ASPECTS OF THE STRUCTURE, FUNCTION, AND APPLICATIONS OF SUPEROXIDE DISMUTASE

Authors: **Joe V. Bannister**
William H. Bannister
Biotechnology Centre
Cranfield Institute of Technology
Cranfield, Bedfordshire, England

Giuseppe Rotilio
Department of Biology
II University of Rome, "Tor Vergata"
Rome, Italy

Referee: Irwin Fridovich
Department of Biochemistry
Duke University Medical Center
Durham, North Carolina

I. INTRODUCTION

The study of superoxide dismutase (SOD) (E.C. 1.15.1.1) started almost half a century ago when the copper known to be present in blood was found to be associated with a protein. This copper protein was isolated from red blood corpuscles and ox liver and named hemocuprein and hepatocuprein, respectively. Purified preparations of hemocuprein and hepatocuprein were found not to combine with oxygen as does hemocyanin, which is the copper-containing respiratory protein of invertebrates. Also, both proteins were found not to promote phosphorylation reactions and not to have polyphenoloxidase, cytochrome oxidase, peroxidase, catalase, or carbonic anhydrase activity. The function of the protein remained unknown until the work of McCord and Fridovich in 1969,[2] when, as a result of various observations carried out at Duke University on the reduction of cytochrome c by the superoxide radical O_2^- generated from the xanthine/xanthine oxidase reaction, hemocuprein (erythrocuprein) was identified as SOD.[3] This copper protein of previously unknown function was found to catalyze the dismutation of superoxide radicals:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

In the intervening period between the first isolation of hemocuprein and the discovery of its function, it was realized that a whole family of cupreins was present in the various organs. Porter and Folch[4] isolated a copper protein from bovine brain and called it cerebrocuprein. A similar protein was isolated from normal human brain.[5] All the cupreins isolated from the various organs in a single species were suggested to be identical.[6] Antibodies raised against the human hemocuprein were found not to cross-react with beef hemocuprein,[7] indicating a species difference. Human heptocuprein, cerebrocuprein, and erythrocuprein were confirmed to be identical.[8] These proteins were observed to have a similar isoelectric point, sedimentation velocity, molecular weight, electron paramagnetic resonance and visible absorption spectra, immunological cross-reactivity, and amino acid composition and gave similar peptide maps following trypsin digestion. Besides copper, the cupreins were also found to contain zinc.[9]

Further investigations after the discovery of copper/zinc SOD led to the realization that

the enzyme exists as a family of metalloproteins. Fridovich and collaborators isolated two other SODs which contained either manganese or iron.[10,11] The iron- and manganese-containing enzymes have been shown to have similarities in amino acid composition and are considered to belong to a class of SODs which is totally different from the copper/zinc-containing class of the enzyme. The distribution of the various SODs is described in Section III.

The best way of distinguishing among the various SODs is to isolate the protein and characterize the metal present. However, each isozyme has been found to have a particular characteristic sensitivity towards a number of reagents. Cyanide was found to inhibit the copper/zinc SOD but not the manganese SOD.[12,13] Furthermore, the product of the dismutation reaction, hydrogen peroxide, inactivates both the copper/zinc- and iron- but not the manganese-containing enzymes,[14-16] while azide inhibits the enzymes in the following order: iron > manganese > copper/zinc SOD.[17] Asada et al.[18] have extensively used these inhibitors to distinguish which isozyme is present in a variety of plant and animal species. Other methods, also based on selective inhibition or reconstitution of a particular form of the enzyme, have been developed. Kirby et al.[19] prepared apo enzymes of the manganese- and iron-containing SODs by treatment with guanidine hydrochloride at low pH. The apo enzymeswere then assumed to be reactivated by the metal characteristic of the native enzyme. Kirby et al.[19] considered that it would be improbable for the apo enzyme to be reactivated by a metal other than that found in the native state. This may, however, not always be the case because Gregory and Dapper[20] have been able to substitute manganese for iron in SOD from *Bacteroides fragilis* without any loss of activity. Geller and Winge[21] found that when crude extracts are treated with 2% sodium lauryl sulfate at 37°C for 30 min only the manganese SOD activity is inhibited in contrast to the copper/zinc activity.

The discovery of SOD opened a whole new area of research not only on the structure and function of the enzymes, but also concerning the roles of free radicals in biology and medicine. A number of texts have been published on the subject.[22-37] This field of research has today come to be of interest to physicists, biochemists, molecular biologists, and clinicians. The aim of this review is to address as many of these diverse interests as possible.

II. ASSAYS FOR SOD

A. Introduction

The dismutation reaction:

$$2O_2^- + 2H + \rightarrow H_2O_2 + O_2$$

is not easily followed by measuring the disappearance of the substrate or by the appearance of the products. The main factor in the development of assays for SOD has been the instability of the substrate, the superoxide radical, O_2^-. The radical spontaneously dismutes, and its lifetime is also drastically shortened by transition metal ions that may be present in the reaction medium. Therefore a direct assay for SOD is not always conveniently possible. Although such direct assays have proved useful for mechanistic studies, they require the use of sophisticated equipment that may not be present in all laboratories. More conventional are the indirect assays in which O_2^- is generated enzymatically or nonenzymatically. In these assays, the flux of O_2^- reacts with an indicator. This type of assay is classified as an indirect negative assay because it records negative measurements, i.e., the inhibition by SOD of a spectrophotometrically measurable product resulting from the reaction between the indicator and O_2^-. In one case, an indirect assay has been developed to record positive measurements. This is the so-called dianisidine assay. Indirect assays offer the advantage of being inexpensive and very sensitive. While direct assays for SOD must be performed in the presence

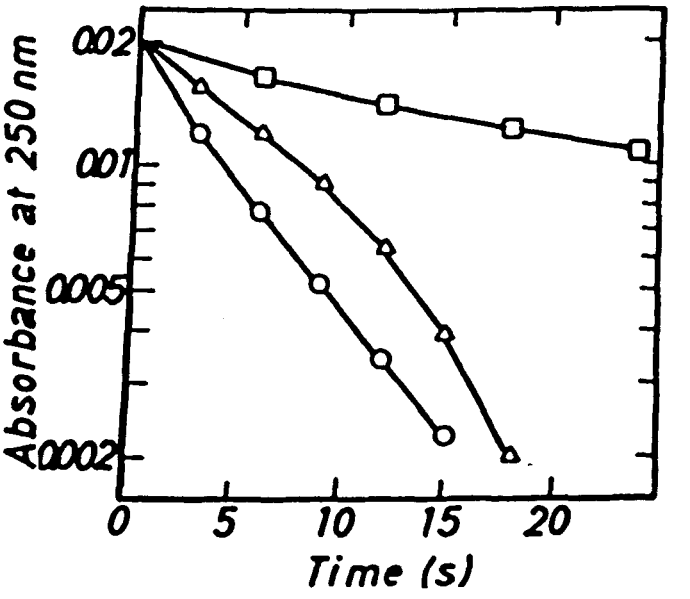

FIGURE 1. The effect of copper/zinc and manganese SOD on the disproportionation of O_2^-. Stock solution of substrate is added to 50 m*M* 2-amino-2-methyl-1-propanol HCl, pH 9.5, + 0.2 m*M* diethylenetriamine pentaacetic acid + 100 m*M* catalase. The decay at A_{250} is followed in the absence and presence of SOD. □, Spontaneous reaction; ○, 85-p*M* human copper/zinc SOD; △, 350-p*M* bovine manganese SOD. (From Marklund, S., *J. Biol. Chem.*, 251, 7504, 1976. With permission.)

of $\sim 10^{-5}$ *M* superoxide, indirect assays can be carried out at steady-state levels of O_2^- in the range of 10^{-10} to 10^{-6} *M*. The indicators are used at concentrations which effectively compete with the dismutation reaction so that virtually all the O_2^- produced can be trapped and detected.

B. Direct Methods of Assaying for SOD

There are two physical properties of O_2^- that are utilized for its direct detection in the presence and absence of enzyme. These are its paramagnetism and its ultraviolet (UV) absorption. The superoxide radical contains one unpaired electron giving rise to an electron paramagnetic resonance (EPR) signal which is unique and which can be extracted from a complicated set of overlapping signals.[38] In frozen aqueous solutions at a temperature below 220 K, the superoxide radical exhibits an axial type of EPR signal with a relatively broad g_{11} component with $g_{11} \simeq 2.1$. However, due to rapid relaxation, the spectrum is evident only at low temperatures. As the temperature is raised, the signal begins to broaden and is no longer visible in aqueous solution at room temperature. Rapid-freeze EPR has been used as a direct assay for SOD.[39] However, because of various technical difficulties, this method has not resulted in a routine assay for SOD. Instead, direct assays are mostly based on the optical properties of O_2^-, which exhibits an absorption maximum around 250 nm, with a molar extinction coefficient of 2000. The main difficulty with this type of direct assay is the high absorption in the UV region of the biological samples to be assayed. The simplest direct assay was described by Marklund.[40] Superoxide was generated from its potassium salt at alkaline pH, where its stability increases. The decay at 250 nm in the presence and absence of SOD is observed spectrophotometically (see Figure 1). One unit of activity was arbitrarily taken as the enzyme activity which results in the dismutation of O_2^- at a rate of 0.1 sec^{-1}. The generation of O_2^- from potassium superoxide and its stabilization in

18-crown-6-polyether was also applied to stop-flow procedures.[41] Although the preequilibrium state cannot be properly investigated because of the time resolution of the instrument (~5 msec), the method has been applied for mechanistic studies. Another spectrophotometric direct assay is based on the decay kinetics of O_2^- generated within 10 msec by flash photolysis of flavin mononucleotide in the presence of *N,N,N',N'*-tetramethylethylenediamine and oxygen.[42]

