Iron, particularly in the ferrous state plays a role in regulating the biosynthesis of the manganese superoxide dismutase (MnSOD) in *Escherichia coli*. Addition of iron has a repressive effect on the synthesis of MnSOD under normal or inducing conditions (i.e., in the presence of paraquat). Addition of manganese to cultures already derepressed for MnSOD biosynthesis causes a further increase in the amount of active enzyme, however, this effect is also abolished by the addition of iron. Removal of metals from the growth medium by Chelex 100 also derepresses the synthesis of MnSOD but repletion of the medium with iron abolishes this effect. Chelators specific for Fe^2+^, 2,2'-dipyridyl, and 1,10-phenanthroline, cause a 5-7-fold increase in MnSOD. Removal of iron also increases the synthesis of MnSOD in the absence of oxygen. A model is presented to account for the observed effects of oxygen, superoxide anion, and chelators on the increased synthesis of MnSOD. In this model, the regulatory repressor for MnSOD biosynthesis is envisioned as being an iron-containing protein.

Superoxide dismutases are metalloenzymes found in organisms that are exposed to oxygen for transient or long periods of time (1–3). Their sole function is to remove the superoxide anion (O_2^-) formed via the univalent reduction of dioxygen, and thus protect the cells against oxygen toxicity.

*Escherichia coli* has three isoenzymic forms of superoxide dismutase (4): an iron (5), a manganese (6), and a hybrid (4, 7) isoenzyme. FeSOD is synthesized in anaerobically as well as in aerobically grown cells and is therefore considered to be constitutive (4). MnSOD is absent under anaerobic conditions but is rapidly synthesized upon exposure to oxygen (4, 8). HySOD is also found in aerobically grown cells (4).

It has been demonstrated that it is not actually molecular oxygen that is the inducer of MnSOD but a product of its metabolism, O_2^- This conclusion was made when the levels of MnSOD were shown to vary with changing growth rate in *E. coli* grown in a glucose-limited chemostat at constant aeration (9). Furthermore, growth of *E. coli* on carbon sources such as lactate or succinate, which require an oxidative metabolism, resulted in higher levels of superoxide dismutase than when grown fermentatively on glucose (10). It has also been shown that when glucose-grown cells are transferred to trypticase-soy yeast extract (TSY) medium, which contains a low concentration of glucose (i.e., 0.25%), the superoxide dismutase levels remain low until the glucose is exhausted and then increase rapidly when the cells utilize other components in the medium (10). Furthermore, redox active compounds such as paraquat, when added to the growth media also increase MnSOD levels. These higher levels of MnSOD are attributed to increased intracellular O_2^- production, generated by cyclic oxidation/reduction of the compounds at a constant pO_2 (11, 12).

The induction of MnSOD is prevented by inhibitors of transcription or of translation, but not by inhibitors of replication (12). This was concluded when chloramphenicol and rifampicin inhibited the increase in MnSOD biosynthesis whereas hydroxyurea did not. In a similar study, a 2-fold increase in MnSOD was noted when nalidixic acid was used instead of hydroxyurea (24). Further studies have shown that the inductive effect of nalidixic acid is partially related to its iron-chelating capacity. In this report we present data on the increased biosynthesis of MnSOD by ferrous iron chelators and show that this increase is independent of O_2^- A model is proposed to account for these findings.

**MATERIALS AND METHODS**

*Chemicals*—2,2'-Dipyridyl, 2,6-pyridine dicarboxylic acid, quinolinic and picolinic acid were purchased from Aldrich. 1,10-Phenanthroline, ferrocynphen (dicyano-bis(1,10-phenanthroline)-ferroso-II complex), bathophenanthroline sulfonate, isonicotinic acid hydrazide, diethylthiienamipentaoctacid, 2-hydroxyquinoline, and methyl viologen (paraquat) were purchased from Sigma. Chemicals were generally added to the growth media as solid. Stock solutions of 2,2'-dipyridyl (50 mM) and 1,10-phenanthroline (10 mM) were prepared by adding 0.5 ml of 96% ethanol followed by 4.5 ml of sterile water before adding to the growth medium. Ferroc ammonium sulfate, ferrous ammonium sulfate, and manganese sulfate (Fisher) were prepared in 100 mM stock solutions. Paraquat (100 mM) was dissolved in sterile distilled water before use. Chloramphenicol, rifampicin, and hydroxyurea were purchased from Sigma and were added to the growth media as solids.

