at the ordinate. The concentration dependence as shown in Fig. 2 suggests that this autoxidation process may be saturable, although the very limited solubility of NF³

³ The solubility of NF in the absence of protein is 840 μM. Thus, when the mixing cell technique is employed, the maximum concentration of NF that could be attained is 420 μM.

Fig. 1. The difference spectrum of HbO₂ in the presence of NF. The final concentrations of HbO₂ and NF were 0.5 μM and 83.3 μM, respectively. The scanning interval was 17.2 min. The first and last scans are indicated.

Fig. 2. The rate of HbO₂ autoxidation in nanomoles of MetHb formed per min per amol of HbO₂ as a function of NF concentration. The concentration of HbO₂ was 0.5 μM. Each point represents the mean of at least three determinations. Standard errors were less than 5%. The inset shows the double reciprocal plot of the data corrected for the rate of HbO₂ autoxidation in the absence of NF.
partial inhibition of HbO₂ autoxidation. Furthermore, there appeared to be a slight increase in the degree of inhibition when superoxide dismutase or catalase were present in combination as compared to either being present singularly. To take into consideration the possibility that the inhibitory effects of superoxide dismutase or catalase result from non-specific protein effects, similar experiments were performed using bovine serum albumin in a protein concentration (mg/ml) comparable to that present with superoxide dismutase. When such an experiment was performed, bovine serum albumin (200 μg/ml) was found to inhibit NF-induced autoxidation by approximately 15% (Table I). Such an inhibitory effect may occur either as a result of binding of NF to bovine serum albumin, thereby lowering the effective NF concentration, or by bovine serum albumin stabilizing HbO₂ against NF-induced autoxidation. In contrast, however, superoxide dismutase (Table I) at an equivalent protein concentration of 200 μg/ml, was able to inhibit HbO₂ autoxidation by 48%, some 2.5-fold greater than that produced by bovine serum albumin. The inhibition of HbO₂ autoxidation by catalase (41%) or superoxide dismutase (48%) (Table I) suggests that as HbO₂ is converted to MetHb, oxygen is converted to superoxide. Superoxide thus formed may dismute to yield hydrogen peroxide, or considering the inhibitory effects of superoxide dismutase, cause further oxidation of HbO₂ to MetHb. Furthermore, the inhibitory effects of catalase alone, or in combination with superoxide dismutase, imply that hydrogen peroxide formed as a result of the dismutation or reduction of superoxide may also be able to convert HbO₂ to MetHb in this system.

To further examine the hypothesis that superoxide may be released during the interaction of NF with HbO₂, superoxide generation was measured using acetylated cytochrome c. Fig. 3 shows the dependence on NF concentration for the rate of superoxide generation, measured by the reduction of acetylated cytochrome c. In these experiments a higher concentration of HbO₂ (40 μM) was used, as compared to the autoxidation experiments, in order to generate measurable amounts of superoxide. Thus, although a higher maximal concentration of NF could be used (1.68 mM), the ratio of the concentrations of NF to HbO₂ (i.e. 42 versus 840) was not large enough to achieve saturation, and the rate of superoxide generation remained linear over the NF concentration range employed. Table II shows that the reduction of acetylated cytochrome c could be inhibited by superoxide dismutase or by complexing the heme iron of HbO₂ with ethyl isocyanide. The inhibition of acetylated cytochrome c reduction was dependent on the concentration of superoxide dismutase with a maximum of 83% inhibition of superoxide measured at 2600 units of superoxide dismutase/ml, while the complete conversion of HbO₂ to ethylisocyanoferrohemoglobin decreased superoxide formation by 94%. A relatively large concentration of superoxide dismutase was required to inhibit acetylated cytochrome c reduction. For example, as shown in Table II, 520 units of superoxide dismutase/ml resulted in only 18% inhibition, while 1300 and 2600 units/ml resulted in 53% and 83% inhibition, respectively. In order to preclude a generalized protein effect from being responsible for this inhibition, experiments were performed in which bovine serum albumin was present at an equivalent protein concentration as superoxide dismutase at 2600 units/ml (i.e. 1 mg/ml). Bovine serum albumin, at 1 mg/ml, had no measurable effect on superoxide generation as evidenced by acetylated cytochrome c reduction. The relatively slow rate of superoxide generation, however, may require a larger ratio of superoxide dismutase to HbO₂ in order to trap the superoxide formed before it can either participate in the further oxidation of HbO₂ to MetHb or undergo dismutation to hydrogen peroxide (vide infra).