Absorption spectroscopy has also been applied to the detection of radiolytically generated superoxide radicals. The technique of pulse radiolysis has provided an important assay for SOD which has proven useful for detailed mechanistic studies on all three isozymes of SOD.[43-50] Several accounts of the technique have appeared in the literature.[51,52] The passage of a single pulse of duration 10^{-8} to 10^{-6} sec of ionizing radiation, usually in the 2- to 10-MeV range, in water results in the formation of a number of species:

$$H_2O \rightarrow OH^{\cdot}, e_{aq}^-, H^{\cdot}, H_2, H_2O_2$$

In the presence of an oxygenated buffer solution and 100 m*M* formate or ethanol, O_2^- is the predominant species because of the following reactions:

$$OH^{\cdot} + HCOO^- \rightarrow H_2O + COO^{\cdot -}$$

$$COO^{\cdot -} \xrightarrow{O_2} O_2^- + CO_2$$

$$OH^{\cdot} + CH_3CH_2OH \rightarrow H_2O + CH_3\dot{C}HOH$$

$$CH_3\dot{C}HOH \xrightarrow{O_2} CH_3\dot{C}HO_2OH$$

$$OH^{\cdot} + CH_3CH_2OH \rightarrow H^+ + CH_3CHO + O_2^-$$

$$e_{aq}^- + O_2 \rightarrow O_2^-$$

$$H^{\cdot} + O_2^- \rightarrow HO_2^{\cdot} \rightleftharpoons O_2^- + H^+$$

All the reactions are rapid, and a maximum O_2^- concentration is reached within 5 μsec of the radiation pulse, allowing reaction times as short as 2×10^{-9} sec to be measured. The decay of O_2^- in the presence and absence of enzyme is followed spectroscopically. In common with the direct assays which utilize the UV absorption of O_2^-, the problem of sample absorption in this region arises. Also the maximum single pulse yield of O_2^- (~200 μ*M*) is less than that obtained from a solution of potassium superoxide.

Direct methods of assaying for SOD have also been applied to the measurement of products, particularly oxygen. Oxygen can be measured electrochemically with a variety of electrodes. However, a peculiar property of the electrodes used for polarographic assays of SOD is that they are able to produce convenient amounts of O_2^- besides being sensitive to the oxygen concentration. Rigo et al.[53] described a dropping mercury electrode which, when coated by a surfactant, was a very convenient source of O_2^-. Although 10^{-11} *M* SOD could be assayed, the method could only be utilized at pH values ≥ 10. Argese et al.[54] found that the enzyme could be assayed at neutral pH by utilizing an appropriately modified rotating-disk electrode. The sensitivity of the rotating-disk electrode is, however, three orders of magnitude lower than that of the dropping mercury electrode. Both methods are direct assays and are not limited by sample turbidity or high UV absorption of the sample. The rotating-disk electrode is particularly convenient for mechanistic studies on SODs whose

activity is pH dependent.[55] It is however not suitable for assaying the SOD activity of a biological sample because of its low sensitivity.

C. Indirect Assays for SOD

The indirect methods of assaying for SOD are the most widely used for routine assays. In these assays, a unit of enzyme activity is generally defined as the amount of enzyme which inhibits the reaction of O_2^- with the indicator by 50%. The simplest of the indirect negative assays are those in which O_2^- is produced during the autoxidation of a compound. The O_2^- produced acts as the chain-propagating species, and the end product is usually colored and its rate of formation can be followed spectroscopically. The effect of SOD is to inhibit end-product formation. Such assays have been developed based on epinephrine,[56,57] hydroxylamine,[58,59] pyrogallol,[60] and 6-hydroxydopamine.[61] Photooxidation sensitized by riboflavin,[62] phenazine methosulfate,[63] flavins,[64] and tetra-hydropteridine[65] have also been developed as assays for SOD. Photoreduction of the dyes to an autoxidizable semiquinone leads to the formation of O_2^- on reoxidation. The indicator utilized in these cases has been nitroblue tetrazolium, which reacts with O_2^- to form blue formazan, although some caution about its use has been advised by Auclair et al.[66] In the absence of oxygen, SOD was observed to have no effect on formazan formation, indicating that the reduced dyes can directly reduce the nitroblue tetrazolium. However, Nishikimi[65] observed that only 7% of the nitroblue tetrazolium was reduced by an oxygen-independent pathway, and Fridovich[67] reported that in air-equilibrated solutions 80% of the nitroblue tetrazolium reduction is mediated by O_2^- generated from the xanthine/xanthine oxidase reaction. The photoxidation sensitized by riboflavin and phenazine methosulfate was developed into an assay for measuring levels of SOD in crude extracts following polyacrylamide gel electrophoresis (PAGE). The following are the reaction conditions utilizing riboflavin as the O_2^- generator.[62] Gels are soaked in a solution of 2.45×10^{-3} *M* nitroblue tetrazolium for 30 min followed by an immersion for another 30 min in a solution containing 0.028 *M* *N,N,N′,N′*-tetramethylenediamine, 2.8×10^{-5} *M* riboflavin, and 0.036 *M* phosphate buffer, pH 7.8. The gels are then removed from the solution and illuminated for 30 min. During the illumination period, the gels become uniformly blue, except at positions containing the enzyme activity. Scanning of gels loaded with crude extracts and of gels loaded with a known concentration of purified enzyme allow the concentration to be determined from the peak height.

The generation of O_2^- from the xanthine/xanthine oxidase reaction and its ability to reduce cytochrome c formed the basis of the first indirect assay to be developed for measuring the activity of SOD.[2] The assay mixture consists of 1×10^{-5} *M* oxidized cytochrome c, 5×10^{-5} *M* xanthine, sufficient xanthine oxidase, and 50 m*M* phosphate buffer at pH 7.8 containing 10^{-4} *M* EDTA in a total volume of 3 mℓ. The reduction of cytochrome c is followed at 550 nm. One unit of SOD is defined as the amount that causes 50% inhibition of the rate of reduction of cytochrome c. Nitroblue tetrazolium can also be used as the indicator. The dye provides the basis for an assay which is free of interference by other catalytic activities which may be present in crude extracts such as cytochrome oxidase and cytochrome c peroxidase and which are capable of oxidizing ferrocytochrome c. Acetylation and succinylation of the cytochrome c was found to diminish its ability to be utilized as a substrate following its reduction by O_2^-.[68-70] Fresh xanthine oxidase should be used because preparations which have been stored for long periods, frozen and thawed repeatedly, or exposed to high salt concentrations may be contaminated with the deflavo derivative, which will directly reduce cytochrome c.[67]

Riboflavin was observed to photosensitize the oxidation of dianisidine, and all the SOD isozymes were found to augment the oxidation.[71,72] This result has formed the basis of an assay which has been called an indirect positive assay. The assay was also found applicable to polyacrylamide gels.[73] The oxidation of dianisidine is monitored at 460 nm, and the assay

proceeds by a mechanism which is initiated by the photoxidation of riboflavin. The electronically excited state of riboflavin oxidizes the dianisidine, yielding the flavin semiquinone and a dianisidine radical which, in the absence of competing reactions, would dismute to yield the divalently oxidized dianisidine:

$$Rb^* + DH_2 \rightarrow RbH + DH^\cdot$$

$$DH^\cdot + DH^\cdot \rightarrow D + DH_2$$

However, the flavin semiquinones RbH can reduce oxygen to O_2^-, and this radical can in turn reduce the dianisidine radical $DH^\cdot$, preventing its net oxidation:

$$RbH + O_2 \rightarrow Rb + H^+ + O_2^-$$

$$H^+ + O_2^- + DH^\cdot \rightarrow DH_2 + O_2$$

SOD will scavenge the O_2^- formed and therefore will inhibit the reduction of the dianisidine radical, which can then dismute to yield the divalently oxidized dianisidine. The possibility that O_2^- could reduce the final product of dianisidine oxidation and reverse the change in absorbance at 460 nm was tested and excluded. The assay has been used to determine the rate constant for purified swordfish liver copper/zinc SOD[74] and could be applied to crude extracts.

In principle, any chemical method for trapping O_2^- is an indirect assay. An EPR approach of this type is the spin-trapping technique, which overcomes the difficulties encountered with the direct detection of O_2^- by EPR. Spin trapping is an integrative method in which the unstable free radical, in this case O_2^-, reacts with a scavenger to yield a stable free radical which can be monitored by EPR. The spin-trapping agents used for O_2^- detection are usually nitrones. Details of the technique have recently been published.[75] The spin-trapping agent commonly used for O_2^- is 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) and the spin adduct formed is 5,5-dimethyl-2-hydroperoxypyrroline-*N*-oxide (DMPO-OOH). This gives a characteristic EPR signal. The superoxide radical can be generated from the xanthine/xanthine oxidase reaction. The inhibition of the rate of spin adduct formation by SOD can be developed as an assay for the enzyme. The method would, however, only be useful for pure enzyme since the spin trap may interact with the system under investigation and give erroneous results. Recently Rosen et al.[76] observed that a particular hydroxylamine, 2-ethyl-1-hydroxy-2,5,5-trimethyl-3-oxazolidine (OXANOH), reacts with O_2^- to form its corresponding nitroxide, 2-ethyl-2,5,5-trimethyl-3-oxazolidinoxyl (OXANO), which is a stable product. OXANOH oxidation was used to detect O_2^- formation in a number of biological extracts and no interferences were found. Product formation could be inhibited by SOD, leading to the development of an assay.