**Bacterial Strains**—*E. coli* B_874_ (ATCC 29682) was used throughout.

*Media*—A glucose-minimal medium containing, per liter, 0.2 g of MgSO_4_ 7H_2O, 2.0 g of citric acid/H_2O, 10 g of K_2HPO_4, 3.5 g of Na_2HPO_4, 0.05 mg of vitamin B_6, and 5.0 g (g) of glucose, or trypticase-soy yeast extract medium containing, per liter, 30 g trypticase broth, and 5 g yeast extract (Baltimore Biological Laboratories) were used. Metals were removed from the TSY medium by Chelex 100 (Bio-Rad). Chelex-100 (100 g/1200 ml) was added to the medium and stirred at room temperature for 3 h. The medium was then filtered 3 times, to remove the resin, using Whatman No. 5 filter paper.

*This work was supported in part by Grant PCM-8213853 from the National Science Foundation. This is Paper 9384 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27606. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: FeSOD, iron superoxide dismutase; MnSOD, manganese superoxide dismutase; HySOD, hybrid superoxide dismutase; O_2^-_max, optical density at 600 nm; PQ^- (paraquat), 1,1'-dimethyl-4,4'-bipyridinium; RP, repressor protein.
paper and then refiltered through a Millipore filtering apparatus using Gelman type A-E 47-mm glass fiber filter paper (Gelman Sciences Inc., Ann Arbor, MI). The pH of the medium was adjusted to 7.0 with concentrated HCl before use. Metal analysis was determined by atomic absorption spectrophotometry using a Perkin-Elmer Spectrophotometer model 5000. Metal concentrations differed with different batches of media as indicated.

**Growth Conditions**—Overnight cultures grown in glucose-minimal medium for 15-17 h were used as the inocula. In all cases steps were taken to ensure that the inocula used were in the logarithmic phase of growth and utilizing glucose (10) unless otherwise stated. To maintain sufficient aeration, liquid cultures were shaken at 200 rpm where the flask to culture volume ratio was 5:1. Experimental cultures were inoculated to an initial optical density at 600 nm equal 0.05-0.1 and allowed to grow (37 °C, 200 rpm) for 4-5 generations, except where otherwise specified, before harvesting. Anaerobic growth conditions were maintained by growing the slant, the overnight and the experimental cultures in the designated media equilibrated in an anaerobic environment maintained in a Coy Anaerobic Chamber (Coy Laboratory Products, Ann Arbor, MI). Dissolved oxygen was determined to be less than 0.03 ppm in anaerobically equilibrated distilled water and was undetectable in the medium during anaerobic growth using a dissolved oxygen meter (Yellow Springs Instrument Co.). Culture flasks were closed with rubber stoppers after inoculation to prevent further exchange with the limited experimental flasks to an initial OD, end of the specified growth period, chloramphenicol was poured onto crushed ice and removed from the anaerobic chamber.

**Assays**—Cells were harvested at 4 °C by centrifugation for 20 min at 10,000 × g, resuspended in 0.05 M potassium phosphate plus 0.1 mM EDTA, pH 7.8 (KP/EDTA buffer), and disrupted for 6 min using a Heat Systems W-370 sonicator equipped with a cup horn, operated at an output of 60 W. Temperature was maintained at 4 °C by using a circulating refrigerated water bath and the sonifier power was applied intermittently at 45-s pulses. Cell debris was removed by centrifugation at 27,000 × g for 30 min. Cell-free extracts were dialyzed for 48 h against the KPJEDTA buffer with at least three changes of buffer. Protein was assayed by the method of Lowry et al. (13), using bovine serum albumin as a standard. Superoxide dismutase isoenzymes were separated by electrophoresis on 10% polyacrylamide gels (15), visualized using an activity stain (16) and quantitated by linear scanning densitometry (4).

**RESULTS**

**Induction of MnSOD—E. coli** grown under aerobic conditions has three isoenzymic forms of superoxide dismutase and only FeSOD under anaerobic conditions. Exposure to paraquat, an intracellular generator of O₂⁻ (11, 12), results in a significant increase in total superoxide dismutase with the major induction being due to an increase in the Mn isoenzyme. In the presence of 0.5 mM PQ, approximately a 4-fold increase in total superoxide dismutase is seen, whereas under anaerobic conditions PQ²⁻ has no effect (Table I).