**DISCUSSION**

The Interaction of NF with HbO₂—The interaction of NF and HbO₂ results in the production of MetHb and superoxide anion, and several plausible mechanisms may exist to account for this observation. Nitrofurantoin may interact with HbO₂ to facilitate the release of bound oxygen from the ferrous

3 NF solubility was increased by the presence of 40 μM HbO₂ and because it was added as a solution in dimethylformamide.
heme iron atom as superoxide anion resulting in the formation of MetHb.

\[
\text{HbO}_2 + \text{NF} \rightarrow \text{Hb}^{3+} + \text{NF} + \text{O}_2^- \]

In this mechanism NF is not altered but in interacting with Hb, serves to decrease the affinity of Hb for O_2. Such an alteration in oxygen affinity may be produced either by a direct interaction of NF with or near the heme iron atom or by an allosteric mechanism whereby NF in interacting with Hb affects the quaternary structure of the apoprotein or by a combination of both effects. We have obtained preliminary evidence in support of an interaction of NF in proximity to the paramagnetic heme iron atom in ferrihemoglobin (15). In these studies, using T_1 relaxation rate measurements, NF was found to interact with MetHb at a site distinct from, but in proximity to, the heme iron atom, and the interaction, as evidenced by the paramagnetic effect, continued to occur in the presence of directly coordinating ligands such as fluoride and cyanide (i.e. fluoride enhanced the paramagnetic effect while cyanide diminished but did not completely remove the paramagnetic effect). Since Hb is not paramagnetic, however, a similar study could not be performed with NF and HbO_2.

Further support for such an interaction may be derived by analogy from studies on aniline-catalyzed HbO_2 autoxidation by Mieyal and Blumer and from T_1 relaxation rate studies on the interaction of aniline, 2,6-dimethylaniline, benzene, and toluene with ferrihemoglobin (16-19). Mieyal and Blumer have proposed that aniline interacts with HbO_2 at a site distinct from the heme iron atom and alters the affinity of Hb for oxygen thus stimulating autoxidation (13). We have found that, at equivalent concentrations, aniline and NF produce comparable rates of HbO_2 autoxidation. Novak and Mieyal have presented NMR evidence in support of an interaction of aniline with MetHb at a site near the heme iron atom (16). These NMR studies demonstrated that aniline continued to interact with hemoglobin in proximity to the paramagnetic heme iron atom in the presence of ligands such as cyanide or fluoride (i.e. fluoride enhanced the paramagnetic effect while cyanide diminished but did not completely remove the paramagnetic effect). Furthermore, these results are also interesting in light of the fact that aniline binds cooperatively to MetHb (20). Thus either a direct interaction of NF with HbO_2 near the heme iron atom or an NF-induced conformational change in the apoprotein or a combination of both effects may produce the NF-mediated increase in the rate of autoxidation of HbO_2 resulting in the generation of superoxide anion.

In an alternative mechanism, NF may facilitate the release of molecular oxygen from HbO_2. NF may then be reduced to the nitro anion free radical via Hb thus yielding superoxide, from the subsequent transfer of one electron from the free radical species to molecular oxygen, and MetHb.

\[
\text{NF} + \text{HbO}_2 \rightarrow \text{NF}^- + \text{Hb}^{3+} + \text{O}_2^- \\
\text{NF}^- + \text{O}_2 \rightarrow \text{NF} + \text{O}_2^- 
\]

Some evidence may also be presented in support of this mechanism (9). When erythrocytes were incubated with NF under anaerobic conditions, cellular GSH levels decreased significantly, as compared to controls, presumably as a result of GSH acting as a scavenger of the NF free radical. This NF-produced depletion of GSH could be partially inhibited (50%) by ethyl isocyanide, thus implicating Hb in the reduction of NF. In general, however, the above mechanism is not thermodynamically favorable. The standard 1-electron reduction potentials of NF (-264 mV) (21) and Hb (+150 mV) (22) do not favor the direct reduction of NF by ferroheme. The interaction of NF with Hb, however, especially at the concentration ratios employed, may substantially alter the standard reduction potential of the Hb^{3+}/Hb^{2+} couple thereby making this reaction thermodynamically favorable. To date, no information is available on the 1-electron reduction potential of the Hb^{3+}/Hb^{2+} couple in the presence of NF.

An equally plausible explanation for the apparent protective effect of ethyl isocyanide in erythrocytes under anaerobic conditions may be obtained from the work of Harada and Omura (5). The reduction of NF to the nitro anion free radical may occur via a flavoprotein reductase, and further reduction of the free radical species may be catalyzed by Hb. The subsequent reduction product(s) may then be responsible for GSH depletion via conjugation. Thus, ethyl isocyanide may effectively inhibit further reduction of the NF nitroaromatic anion free radical by Hb and thereby prevent GSH depletion.