III. DISTRIBUTION OF SODs

A. Introduction

All living cells are prone to oxygen toxicity. This arises from the production of reduction products of oxygen or the excited singlet state. The univalent reduction of oxygen leads to the formation of O_2^-, which can react further to produce hydrogen peroxide (H_2O_2) and hydroxl radicals ($OH^\cdot$). Superoxide radicals are generated in many biological oxidations. These include the autoxidation of flavins,[39] hydroquinones,[77] catecholamines,[78] thiols,[79,80] tetrahydropterines,[81] hemoproteins,[82,83] and reduced ferrodoxin.[84] Subcellular organelles such as chloroplasts[85] and mitochondria[86] also produce superoxide radicals. The radical is also formed by several oxidative enzymes — xanthine oxidase, aldehyde oxidase, dihydroorotic

acid dehydrogenase, and by several flavin dehydrogenases.[87] The radical may occur as an intermediate in the reaction catalyzed by galactose oxidase,[88] indoleamine dioxygenase,[89] and 2-nitropropane dioxygenase and other flavin-containing oxidases.[90,91] Finally superoxide radicals are formed by neutrophils and macrophages during phagocytosis.[92] The toxicity of the superoxide radical has been considered in terms of the possible indirect reactions it can undergo with other cellular constituents, with the most important being hydrogen peroxide.[93] This reaction in the presence of trace-metal ions leads to the formation of hydroxyl radicals. Clearly, therefore, the presence of SOD is to be considered as the first line of defense against the toxicity of oxygen-centered radicals. This implies that the enzyme is essential to aerobic organisms and is not required by anaerobic organisms. McCord et al.[94] proposed an enzyme-based theory of obligate anaerobiosis, whereby the aerobic existence of an organism depends upon its ability to produce SOD, following their finding that obligately anaerobic bacteria, i.e., species which exhibit sensitivity to oxygen at 0.2 atm or less, did not possess any SOD activity. However, Loesche[95] had found a wide range of oxygen tolerance which he defined as maximum oxygen content in the atmosphere in which an anaerobic organism would display growth. This meant that there exists a wide spectrum of oxygen tolerance among anaerobic organisms, and detailed experimentation showed that the theory does not always apply. In 1973, Bell[96] reported that SOD activity was present in certain strains of anaerobic sulfate-reducing bacteria, and certain aerobic bacteria have been shown to lack SOD activity.[97-99] The absence of SOD activity has to be treated with circumspection because the levels of the enzyme may be so low that it may escape detection. Norrod and Morse[97] reported that only some strains of the obligate aerobe *Neisseria gonorrhoeae* lacked detectable levels of SOD activity, and in a further investigation they reported the presence of the enzyme in other *Neisseria* species.[100] The absence of any SOD activity could also be due to the fact that the organism has selected another form of defense against oxygen-mediated damage. The aerotolerant bacterium *Lactobacillus plantarum* was shown to lack SOD activity.[101] However, a careful study demonstrated that the bacterium maintains a high level (20 to 25 m*M*) of manganese(II) in a dialyzable form which takes the place of SOD in scavenging superoxide radicals.[102]

Recent investigations concerning manipulation of the genes have confirmed a physiological role for SOD. *Escherichia coli,* with defects in both the manganese and iron SOD gene, was found to be hypersensitive towards paraquat and oxygen[102], as was a mutant of yeast with a defect in the manganese SOD gene.[102b] Conversely, when the gene for human copper/zinc SOD is transfected into mouse L cells and human HeLa cells, a higher degree of resistance to paraquat was observed. The increased resistance was considered to be due to overproduction of SOD.[102c]

B. Distribution in Bacteria, Animals, and Plants

The distribution of the three forms of SOD has been extensively studied. The combination of sensitivities and insensitivities of the three enzymes to cyanide, hydrogen peroxide, and azide reported in Section I has allowed the determination of which form of the enzyme is present in a crude extract. In general, such investigations have often been confirmed by the isolation and characterization of the enzyme from the organism. The first detailed investigation on the presence of SOD in obligate anaerobes was carried out by Hewitt and Morris,[103] who reported the presence of the enzyme in anaerobic photosynthetic bacteria, sulfate-reducing bacteria, and clostridia. Activity was also detected in *Clostridium acetobutylicum* and *C. pastuerianum,* which McCord et al.[94] had reported to be devoid of the enzyme. It appears that the level of the enzyme in anaerobic species is low and that it can avoid detection. Preliminary characterization indicated that the enzyme in *C. perfringens* was not inhibited by 10^{-3} *M* cyanide, implying that it is either the iron or manganese form of the enzyme. SOD activity was also demonstrated in other anaerobic species. Lumsden and Hall[104] and

Asada el al.[105,106] demonstrated the occurrence of a cyanide-insensitive activity in the anaerobic green and purple sulfur bacteria *Chlorobium thiosulfatophilum* and *Chromatium vinosum*. Cyanide-insensitive activity was also found in various species of the anaerobe *Bacteroides*.[107,108] Tally et al.[109] and Gregory et al.[110] reviewed the presence of SOD in various anaerobic bacteria of clinical importance, and in all the organisms investigated the activity was detected. Various strains of halophilic vibrios[111] and ruminal bacteria[112] were also found to possess SOD activity. The insensitivity to cyanide indicates that the form of the enzyme present in anaerobic bacteria is either the manganese or the iron isozyme. However, the activity present in *B. fragilis* was rapidly inactivated by exposure to 5 m*M* hydrogen peroxide and was also inhibited by 1 m*M* sodium azide, suggesting that the SOD in *B. fragilis* is an iron enzyme. This was further confirmed by isolation and characterization of the enzyme from the same species.[20] An iron form of the enzyme has also been purified from the anaerobes *Desulfovibrio desulfuricans* (Norway, 4),[113] *Chromatium vinosum*,[114] *Chlorobium thiosulfatophilum*,[115] *Propionibacterium shermanii*,[116] and the methanogen *Methanobacterium bryantii*.[117] Therefore, the most primitive form of SOD appears to be the iron enzyme, although a manganese SOD has also been isolated from the anaerobes *P. shermanii*[118] and *B. fragilis*.[119]

In contrast to anaerobic bacteria, aereobic and facultative anaerobic species contain either the iron or the manganese enzyme or both. The reason for this is unclear and may depend on a variety of conditions as explained in Section IV. Facultative anaerobic bacteria such as *E. coli* contain only the iron enzyme when grown anaerobically but, in the presence of oxygen, the manganese enzyme is also present.[120] Aerobic diazotrophs possess both forms of the enzyme. An iron SOD was isolated from the free-living *Rhizobium japonicum*[121] and from *Azospirillum brasiliense*.[122] However, in *R. phaseoli* both forms of the enzyme were present, with the manganese enzyme being the predominant form.[123] A manganese SOD was also isolated from *Azotobacter chroococcum*.[124] However, a recent survey of various *Azotobacter* species indicated they can possess either form of the enzyme.[125] The presence of SOD in nitrogen-fixing bacteria was proposed to be a defense mechanism against oxygen inactivation of the nitrogenase.[126,127]

SOD has been isolated and characterized from a number of bacterial species. An iron enzyme has been purified from *E. coli*,[11,128] *Mycobacterium* sp.,[129,130] *Pseudomonas ovalis*,[131] *Bacillus megaterium*,[132] *Photobacterium* sp.,[133] *Pseudomonas putida*,[134] and *Thiobacillus denitrificans*,[135] while a manganese SOD has been isolated from *E. coli*,[10] *Streptococcus mutans*,[136] *Mycobacterium phlei*,[137] *Mycobacterium* sp.,[129] *Rhodopseudomonas spheroides*,[138] *M. lepraemurium*,[139] *Bacillus stearothermophilus*,[140] *Thermus acquaticus*,[141] *Streptococcus faecalis*,[142] *Thermus thermophilus*,[143] *Paracoccus denitrificans*,[144] *Bacillus subtilis*,[145] and *Serratia marcescens*.[146] The content of manganese SOD in *M. lepraemurium* constitutes about 6% of the soluble protein and appears to be the highest level of the enzyme in any bacterial species.

Algae have also been found to possess iron and manganese SODs.[147] Although the form of the enzyme purified from the blue-green algae (cyanobacteria), which are considered to be among the most advanced prokaryotes, is the iron isozyme,[138,148-150] the manganese enzyme has been isolated from the cytosol of the unicellular alga *Porphyridium cruentum*, which is considered to be most primitive eukaryotic species.[151] Both forms of the enzyme have been isolated from the eukaryotic *Euglena gracilis*.[152] The enzyme isolated from the protozoan *Crithidia fasciculata* contains iron.[153] However, two cyanide-insensitive activites which could be separated subcellularly have been reported for the facultative anaerobic flagellates *Tritrichomonas foetus* and *Monoceromonas* sp. and the aerobic ciliate *Tetrahymena pyriformis*.[154] While a copper/zinc SOD is still lacking in protozoa, its presence in fungi and slime molds and the rest of the animal and plant kindgom has been definitely established, as has the presence of the manganese SOD. Asada and Kanematsu[147] report testing for SOD activity

in two species of *Mycomyceta* (slime molds) and 43 species of *Eumyceta* (fungi). In all the species, the presence of the cyanide-sensitive copper/zinc SOD could be demonstrated, as could a cyanide-insensitive activity. This is probably the manganese SOD since this form of the enzyme has been isolated from *Saccharomyces cerevisiae*[155] and the luminescent fungus *Pleurotus olearius*.[156] The copper/zinc SOD has been isolated from *S. cerevisisae*,[157,158] *Neurospora crassa*,[159] and *Dactylium dendroides*.[160] Bryophytes (mosses), Pteridophytes (ferns), and gymnosperms and angiosperms (both seed plants) have been demonstrated to contain the copper/zinc enzyme in addition to a cyanide-insensitive form, which is probably the manganese enzyme.[147] The copper/zinc enzyme has been purified from green peas,[161,162] wheat germ,[163] spinach leaves,[164] kidney beans,[165] tomato fruit,[166] and maize.[167,168] Various plant extracts have been shown to contain cyanide-sensitive activity.[169-172] In the plant kingdom, a manganese SOD has only been purified from green peas[173,174] and maize.[167] Cyanide-insensitive activity has, however, been detected in extracts from wheat germ,[163] kidney beans,[165] corn,[169] tea,[175] spinach,[176] Jerusalem artichoke,[177] and in several mosses and ferns.[105,147]