**Effects of Metals on the Biosynthesis of MnSOD**—The finding that the induction of MnSOD by nalidixic acid is partially related to its iron chelating capacity prompted the investigation of the effects of adding different metals to TSY medium on the biosynthesis of MnSOD under normal as well as inducing growth conditions (Table II). Adding Fe²⁺, Fe³⁺, or Mn²⁺ to TSY medium without PQ²⁻ had no significant effect on total superoxide dismutase, however, adding Mn²⁺ caused a 200% increase in MnSOD while it had no effect on FeSOD or HySOD. Adding Fe⁺ or Fe³⁺ decreased MnSOD by 84% and increased FeSOD by 64%. When Fe³⁺ or Fe²⁺ was added in conjunction with Mn²⁺ the stimulatory effect (200%) originally seen with Mn²⁺ alone was completely abolished with Fe⁺ being more effective than Fe³⁺. Similar but more dramatic results were seen under inducing conditions (i.e. in the presence of 0.2 mM PQ²⁻). Again, Mn²⁺ stimulated the inductive effect of PQ²⁻ on MnSOD and iron abolished this effect. Of greater interest is the finding that iron completely abolished the inductive effect of PQ²⁻ (Table II).

If iron has a repressive effect on the synthesis of MnSOD, then iron would be expected to be less effective in inducing growth conditions (Table II).

**TABLE I**

| Conditions | Superoxide dismutase | | |
| --- | --- | --- | --- |
| | Total | Mn⁺ | Hy⁺ | Fe⁺ |
| units/mg | | | | |
| TSY (aerobic) | 16.7 | 4.9 | 2.7 | 9.1 |
| TSY (anaerobic) | 9.9 | 0 | 0 | 9.9 |
| TSY + PQ²⁻ (aerobic) | 64.1 | 57.5 | 5.5 | 1.1 |
| TSY + PQ²⁻ (anaerobic) | 10.2 | 0 | 0 | 10.2 |

**TABLE II**

| Metal added | TSY | FeSOD | PQ²⁻ |
| --- | --- | --- | --- |
| | units/mg | units/mg | units/mg |
| None | 6.1 | 1.9 | 1.4 | 2.8 | 29.8 | 26.5 | 2.5 | 0.8 |
| Fe²⁺ | 5.9 | 0.3 | 0.9 | 4.7 | 6.9 | 4.8 | 1.2 | 0.9 |
| Fe³⁺ | 6.2 | 0.3 | 1.4 | 4.5 | 6.3 | 2.7 | 1.3 | 2.3 |
| Mn²⁺ | 7.4 | 3.8 | 1.2 | 2.4 | 49.9 | 44.1 | 5.2 | 0.6 |
| Fe⁺, Mn²⁺ | 7.4 | 0.6 | 1.9 | 4.9 | 8.1 | 5.1 | 1.1 | 1.9 |
| Fe⁺, Mn³⁺ | 6.8 | 1.1 | 1.5 | 4.2 | 9.6 | 6.2 | 1.7 | 1.8 |

* Fe(NH₄)₂SO₄·6H₂O, FeNH₂SO₄·12H₂O, and MnSO₄·H₂O were added at 1 mM each.

**TABLE III**

| Metal added | Final OD₆₀₀ | Superoxide dismutase |
| --- | --- | --- |
| | units/mg | units/mg |
| None | 0.30 | 24.7 | 17.5 | 4.9 | 2.4 |
| Fe²⁺ | 0.30 | 11.4 | 4.6 | 3.8 | 3.0 |
| Fe³⁺ | 0.30 | 8.9 | 3.5 | 1.0 | 4.4 |
| Mn²⁺ | 0.30 | 24.4 | 23.6 | 8.0 | 0.0 |
| Fe⁺, Mn²⁺ | 0.35 | 7.0 | 5.5 | 1.0 | 0.5 |
| Fe⁺, Mn³⁺ | 0.34 | 6.6 | 5.3 | 0.6 | 0.7 |

* Metals were added at 1 mM each as indicated in Table II.
then removing iron from the medium by using Chelex 100 should have the opposite effect (i.e., induce MnSOD). This was found to be the case (Table III). Chelex 100 is known to remove other divalent ions which probably explains the poor growth noted in the Chelex-treated media.