Of the two mechanisms suggested for NF-stimulated autoxidation of HbO_2 and generation of superoxide, that which proposes the formation of superoxide via interaction of NF with HbO_2 appears to be the more thermodynamically favorable. The interaction of NF with HbO_2 generates a steady state of superoxide anion as a function of NF concentration. Hence the rate of autoxidation of HbO_2 is linear with time (1%-2 h), and the initial rate is a function of NF concentration (Fig. 2). A certain amount of superoxide thus generated may serve to further oxidize HbO_2 to MetHb or dismute to form hydrogen peroxide which is also capable of oxidizing HbO_2 to MetHb (vide infra). The effect of either superoxide dismutase or catalase, therefore, is to inhibit HbO_2 autoxidation via conversion of superoxide to hydrogen peroxide or hydrogen peroxide to water, respectively. When superoxide dismutase and catalase are present simultaneously, a slightly greater inhibitory effect on HbO_2 autoxidation was observed (Table I). Although the systems are not directly comparable since substantially larger concentrations of NF and HbO_2 were employed, the same effect is also observed for NF-mediated superoxide generation where addition of superoxide dismutase was able to markedly inhibit the reduction of acetylated cytochrome c (Table III). Thus, in the absence of a 1-electron reduction potential for the Hb^{3+}/Hb^{2+} couple in the presence of NF, the NF-stimulated release of superoxide from HbO_2 appears to be a viable mechanism.

**Relationship to Other Results Involving Hb-Ligand Interactions**—Hirota and Itano have studied the affinity of a series of substituted nitrosobenzenes for ferrohemoglobin (23). The nitroso species is an intermediate in the reduction pathway converting nitro-containing compounds to amines (e.g. nitrobenzene to aniline). The series of nitrosobenzenes studied was found to have relatively large affinities for ferrohemoglobin and in all cases bound cooperatively (Hill coefficients of 1.8-3.8). These investigators proposed a model of nitrosarene-ferrohemoglobin interaction in which the iron of the ferroheme is bonded to the nitrogen atom of the nitroso group. Formation of the 1-electron reduced nitrosobenzeno radical as the product in the spontaneous conversion of ferrohemoglobin to MetHb by nitrobenzene was suggested (23).

Menadione may also generate superoxide anion via an analogous interaction with Hb. Goldberg and Stern have proposed that Hb catalyzes the 1-electron reduction of menadione to its free radical semiquinone (24). This semiquinone then reduces oxygen to superoxide.

Many other classes of compounds have been shown to oxidize HbO_2 to MetHb via mechanisms that do not appear to be applicable to NF. Wallace and Caughey (25) and Kawashima and Caughey (26) have presented evidence for a mechanism of MetHb formation produced by agents capable of acting as 1-electron reductants. In this model, agents such as phenols and ferrocyanides donate a single electron to dioxygen bound
as HbO₂. A second electron is donated from the ferrous iron of HbO₂ thus yielding MetHb and hydrogen peroxide as the products. Castro et al. have proposed a similar mechanism to explain the oxidation of HbO₂ by various organic reductants (27). In their model, HbO₂ reacts with the organic reductant via the abstraction of a proton yielding a free radical as well as peroxide and MetHb. It appears unlikely that NF acts via an analogous mechanism. First, such a mechanism does not explain the generation of superoxide as has been observed with NF. Second, a greater inhibitory effect of catalase on the rate of HbO₂ autoxidation would be expected than what we have observed. Third, if NF were to act as a reducing agent via the donation of a proton or an electron, it is difficult to imagine a stable product resulting from such an oxidation of NF.

Participation of Superoxide in NF-mediated HbO₂ Autoxidation—Superoxide anion is generally thought of as a reducing agent, donating a single electron and being oxidized to molecular oxygen. Our data showing that superoxide dismutase diminishes the rate of NF-mediated HbO₂ autoxidation implies that superoxide anion generated during the autoxidation reaction may actually oxidize HbO₂ to MetHb. Other investigators have also reported such an effect of superoxide. Winterbourn et al. have shown that generated superoxide can both convert HbO₂ to MetHb and reduce MetHb to HbO₂ (28). The oxidation reaction is favored when the absolute concentration of MetHb is low, while if the MetHb concentration is large relative to HbO₂, MetHb reduction by superoxide will occur. Lynch et al. found that superoxide could convert HbO₂ to MetHb (29).