In invertebrate species, the distribution of SOD has been poorly investigated. SOD activity has been demonstrated in a limited number of helminth tissues,[178,179] in the hemerythrocytes of sipunculids,[180] in the sea anemone,[182] and in the cephalochordate *Amphioxus lanceolatus*.[182] The presence of the copper/zinc and manganese SODs has been reported in the fruit flies *Ceratitis capitata*[183] and *Drosophila melanogaster*.[184,185] The copper/zinc enzyme has been isolated and characterized from the nematode *Trichinella spiralis*,[186] the hepatopancreas of the lamelli-branch mollusk *Mytilus galloprovincialis*,[187] and from *D. melanogaster*.[188,189] No manganese enzyme has been characterized from invertebrates or from lower vertebrates, where the copper/zinc enzyme has been isolated and characterized from the liver of the swordfish *Xiphias gladius*[190] and from various teleost fish.[191] The swim bladder of the toadfish *Opsanus tau* was found to have the highest concentration of SOD compared to its other organs.[192] Copper/zinc and manganese SOD activities were reported in carp erythrocytes,[193] in heart muscle extracts of the pike *Esox lucius*,[194] and total activity has been determined in various fish species.[195]

In the other vertebrates, copper/zinc and manganese SODs appear to be present in all the tissues investigated. A report that enzyme is absent from adipocytes[196] has been shown to be in error.[197] The copper/zinc SOD has been purified from bovine erythrocytes,[2,198] human erythrocytes,[199,200] horse[201] and pig livers and erythrocytes,[202,203] bovine retina,[204] and bullfrog *Rana catesbeiana* liver.[205] The manganese enzyme has been purified from the livers of chickens,[12] humans,[206] and rats.[207]

C. Anomalous Distribution of SOD

The general pattern of the SODs indicates that the copper/zinc SOD is essentially a eukaryotic enzyme, while the iron SOD is essentially a prokaryotic enzyme. The manganese enzyme is present in both prokaryotes and eukaryotes. However, a number of exceptions to this rule have been discovered. Eukaryotic algae do not possess the copper/zinc enzyme, while this form of the enzyme was found in two bacterial species, *Photobacterium leiognathi*[208] and *Caulobacter crescentus*.[209] The presence of copper/zinc SOD in the free-living *C. crescentus* remains a mystery, as well as does a recent report indicating the presence of the enzyme in two *Pseudomonad* species.[210] However, its presence in *P. leiognathi*, which is a symbiont on the ponyfish *Leiognathus*, was proposed to be a case of eukaryote to prokaryote gene transfer.[191,211] It is unclear why gene transfer has taken place and has been preserved because the bacterium already possesses an iron SOD.[133] Not all symbionts gain access to the genetic information contained in the host cells. None of the nitogen-fixing bacteria present in the roots have been found to acquire the host cell enzyme. The malarial parasite *Plasmodium berghei* was found to have the cyanide-sensitive copper/zinc SOD activity when

present in mice red blood cells (RBC). However, a concomitant decline in host cell SOD with an increase in parasite-associated SOD activities was observed.[212] This indicates that the parasite adopts the host cell enzyme. A similar phenomenon appears to be present in the spirochete *Treponema pallidum* which is extracted from rabbit testicles.[99] A cyanide-sensitive SOD activity was also demonstrated in the prokaryote *P. denitrificans*.[213] However, the isolated and characterized enzyme was found to have different molecular properties and much lower enzymatic activity than the copper/zinc SOD.[214]

Eukaryotic algae are not the only eukaryotic species to contain an iron SOD. Several iron SOD isoenzymes were identified in the leaves of lemon trees.[215] The enzyme was also isolated and characterized from the leaves of the mustard *Brassica campestris*,[216] and a survey of 43 plant families showed that the iron SOD is present in three families: Gingkoaceae, Nymphaceae, and Cruciferae.[172] The enzyme has also been purifed from the water lily *Nuphar luteum*,[217] tomato (*Lycipersicon esculentum*) leaves,[218] and from the most primitive of higher land plants, *Ginkyo biloba*.[219]

D. Subcellular Distribution of SOD

Copper/zinc SOD was exclusively localized in the soluble fraction of rat liver homogenates.[220] However, a detailed investigation of the subcellular distribution of the eukaryotic SOD by Weisiger and Fridovich[12,221] resulted in the first evidence of compartmentalization of the eukaryotic SODs. Intact mitochondria were found to contain the manganese SOD in the matrix space, and the copper/zinc SOD previously thought to be exclusively localized in the cytosol was also found in the mitochondrial intermembrane space. A similar subcellular distribution of the two enzymes was subsequently found in rat liver,[196,222,223] human neutrophils,[224,225] and in *N. crassa*.[226] In rat liver, copper/zinc SOD was also found in the lysosomes, with the amount varying with the nutritional state of the animal.[227] An examination of the subcellular distribution of the manganese SOD in human liver[206,228] revealed that human liver was found to contain 20 times more of the manganese enzyme than an equivalent weight of rat liver and that it is not exclusively located in the mitochondria. The possibility that mitochondrial rupture may have occurred between the time of death and autopsy could not be discounted. However, detailed investigations on fresh liver from a primate, the baboon *Papio ursinus*, also showed that the manganese SOD is present in the cytosol as well as in the mitochondria.

The subcellular distribution of SODs in plants is a matter of debate. Cyanide-sensitive copper/zinc SOD has been found to be localized in the cytosol,[229,230] chloroplasts,[164,229-231] and mitochondrial intermembrane space.[177,231,232] The distribution of the manganese SOD remains unclear. Baum and Scandalios[229] and Salin and Bridges[232] found the manganese SOD in the matrix space of mitochondria isolated from maize and mustard leaves. However, Del Rio et al.[233] claimed that the enzyme is also localized in the peroxisomes in green pea leaves. Various investigators have reported that chloroplasts also contain manganese SOD,[165,234,235] while others have disagreed with this finding.[229,230,236] Jackson et al.[176] found that intact chloroplasts isolated from the leaves of spinach and other C_3 plants have cyanide-insensitive activity presumed to be from the manganese enzyme, but the activity was located on the outside of the chloroplast and was presumed to be absorbed during the preparation. Chloroplasts isolated from the leaves of the mustard *B. campestris* were found to contain the copper/zinc SOD, the iron SOD, which is unique to this species, and an unidentified protein exhibiting SOD activity.[231] It was unclear whether this protein is the manganese SOD or not. All the activity was localized in the chloroplast stroma. This is in contrast to the earlier observation of Okada et al.,[237] who found that in three species of blue-green algae — *Plectonema boryanum*, *Anabaena variabilis*, and *Anacystis nidulans* — the manganese SOD is localized in the thylakoids. In the blue-green algae, the iron SOD is localized in the cytosol. Bacterial iron SOD is entirely localized in the cell matrix. A report indicating its presence in the periplasmic space in *E. coli*[238] was withdrawn.[239]

The SODs are regarded as intracellular enzymes, however, in recent years evidence has been accumulating that they may be present in the extracellular fluid. The nematode *T. spiralis* was found to excrete into the culture fluid the intracellular copper/zinc SOD.[186] In mammals and in *Nocardia asteroides*, a new class of extracellular SOD has been demonstrated. In man, pig, sheep, cow, rabbit, and mouse tissues, a high molecular weight cyanide-sensitive copper-containing SOD was demonstrated,[240,241] while *N. asteroides* possessed an iron-, manganese-, and zinc-containing high molecular weight SOD which was associated with the outer cell wall.[242] This enzyme was shown to be selectively secreted into the culture medium. The mammalian extracellular enzyme has been extensively purified and its properties investigated.[243,244]

IV. BIOSYNTHESIS OF SODs

A. Introduction

The discovery of SOD led to several investigations concerning the biosynthesis of the enzyme. This is because in some organisms the manganese SOD is inducible while in others it is the iron SOD which is inducible. In addition, although much effort has been devoted to the effect of oxygen and oxygen radicals on the biosynthesis of the enzyme, it is also clear that the active site metals do play a significant role in this process.

B. Induction of SOD by Oxygen

There is compelling evidence to indicate that SOD in prokaryotes and eukaryotes is induced in response to oxidative stress. The degree of oxygenation has been shown to reflect the level of SOD in *Streptococcus faecalis*,[245] *E. coli*,[238,246,247] *S. cerevisiae*,[248] rat lung,[249,250] guinea pig leukocytes,[251] *B. fragilis*,[252] blue-green algae,[253] and green algae.[254] Exposure to air of *E. coli* anaerobically grown and containing only the iron SOD led to the induction of the manganese SOD.[238,247,255] No response was, however, observed in cotton leaves.[256] Cotton leaves contain a high level of copper/zinc SOD, which was considered sufficient to allow them to tolerate large fluctuations of oxygen in the atmosphere. The sea anemone *Anthopleura elegantissima* containing the symbiotic dinoflagellate *Symbiodinium microadriaticum* in its gastrodermal tissues has SOD activities nearly two orders of magnitude greater than in individuals totally lacking symbiotic organisms.[257] The increase in the level of SOD activity in these animals was ascribed to the fact that high oxygen levels may occur in tissues of organisms that harbor symbionts which in the presence of light generate more oxygen than is consumed by the combined host and symbiont.