Effect of Chelators on the Synthesis of MnSOD—It appeared that iron played a definite role in regulating the induction of MnSOD, therefore different ion chelators were used to gain greater insight into this phenomenon (Table IV). It is clear that 8-OH-quinoline, 2,2' -dipyridyl, 1,10-phenanthroline, and ferrocyphen were the most effective in increasing MnSOD. 8-OH-Quinoline was very inhibitory to the growth of the organism nor increase the synthesis of MnSOD. The poorly chelating pyridine derivatives (nicotinamide, isonicotinic acid, 2,6-pyridinedicarboxylic acid, picolinic acid, and quinaldic acid) did not affect the growth of the organism nor increase the synthesis of MnSOD. The highly charged chelating agents, diethylenetriaminepentaacetic acid and EDTA greatly inhibited the synthesis of total superoxide dismutase as well as MnSOD. The results suggested that compounds capable of chelating iron within the cell are more effective in increasing total superoxide dismutase as well as MnSOD than compounds that chelate iron from the medium. Furthermore, the stimulatory effects of the chelators on MnSOD biosynthesis were not due to their ability to increase the intracellular generation of O_2 because none were found to increase cyanide-resistant respiration (an indication of O_2 production (11, 12)).

The nature of the iron involved in the repression of MnSOD biosynthesis was further deduced by adding Fe^{2+} or Fe^{3+} to the cultures along with 2,2'-dipyridyl (Table V). The results clearly show that ferrous ion completely abolished the stimulatory effect of 2,2'-dipyridyl on MnSOD biosynthesis.

Induction or Activation—The results clearly showed that ferrous ion chelators caused large increases in the specific activity of MnSOD, however, it cannot be concluded from these data whether this increase is due to de novo synthesis or to activation of some apo-protein or of a precursor polypeptide. The data in Table VI, however, provides the answer. Hydroxyurea (33.3 mM), an inhibitor of DNA replication (17, 18) did not prevent the increase in MnSOD caused by 2,2'-dipyridyl or 1,10-phenanthroline. It did, however, prevent cell division and cause filamentous growth as observed by phase contrast microscopy. Rifampicin, an inhibitor of transcription (19, 20), inhibited the growth of the organism and prevented the synthesis of MnSOD in response to the chelators. Chloramphenicol, an inhibitor of translation, also prevented the increase in MnSOD caused by the chelators. It was concluded, therefore, that the increase in MnSOD in response to the iron chelators was due to de novo protein synthesis and that the control was at the level of transcription. This agrees with previous findings which showed that the induction of MnSOD upon exposure to oxygen (4) or to redox-cycling compounds (12) is also due to de novo synthesis of the enzyme.

### TABLE IV

| Chelator       | mM | Final OD_600 | Superoxide dismutase |
|----------------|----|--------------|----------------------|
| None (control) | 1.0| 3.52 | 17.1 9.7 1.8  5.5
| Nicotinamide   | 1.0| 3.14 | 16.9 5.5 1.8  9.6
| Isonicotinic acid | 1.0 | 2.93 | 18.2 5.6 1.6  11.0
| Picolinic acid  | 1.0| 2.19 | 8.7 6.7 0.9  1.1
| 2,6-Pyridine   | 1.0| 3.12 | 15.6 2.7 1.4  11.5
| dicarboxylic acid | 1.0 | 2.40 | 10.8 6.2 1.5  3.1
| Quinaldic acid  | 1.0| 2.14 | 6.3 5.9 0.3  0.1
| Bathophenanthroline | 1.0 | 2.67 | 1.8 1.6 0.1  0.1
| DETAPAC        | 0.5| 1.37 | 12.6 3.6 1.0  1.0
| EDTA           | 0.5| 0.35 | 24.2 21.8 1.3  1.2
| 8-OH-Quinoline | 0.5| 0.35 | 24.2 21.8 1.3  1.2
| 2,2'-Dipyridyl | 0.5| 0.35 | 57.2 45.3 12.0  1.9
| 1,10-Phenanthroline | 0.4 | 0.67 | 89.9 69.4 10.3  2.9
| Ferrocyphen    | 0.5| 1.38 | 74.9 74.9 6  0

### Table V

| Conditions | Final OD_600 | Superoxide dismutase |
|------------|-------------|----------------------|
|            | Total Mn-Hy-Fe-units/mg | 12823 |
| TSY        | 16.2 5.5 2.6 8.1 |
| TSY + 2,2'-dipyridyl | 145.0 143.0 0 0 |
| TSY + 2,2'-dipyridyl + Fe^{2+} | 14.6 6.9 2.9 5.7 |
| TSY + 2,2'-dipyridyl + Fe^{3+} | 148.0 148.0 0 0 |