The rate of HbO₂ autoxidation cannot be directly compared to the rate of superoxide generated since the two systems differ significantly in HbO₂ concentration. The experimental limitation imposed by present instrumentation⁴ precludes a thorough study of the concentration dependence of HbO₂ at a fixed concentration of NF, on the rate of autoxidation. Similarly, an analogous series of experiments using 40 μM HbO₂ and NF in order to compare rates of autoxidation and superoxide generation could not be performed. In this regard no definitive conclusion may be drawn from these experiments regarding the ability of superoxide anion to participate in further reduction or oxidation of Hb. These studies have, however, demonstrated that NF interacts with HbO₂ and is capable of producing MetHb and superoxide. Such an interaction in the erythrocyte would cause an increase in NADPH oxidation and may represent one mechanism by which NF causes red cell toxicity.

REFERENCES

1. Mason, R. P., and Holtzman, J. L. (1975) Biochem. Biophys. Res. Commun. 77, 253-260
2. Sasame, H. A., and Boyd, M. R. (1975) Life Sci. 24, 1091-1096
3. Dershwitz, M., and Novak, R. F. (1981) Biochem. Biophys. Res. Commun. 83, 31a
4. Boyd, M. R., Stjko, A. W., and Sasame, H. A. (1979) Biochem. Pharmacol. 28, 601-606
5. Harada, N., and Onura, T. (1980) J. Biochem. 87, 1539-1554
6. Kimbro, L. Jr., Sachs, M. V., and Torbert, J. V. (1957) Bull. Johns Hopkins Hosp. 101, 245-257
7. Bazard, A. A., Kopke, F., and Paul, M. F. (1960) J. Lab. Clin. Med. 56, 884-890
8. Dershwitz, M., and Novak, R. F. (1979) Pharmacologist 21, 170
9. Dershwitz, M., and Novak, R. F. (1981) Fed. Proc. 40, 667
10. Reisberg, P. I., and Olson, J. S. (1980) J. Biol. Chem. 255, 4144-4150
11. Sidwell, A. A., Munch, R. H., Guzman Barron, E. S., and Hogress, T. R. (1939) J. Biol. Chem. 123, 339-350
12. Azzi, A., Montecucco, C., and Richter, C. (1975) Biochem. Biophys. Res. Commun. 65, 591-603
13. Mieyal, J. J., and Blumer, J. L. (1976) J. Biol. Chem. 251, 3442-3446
14. Chance, B., and Williams, G. R. (1956) Adv. Enzymol. 17, 65-134
15. Dershwitz, M., and Novak, R. F. (1979) Fed. Proc. 38, 544
16. Novak, R. F., and Mieyal, J. J. (1976) Pharmacologist 18, 242
17. Novak, R. F., Kapetanovic, I. M., and Mieyal, J. J. (1977) Mol. Pharmacol. 13, 15-30
18. Novak, R. F., Dershwitz, M., and Novak, F. C. (1978) Biochem. Biophys. Res. Commun. 82, 634-640
19. Novak, R. F., Dershwitz, M., and Novak, F. C. (1979) Mol. Pharmacol. 16, 1046-1058
20. Mieyal, J. J., and Freeman, L. S. (1976) Biochem. Biophys. Res. Commun. 69, 143-148
21. Adams, G. E., Stratford, J. J., Wallace, R. G., Wardman, P., and Watts, M. E. (1980) J. Nat. Cancer Inst. 64, 555-560
22. Antonini, E., Wyman, J., Brunori, M., Taylor, J. F., Rossi-Fanelli, A., and Caputo, A. (1964) J. Biol. Chem. 239, 907-912
23. Hirota, K., and Itano, H. A. (1978) J. Biol. Chem. 253, 3477-3481
24. Goldberg, B., and Stern, A. (1976) Biochem. Biochem. Acta 347, 628-632
25. Wallace, W. J., and Caughey, W. S. (1975) Biochem. Biophys. Res. Commun. 62, 561-567
26. Kawaniishi, S., and Caughey, W. S. (1979) Biochem. Biophys. Res. Commun. 88, 1203-1208
27. Castro, C. E., Wade, R. S., and Belser, N. O. (1978) Biochemistry 17, 225-231
28. Winterbourn, C. C., McGrath, B. M., and Carrell, R. W. (1976) Biochem. J. 155, 493-502
29. Lynch, R. E., Thomas, J. E., and Lee, G. R. (1977) Biochemistry 16, 4663-4667
30. McCord, J. M., and Fridovich, I. (1969) J. Biol. Chem. 244, 6949-6955
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M Dershwitz and R F Novak

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