Extensive investigations on the induction of SOD have been carried out using the redox active compounds streptonigrin and paraquat (methyl viologen). Inside the cells, these compounds are reduced to a form which reacts with oxygen to produce superoxide radicals. Increased levels of manganese SOD were observed in *E. coli* B in the presence of streptonigrin;[246] similar effects were observed in *E. coli* K12.[247]

A third form of SOD was also found to be induced.[120] This form was also shown to be induced under aeration.[120,255] This third form was found to have an electrophoretic mobility midway between that of the manganese and the iron SODs and when isolated was found to contain one subunit from the manganese SOD and one from the iron SOD and to contain 0.8 atoms of iron per mole but negligible manganese.[120] However, a recent examination of the hybrid SOD in *E. coli* has shown that it may contain manganese as well.[258]

Paraquat was also shown to increase the rate of biosynthesis of the manganese SOD in *E. coli*.[259,260] A marked decrease in the cellular content of this enzymic activity was observed within 30 min after addition of the paraquat to the medium. Cells whose content of manganese SOD had been augmented by paraquat or streptonigrin were found to exhibit increased resistance towards oxygen toxicity.

The induction of manganese SOD in prokaryotes is, however, by no means universal, and a clear picture of the processes involved still remains to be elucidated. In the presence of increased oxygen or superoxide radical levels, the inducible enzyme in *R. japonicum*[123] and *A. brazilense*[122] is the iron enzyme as determined from inhibition studies, while in *B. fragilis* it is the manganese enzyme.[119] In the obligate aerobe *Bdellovibrio stolpii*, two iron SOD isozymes which are structurally distinct from the constitutive iron SOD are induced.[261] Also wild-type and paraquat-resistant mutants of *E. coli* were found to possess comparable levels of SOD.[262] In the presence of paraquat, the wild type responded to the addition of paraquat by inducing more manganese SOD than the mutant. This result has been taken to indicate that the mutant strains must have an alternative mechanism that allows them to grow in the presence of higher concentrations of paraquat without increasing their level of SOD. A decreased paraquat uptake by these cells which in turn affects their rate of intracellular superoxide generation and induction of the manganese SOD was demonstrated.

C. Effect of Active Site Metals in the Biosynthesis of SOD

The effect of metals on the biosynthesis of SOD was first investigated by Shatzman and Kosman,[160,263] who observed that removal of the copper from the growth medium of cultures of the fungus *D. dendroides*, in which 80% of the total activity is the copper/zinc and 20% is the manganese SOD activity, resulted in the decreased synthesis of the copper/zinc enzyme. However, in copper-poor medium, the fungus was observed to increase the level of the manganese enzyme such that the total level of the cellular SOD activity remained the same. A switching back to normal synthesis (80% copper/zinc and 20% manganese SOD activity) was observed when copper was restored to the growth medium.

Injection of copper sulfate solution subcutaneously in the rat (5 mg copper per kilogram body weight) caused an increase in SOD activity in the cytosol and mitochondrial intermembrane spaces where the copper/zinc enzyme is localized.[264] The appearance of new electrophoretic SOD activity in the intermembrane space preparations was also observed. The level of activity observed indicated that in the cytosol it was augmented by 35% and in the intermembrane space by 100%.

The metal supply was also found to influence the synthesis of the iron and manganese SODs. The anaerobic bacterium *P. shermanii* was found to produce either the iron or manganese SOD, depending on the metal supplied.[118] Data consistent with synthesis by the anaerobic *B. fragilis* of a single SOD apoprotein capable of binding either manganese or iron have also been obtained.[20] A similar effect has been observed in *S. mutans*[265] and *P. shermannii*.[118]

A detailed study on the role of iron and manganese on the biosynthesis of the enzyme in *E. coli* has recently been carried out.[266,267] These investigations have shown that the evidence that the iron SOD is constitutive whereas the manganese SOD is under repression control is an inadequate interpretation of the controls of biosynthesis of these enzymes. The previously observed induction of the manganese SOD by paraquat has been found to be inhibited by inhibitors of transcription or of translation but not by inhibitors of replication.[268] Chloramphenicol and rifampicin inhibited the increase in manganese SOD biosynthesis whereas hydroxyurea did not. A twofold increase in manganese SOD biosynthesis was observed in the presence of nalidixic acid. This effect was interpreted to be due to its ability to chelate iron(II) rather than to an increase in the intracellular flux of superoxide radicals.[260] Other chelating agents were found to have a similar effect.

Two models have been proposed to account for this oxygen-independent control of SOD biosynthesis. Moody and Hassan [266] have proposed a scheme (Figure 2) whereby the biosynthesis of the manganese SOD is under the control of iron-containing regulatory protein and that removal of this iron "turns on" the biosynthesis of the manganese SOD. The active regulatory protein or repressor has iron(II) associated with it ($RP\text{-}Fe^{2+}$), while the inactive

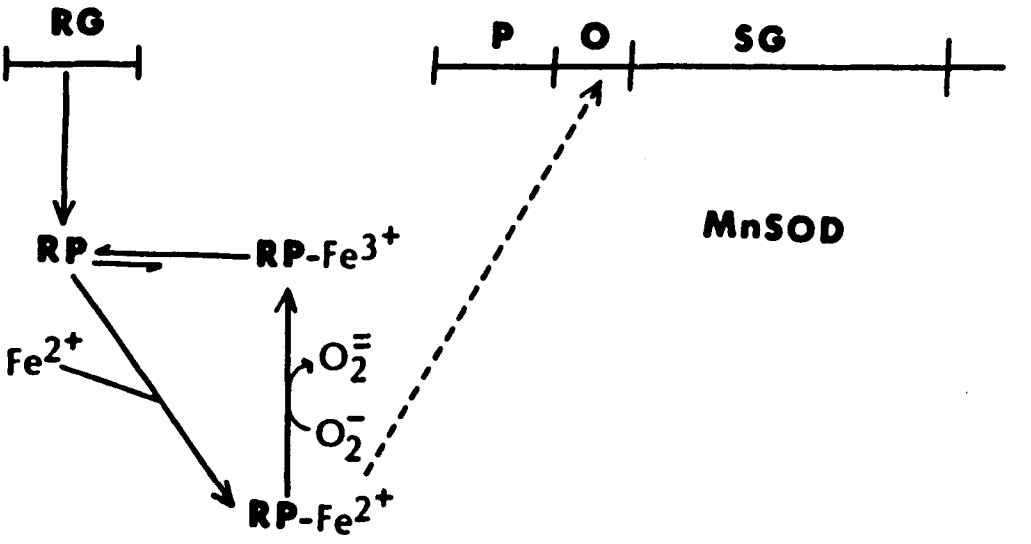

FIGURE 2. Schematic model for the regulation of manganese SOD by oxygen super radicals and iron chelators. RG, regulatory gene; P, promotoer; O, operator; SG, structural gene (manganese SOD); RP, apo-repressor protein (inactive); RP-Fe, ferric repressor (inactive); RP-Fe, ferrous repressor (active). (From Moody, C. S. and Hassan, H. M., *J. Biol. Chem.*, 259, 12821, 1984. With permission.)

repressor has either iron(III) ($RP\text{-}Fe^{3+}$) or no iron at all (RP). Superoxide radicals play a role in the scheme by changing the valence state of the iron associated with the repressor protein.

What is perhaps a more simple model has been proposed by Pugh and Fridovich.[267] In this scheme (Figure 3), the manganese SOD is not synthesized anaerobically because the manganese(II) cannot compete with the iron(II) for the apomanganese SOD. This means that the manganese-containing enzyme will only be synthesized anaerobically in the presence of chelators selective for iron(II). This has in fact been shown to be the case.[269] This scheme also proposes autogenous repression by the apo enzymes and by the incorrectly metal-substituted enzymes.

D. Characterization of the Genes Encoding for SOD

A detailed view of the mechanism controlling the biosynthesis of SOD will probably only become known from studies involving in vitro transcription and translation of the corresponding genes. A number of investigations have already been carried out in this direction. Isolated mRNA from human placenta[270] and from human cell cultures[271] when translated in a rabbit reticulocyte cell-free system was shown by immunoprecipitation to produce the copper/zinc SOD. The mRNA for this isozyme was found to have a molecular weight of 130,000. A cDNA clone was constructed and was utilized for determining the expression in different cells.[272] The nucleotide sequence of one clone ps61-100 was determined (Figure 4).[273] The sequence was found to contain 459 nucleotides followed by a single stop codon (TAA) and by a noncoding sequence of 95 nucleotides. The amino acid sequence for human copper/zinc SOD determined by Barra et al.[274] was found to agree with the nucleotide sequence rather than the primary structure determined by Jabusch et al.[275]

Hybridization of the clone to mRNA from human cells revealed the presence of two mRNA species of 0.7 and 0.5 kb in size.[276] The two mRNA species were found to code for the same polypeptide chain and were transcribed from the same gene; in addition, the major 0.7 kb mRNA was approximately four times more abundant than the smaller mRNA. The larger mRNA was found to contain 222 additional nucleotides at the 3′-polyadenylated terminus, and both species had multiple 5′ ends.