### Table VI

| Growth conditions | Final OD_600 | Superoxide dismutase |
|------------------|-------------|----------------------|
|                  | Total Mn-Hy-Fe-units/mg | 12823 |
| TSY              | 6.6 2.3 1.1 3.2 |
| TSY + 2,2'-dipyridyl (0.5 mM) | 42.3 38.2 1.7 2.4 |
| TSY + 1,10-phenanthroline (0.1 mM) | 49.4 47.5 0.1 1.9 |
| TSY + CM (250 µg/ml) | 5.2 3.3 1.2 0.7 |
| TSY + 2,2'-dipyridyl + CM | 7.4 5.4 1.3 0.7 |
| TSY + 1,10-phenanthroline + CM | 7.7 5.5 1.2 1.0 |
| TSY + Rif (50 µg/ml) | 5.4 3.5 0.5 1.0 |
| TSY + 2,2'-dipyridyl + Rif | 8.6 6.0 1.8 0.8 |
| TSY + 1,10-phenanthroline + Rif | 8.5 6.3 1.2 1.0 |
| TSY + HU (250 µg/ml) | 8.9 1.7 0.2 7.0 |
| TSY + 2,2'-dipyridyl + HU | 58.2 52.6 0.8 4.9 |
| TSY + 1,10-phenanthroline + HU | 54.2 56.8 1.8 5.7 |

* E. coli B, grown aerobically at 37°C for 17 h in a glucose-minimal medium were used to inoculate TSY medium containing the specified chelators and inhibitors. The cultures were allowed to incubate at 200 rpm for 5 min before being harvested, sonicated, and dialyzed cell-free extracts were assayed. CM, chloramphenicol; Rif, rifampicin; HU, hydroxyurea.
data presented thus far clearly show that the synthesis of MnSOD in E. coli is under the control of ferrous ion. It is possible that the regulatory protein of MnSOD is an iron-containing protein and that removal of the iron "turns on" the biosynthesis of the enzyme. If this is the case, then the induction of MnSOD by iron chelators or by iron-depletion of the medium should be seen in the absence of oxygen. This was found to be the case. Data in Fig. 1 shows that chelating the TSY medium with Chelex 100 induced the MnSOD (Lane 1) and that supplementing the chelated medium with MnSO\textsubscript{4} caused greater induction (Lane 4). This suggested that the Chelex-treated medium was limiting in [Mn\textsuperscript{2+}]. The data also clearly showed that the induction of MnSOD was completely repressed by adding excess Fe\textsuperscript{2+} alone (Lanes 2 and 3) or in conjunction with Mn\textsuperscript{2+} (Lane 5). The iron chelators, 2,2'-dipyridyl and 1,10-phenanthroline were also excellent inducers of MnSOD under anaerobic conditions (Table VII). This induction was also inhibited by chloramphenicol and rifampicin but not hydroxyurea (data not shown).

**DISCUSSION**

The data presented indicate that iron plays a role in regulating the biosynthesis of MnSOD. A schematic model is proposed (Fig. 2) to accommodate the present findings as well as the observations that oxygen (4, 8) and increased intracellular fluxes of O\textsubscript{2} (11, 12) increase the biosynthesis of MnSOD. In this model, the control for MnSOD biosynthesis is envisioned as being a negative control operon, where the regulatory protein exists in either an active or an inactive state. The active repressor state has Fe\textsuperscript{2+} associated with it (RP-Fe\textsuperscript{2+}), while the inactive repressor has either Fe\textsuperscript{3+} (RP-Fe\textsuperscript{3+}) or no iron (RP) associated with it. O\textsubscript{2} plays a role in increasing the biosynthesis of MnSOD by changing the valence of the iron associated with the repressor protein. When the intracellular flux of O\textsubscript{2} is increased by an increased oxidative metabolism or by redox-cycling compounds, it acts to oxidize the Fe\textsuperscript{2+} in the repressor protein. Other oxidants (i.e. H\textsubscript{2}O\textsubscript{2} etc.) may also oxidize RP-Fe\textsuperscript{2+} to RP-Fe\textsuperscript{3+}. The RP-Fe\textsuperscript{3+} is an inactive form of the repressor, which may undergo a conformational change and lose the metal. The repressor is now inactive and MnSOD is synthesized. Under anaerobic conditions, where there is no superoxide or other oxidants to oxidize the Fe\textsuperscript{2+}, the repressor protein is always active and no MnSOD is made. However, the increased synthesis of MnSOD by chelators and by removal of iron from the medium under anaerobic conditions can be explained by Fe\textsuperscript{2+} being unavailable to be associated with the repressor protein and therefore MnSOD is synthesized even in the absence of O\textsubscript{2}, O\textsubscript{2} or other oxidants.

The increased synthesis of MnSOD, aerobically, in the iron-depleted medium or in the presence of chelators can also be explained by the formation of an inactive repressor protein lacking Fe\textsuperscript{2+}.

The identity of the repressor protein is presently being investigated, however, data in Table IV indicated that FeSOD was a likely candidate because increased levels of MnSOD were associated with a decrease in FeSOD. This possibility was ruled out, however, by two lines of evidence in particular: 1) data in Table VI indicated that MnSOD biosynthesis was possible without a concomitant decrease in FeSOD when hydroxyurea was used to inhibit DNA biosynthesis; and 2) recent studies (21) have shown that the level of MnSOD is independent of the amount of FeSOD in the cells.

It was shown (Table II) that excess Fe\textsuperscript{2+} was equally as effective as Fe\textsuperscript{2+} in repressing the biosynthesis of MnSOD. This can be explained by the fact that the cells used for inocula were grown overnight in the glucose-minimal medium which is low in iron content. These growth conditions are known to induce the enterochelin (enterobactin) transport system (22) which transports Fe\textsuperscript{3+} as a ferric-enterochelin complex (23). This allows "ready" transport of Fe\textsuperscript{3+} which,
once inside the cells, is converted to Fe$^{2+}$ and represses the biosynthesis of MnSOD.

REFERENCES

1. Fridovich, I. (1975) Annu. Rev. Biochem. 44, 147–159
2. Fridovich, I. (1978) Science (Wash. D. C.) 201, 875–880
3. Hassan, H. M., and Fridovich, I. (1980) in Enzymatic Basis of Detoxication (Jakoby, W. B., ed) Vol. 1, pp. 311–332, Academic Press, New York
4. Hassan, H. M., and Fridovich, I. (1977) J. Bacteriol. 129, 1574–1583
5. Yost, F. J., Jr., and Fridovich, I. (1973) J. Biol. Chem. 248, 4905–4908
6. Keele, B. B., Jr., McCord, J. M., and Fridovich, I. (1970) J. Biol. Chem. 245, 6176–6181
7. Dougherty, H. W., Sadowski, S. J., and Baker, E. E. (1978) J. Biol. Chem. 253, 5220–5223
8. Gregory, E. M., Yost, F. J., Jr., and Fridovich, I. (1973) J. Bacteriol. 115, 987–991
9. Hassan, H. M., and Fridovich, I. (1977) J. Bacteriol. 130, 805–811
10. Hassan, H. M., and Fridovich, I. (1977) J. Bacteriol. 132, 505–510
11. Hassan, H. M., and Fridovich, I. (1977) J. Biol. Chem. 252, 7667–7672
12. Hassan, H. M., and Fridovich, I. (1979) Arch. Biochem. Biophys. 196, 385–395
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
14. McCord, J. M., and Fridovich, I. (1969) J. Biol. Chem. 244, 6049–6055
15. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427
16. Beauchamp, C., and Fridovich, I. (1971) Anal. Biochem. 44, 276–287
17. Rosenkranz, H. S., Carr, H. S., and Pollak, R. D. (1967) Biochim. Biophys. Acta 149, 228–245
18. Sinha, N. K., and Sunstad, D. P. (1972) J. Bacteriol. 112, 1321–1334
19. Riva, S., and Silvestri, L. G. (1972) Annu. Rev. Microbiol. 26, 199–224
20. McClure, W. R., and Cech, C. L. (1978) J. Biol. Chem. 253, 2849–2856
21. Fee, J. A., Nettleton, C. J., Bull, C., and Baldwin, T. O. (1984) Fed. Proc. 43, 2058
22. Rosenburg, H., and Young, I. G. (1974) in Microbial Iron metabolism: A Comprehensive Treatise (Neilands, J. B., ed) pp. 67–82, Academic Press, New York
23. Langman, L., Young, I. G., Frost, G. E., Rosenberg, H., and Gibson, F. (1972) J. Bacteriol. 112, 1142–1149
24. Hassan, H. M., and Fridovich, I. (1980) Abst. Annu. Meet. Am. Soc. Microbiol. 142, K86