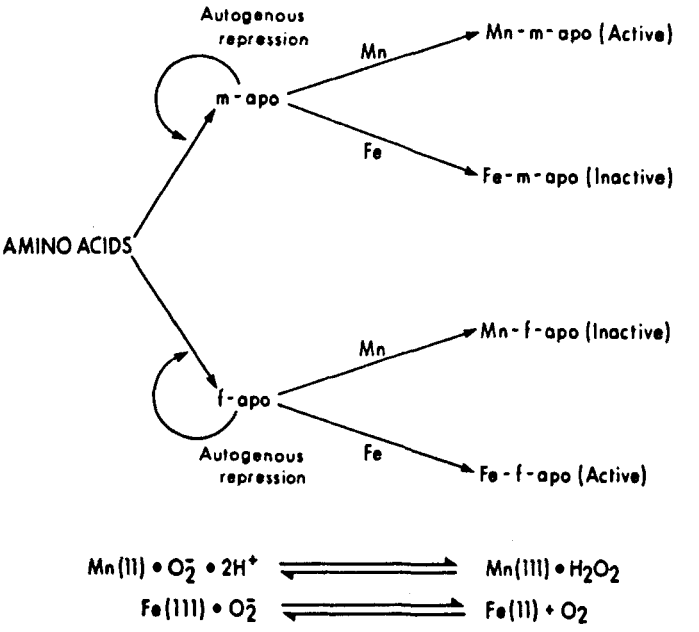

FIGURE 3. Effect of metals on the biosynthesis of manganese and iron SODs. m-apo, apoprotein of manganese SOD; f-apo, apoprotein of iron SOD. The valences of the metals are not shown; however, it is assumed that iron(II) binds to both m- and f-apo more avidly than manganese(II), but not more avidly than manganese(III). The reactions illustrated at the bottom indicate why O_2^- increases the availability of manganese(III). (From Pugh, S. Y. R. and Fridovich, I., *J. Bacteriol.*, 162, 196, 1985. With permission.)

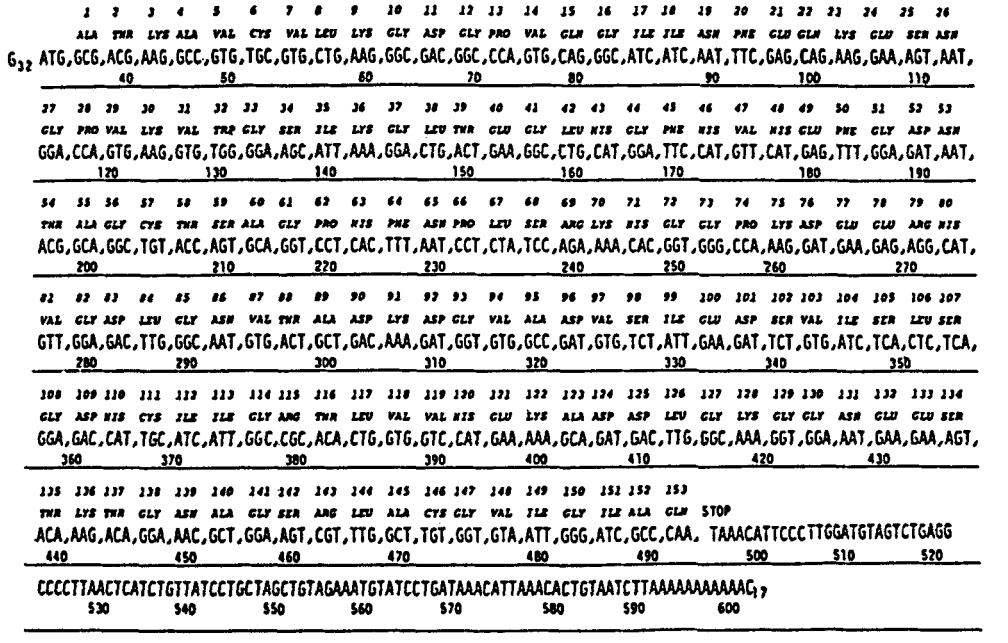

FIGURE 4. The cDNA and predicted amino acid sequence for human copper/zinc SOD. (From Sherman, L., Dafni, N., Lieman-Hurwitz, J., and Groner, Y., *Proc. Natl. Acad. Sci. U.S.A.*, 80, 5465, 1983. With permission.)

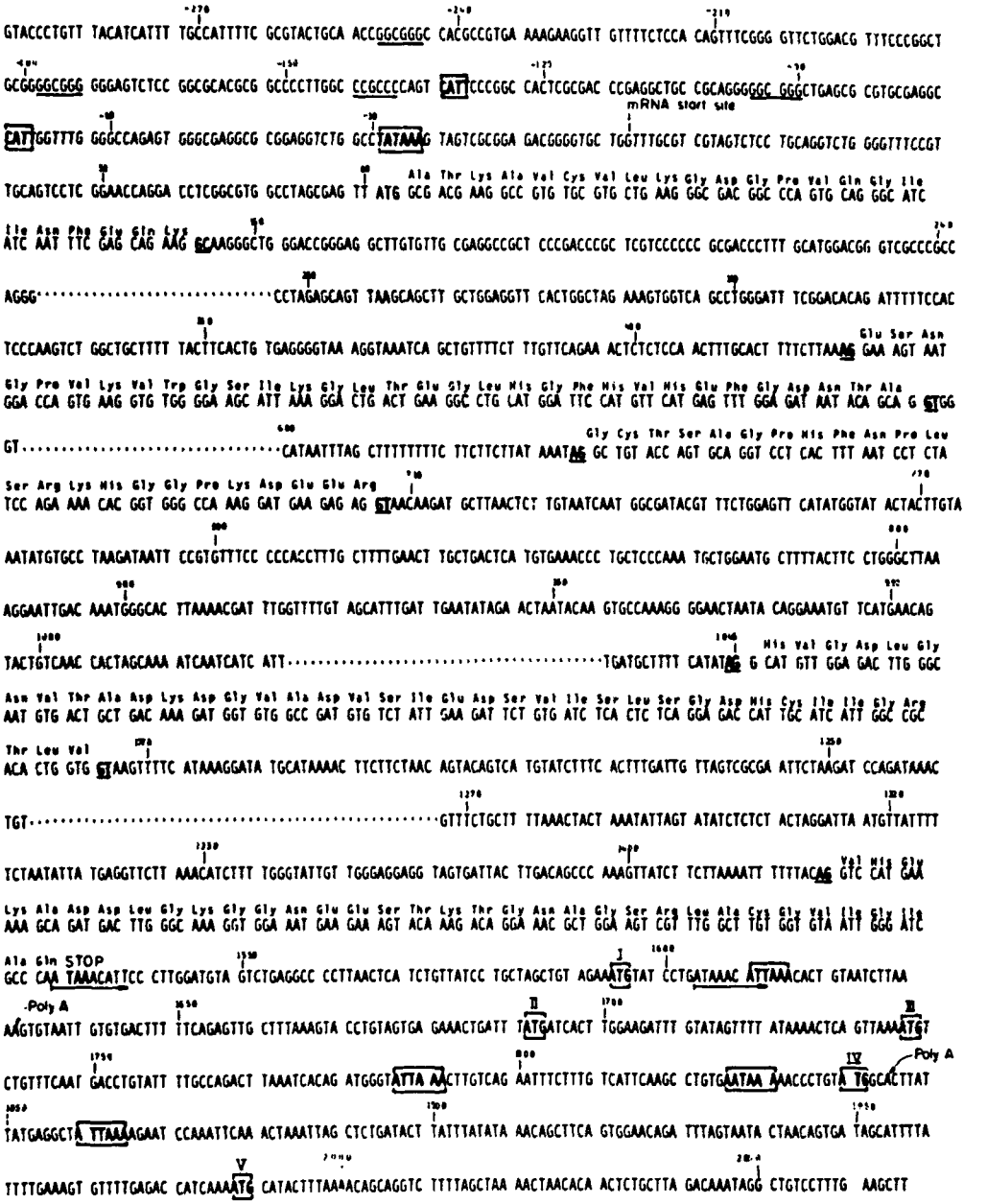

FIGURE 5. Nucleotide and amino acid sequence of the human copper/zinc SOD gene. The sequence of all coding regions and adjacent nucleotides are shown with 110 bases per line. The 'TATA', 'CAT', and polyadenylated sequences are boxed. The splice junctions are underlined. The exons were identified by comparison with the cDNA sequence given in Figure 4. The arrows mark the two 9-nucleotide direct repeats at the 3-noncoding region. (From Levanon, D., Lieman-Hurwitz, J., Dafni, N., Meir, W., Sherman, L., Bernstein, Y., Zehava, L.-R., Dancigen, E., Stein, O., and Groner, Y., *EMBO J.*, 4, 77, 1985. With permission.)

The gene for human copper/zinc SOD located on chromosome 21 was finally isolated and characterized.[277] The gene was found to be around 11 kb in length, and the nucleotide sequence (Figure 5) reveals five exons and four introns. The donor sequence at the first intron was found to contain an unusual variant dinucleotide 5′-GC rather than the highly conserved 5′-GT. In addition to the functional gene, four processed pseudogenes were isolated.

The nucleotide sequence of the gene for the yeast mitochondrial manganese SOD has also been determined.[278] The sequence of this gene was initially inadvertently identified as the gene for the Rieske iron-sulfur protein. The predicted amino acid sequence, however, revealed the primary structure to be identical with the manganese SOD[279] rather than the Rieske protein, except for an N-terminal extension of 27 amino acids and an extra 4 amino acids at the C-terminal end. The plasmid isolated from the genomic clone bank of *S. cerevisiae* FL-100 was found to synthesize in *E. coli* a protein of about 27,000 mol wt. The gene sequence (Figure 6) predicts a molecular mass of 26,123, and the yeast manganese SOD has been shown to be synthesized as a precursor protein which has a 2000-dalton extension.[280] This extension probably corresponds to the 27 amino acid extensions obtained from the gene sequence. The nucleotide sequence is 699 bases long and starts at the ATG codon triplet.[278]

In prokaryocytes, the cloning and mapping of the *E. coli* genes for the iron and manganese SODs has been described.[281,282] The chromosomal manganese SOD locus was found to lie near 87 min on the *E. coli* map,[282] while for the iron SOD it was near 50 min.[281] The possibility of having *E. coli* strains overproducing SOD opened the way for the study of regulation in this bacterium. Nettleton et al.[283] isolated the iron SOD gene from an *E. coli* plasmid library, and strains overproducing this isozyme were obtained. In agreement with previous observations,[245,246,260] oxygen was found to induce the manganese SOD in the strains overproducing the iron isozyme. The level of the manganese isozyme was, however, found to be independent of the iron isozyme. A seven- to tenfold difference in the concentration of the iron protein had no effect on the level of the manganese protein. This result was interpreted to mean that superoxide radicals do not directly increase the level of the manganese SOD since the strain is already overproducing the iron SOD and consequently has sufficient enzyme to remove an excess of superoxide radicals. Increased oxygenation was also found to repress the iron SOD production. The significance of the results is unclear. They do, however, appear to lend credence to the model suggested by Moody and Hassan,[266] whereby an iron-containing repressor protein is probably regulating the biosynthesis of these SODs.

V. CHEMICAL ASPECTS OF THE STRUCTURE OF SODs

A. Introduction

The purification of copper/zinc SOD from bovine erythrocytes was followed by purification of this isozyme from a variety of animal and plant sources. The search for SOD activity also led to the discovery of the iron and manganese isozymes. The purification procedure employed for purifying these isozymes has been described in detail.[284,287] A recent report describes the use of a red dye for the purification of the manganese SOD.[146] However, the exact mechanism of the binding and release of the enzyme from the dye is unknown. The multitude of isozymes purified and characterized has led to a clear picture of the chemical properties of the SODs.

B. Primary Structure of Copper/Zinc SOD

The copper/zinc SODs are homodimers with a molecular weight of around 32,000. The two identical subunits are associated solely by noncovalent interactions and each subunit contains a 1-g atom each of copper and zinc. Initially, the subunits were thought to be covalently linked[288] and to be nonidentical.[289] However, primary structural investigations have confirmed their identity and the noncovalent linkage. The complete amino acid sequence has been determined for 11 copper/zinc SODs (Figure 7).[274,275,290,300] Gaps and insertions have been included to optimize the sequence homologies. Only the enzymes from higher vertebrate species have a block N-terminal group. These enzymes are generally considered to be more stable. However, whether the increased stability is due to acetylation of the

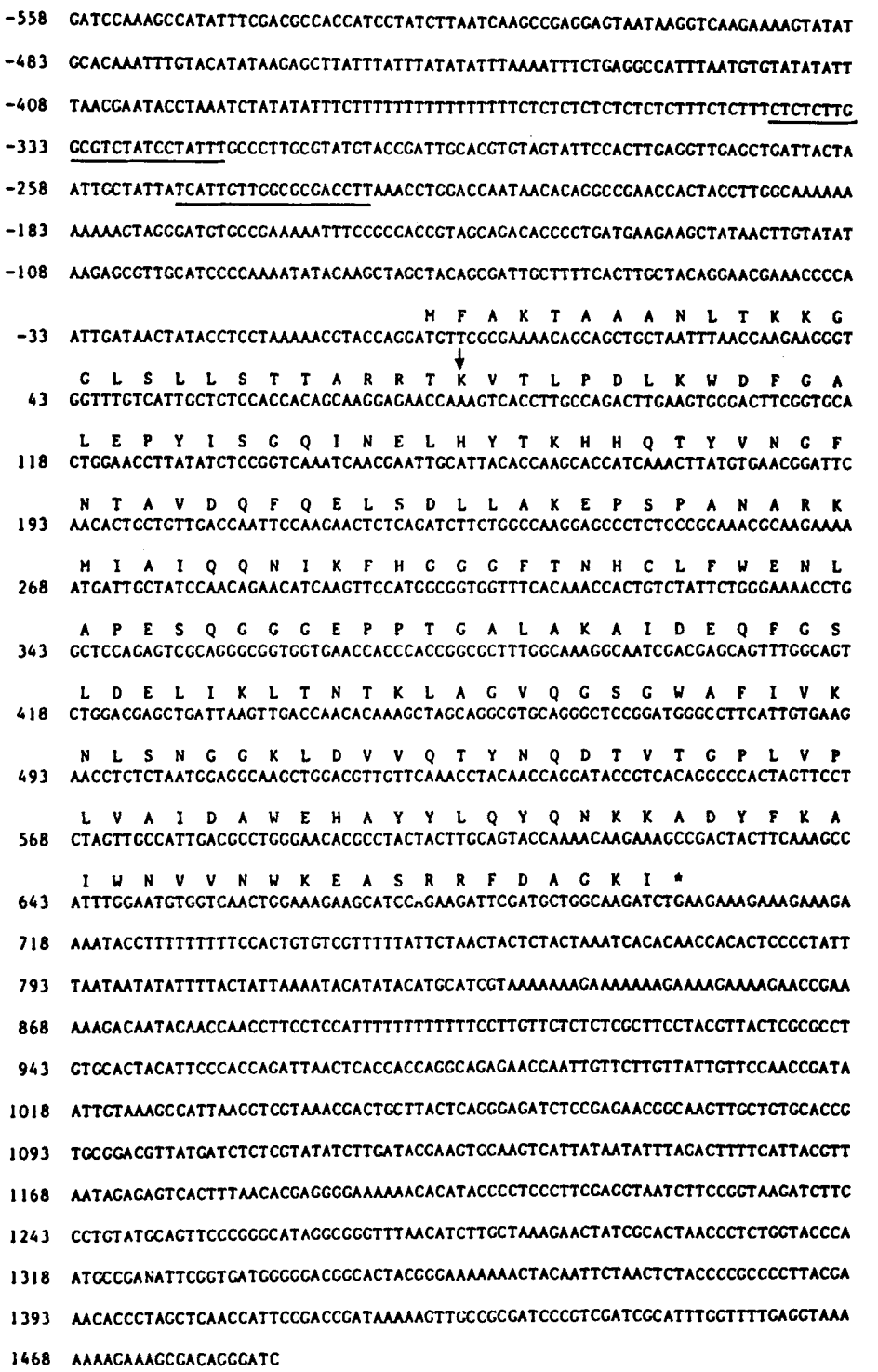

FIGURE 6. Nucleotide sequence and deduced protein sequence of yeast manganese SOD. The sequence shown is that of the entire Bam HI fragment comprising the structural gene and 1300 nucleotides of the flanking regions. The numbers to the left of the DNA sequence represent the first nucleotide in each row relative to the A in the translation-initiation ATG. The first amino acid residue of the mature protein is indicated by an arrow. Sequences similar to the CYCI-UAS sequences are underlined. (From Marres, C. A., Van Loon, A. P. G. M., Oudshoorn, P., Van Steeg, H., Grivell, L. A., and Slater, E. C., *Eur. J. Biochem.*, 147, 153, 1985. With permission.)

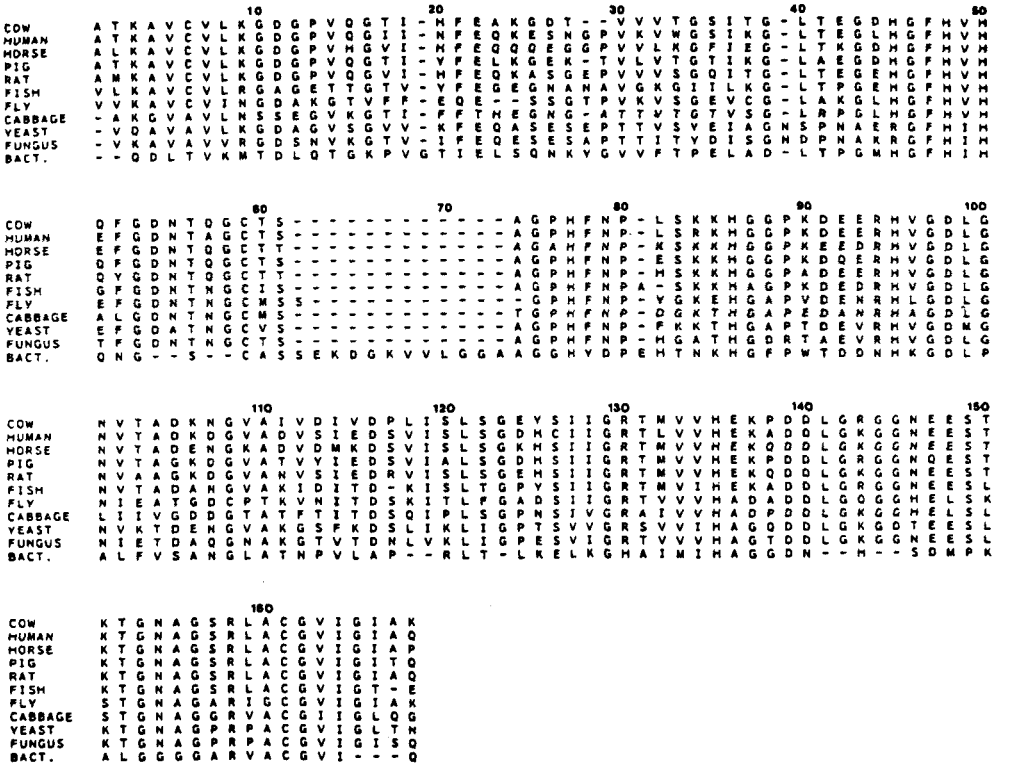

FIGURE 7. Amino acid sequences of copper/zinc SOD.

N-terminal amino acid remains to be investigated. The alignments obtained indicate that the metal-binding sites appear to be conserved in all the enzymes. These are His48, 50, 77, and 135 for the copper and His77, 86, 95, and Asp98 for the zinc. These refer to His44, 46, 61, and 118 for the copper and His 61, 69, 78, and Asp81 for the zinc in the original bovine amino acid sequence.[290] Also conserved are Arg158 (bovine 141), which is considered to be important for activity,[301,302] and the intrachain disulfide bridge formed by Cys60 and 161 (bovine 55 and 144).[300] A high degree of homology is evident between the vertebrate SODs (Table 1). This indicates that the various copper/zinc proteins have diverged very slowly. The sequences conservation is almost as great as that of eukaryotic cytochrome c. The highest degree of homology is observed between the bovine and pig sequences and the lowest identity is between the bacterial and all the other enzymes. Since prokaryotes are generally believed not to possess the copper/zinc SOD, the presence of this enzyme in the luminescent bacterium *P. leiognathi* has been considered to be a case of gene transfer from eukaryotic to prokaryotic species.[191] This is because this bacterial species, although found free living, is also a symbiont of ponyfish. In the absence of any structural information on the ponyfish copper/zinc SOD, the amino acid sequences of the two enzymes from *P. leiognathi* and swordfish liver were compared. The swordfish liver enzyme has been shown to be closely related to the unsequenced enzyme of the bacterial host, the ponyfish.[191] The alignment of the two sequences together with the frequency of substitutions determined according to McLachlan[303] is given in Figure 8. The aligned sequences reveal a relatively low degree of homology. However, when mutations are taken into account, long stretches of closely related sequences are obtained, and the weighted similarity between the two enzymes rises to 44%. The bacterial enzyme, despite having a low sequence homology, also has a similar secondary structure to the other copper/zinc SODs. The amino acid sequence

Table 1
SEQUENCE HOMOLOGIES BETWEEN COPPER/ZINC SUPEROXIDE DISMUTASES*

	Cow	Human	Horse	Pig	Rat	Fish	Fly	Cabbage	Yeast	Fungus	Bacteria
Cow	—	82	78	84	82	71	55	53	53	55	28
Human	82	—	78	82	83	66	58	52	55	55	25
Horse	78	78	—	75	80	66	55	49	56	54	26
Pig	84	82	75	—	80	67	55	56	52	53	28
Rat	82	83	80	80	—	64	57	52	56	54	26
Fish	71	66	66	67	64	—	54	52	53	54	30
Fly	55	58	55	55	57	54	—	56	50	50	24
Cabbage	53	52	49	56	52	52	56	—	50	52	26
Yeast	53	55	56	52	56	53	50	50	—	70	26
Fungus	55	55	54	53	54	54	50	52	70	—	26
Bacteria	28	25	26	28	26	30	24	26	26	26	—

* Values are given as percentage of sequence homologies.

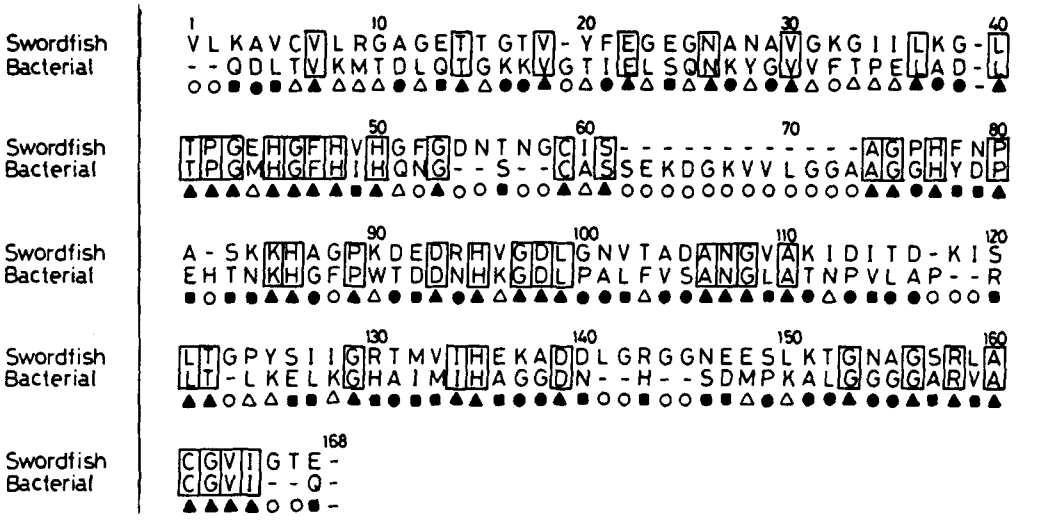

FIGURE 8. Amino acid sequences of swordfish liver and bacterial *(P. leiognathi)* copper/zinc SOD. Identical residues are boxed. Symbols refer to the frequency of substitutions. ▲, Identity; ■, frequently found substitution; ●, medial substitution; △, infrequent substitution; ○, rarely found or no substitution. Common gaps are due to alignment to other eukaryotic enzymes shown in Figure 7. (From Bannister, J. V. and Parker, M. W., *Proc. Natl. Acad. Sci. U.S.A.*, 82, 149, 1985. With permission.)

could also be built into the known bovine erythrocyte tertiary structure without disruption to the fold.[211] All this evidence clearly supports the view that gene transfer from the ponyfish to the *P. leiognathi* may have taken place. However, definitive evidence remains to be obtained at the molecular level. An alternative possibility could be that independent evolution of copper/zinc SOD took place in both eukaryotes and prokaryotes or perhaps that the enzyme originated in prokaryotes and was transferred to eukaryotes. This could also imply that the prokaryote and eukaryote copper/zinc SOD had a common ancestor. This possibility should not be discounted because a second copper/zinc SOD has been discovered in the nonsymbiotic *C. crescentus*[209] and, more recently, in two pseudomonad strains, *Pseudomonas diminuta* and *P. maltophilia*.[211]

Table 2
METAL CONTENTS AND MOL WTS OF VARIOUS SPECIES

Source	Metal content	Mol wt
Escherichia coli B	1-g atom Fe	38,000
Plectonema boryanum	2	41,700
	1	36,500
Bacillum megaterium	1	40,000
Pseudomonas ovalis	1.4	40,000
Desulphovibrio desulfuricans	1.60	43,000
Photobacterium leiognathi	1.61	40,660
Chromatium vinosum	2	41,000
Chlorobium thiosulfatophilum	1.8	43,000
Thiobacillus denitrificans	1.35	43,000
Anacystis nidulans	1	37,000
Crithidia fasciculata	1.4	43,000
Strepthococcus mutans	2-g atoms Mn	39,500
Escherichia coli	1.6	39,500
Pleurotus olearius	2	76,000
Chicken liver mitochondria	2.3	80,000
Saccharomyces cerevisiae	4	96,000
Mycobacterium lepraemurium	1.29	45,000
Thermus aquaticus	2	80,000
Rat liver mitochondria	4	89,000
Bovine heart mitochondria	2	86,000
Pisum sativum	1	94,000
Paracoccus denitrificans	1.34—2	41,500

Table 3
HETEROGENEITY OF SOME BACTERIA SPECIES

Source	Metal content		Mol wt
Mycobacterium phlei	1.7-g atoms Mn 1.2-g atoms Fe	0.7-g atoms Zn	80,000
Thermoplasma acidophilum	2.0-g atoms Fe	1.0-g atoms Zn	82,000
Methanobacterium bryantii	2.7-g atoms Fe	1.7-g atoms Zn	91,000
Nocardia asteroides	1.2-g atoms Fe 1—2-g atoms Mn	1—2-g atoms Zn	100,000

C. Primary Structure of Manganese and Iron SODs

The manganese- and iron-containing SODs have not been as thoroughly investigated compared to the copper/zinc SODs. The iron and manganese enzymes have a subunit molecular weight of about 23,000 daltons and, while the iron enzyme has been shown to exist in dimeric form, the manganese enzyme has been found to form tetramers as well as dimers.[304] The metal content of both isozymes varies between 1- and 2-g atoms per dimer (Table 2). It is as yet unclear whether or not this variation in metal content is due to the loss of the metal during the purification procedure. Crystallographic data on the iron SOD have indicated two metal-binding sites per dimer.[305] A number of SODs have been found to be heterogeneous in the type of metal present (Table 3). The possibility that these enzymes may represent a new class of SODs has to be entertained.

The amino acid sequence has been determined for only four manganese SODs (Figure 9),[279,306-309] and the crystal structure of the enzyme from *Thermus thermophilus* at 2.4-Å resolution has been determined.[310] The ligands to the metal are three histidines and one

aspartic acid residue which, when combined with the primary structure in Figure 9, correspond to His26, His81, Asp175, and His179. There is as yet no complete amino acid sequence for an iron SOD; however, the ligands to the manganese are also present in identical positions in the iron protein,[311] and there are strong indications that the manganese and iron proteins are structural homologs.[312]

VI. RELATIONSHIPS AMONG PRIMARY STRUCTURE, CONFORMATION, AND MECHANISM OF SODs

A. Introduction

The basic structure-function relationship in the SODs is considered to be twofold. The active site metal (copper, manganese, or iron) carries out a one-electron transfer between two superoxide radicals. This fact relates to whether a complex is formed between the metal and the substrate prior to electron transfer and, if this is the case, whether it may be formally considered to be an inner- or an outer-sphere type of interaction. The second problem is the presence around the active site metal of a protein pocket strategically lined by positively charged amino acid residues. This structural feature would create an electrostatic sink for superoxide radicals which enhances the rate of encounter.

This general picture favors a "simplistic" view of the structure-function relationships of these enzymes. It has, however, been generated by a great deal of spectroscopic, mechanistic, chemical, and crystallographic studies over more than a decade. These were mostly carried out on the bovine copper/zinc isozyme. Recently, data have become available in comparable detail for the manganese and iron isozymes. At the same time, however, questions have been posed that challenge the "simplistic" aspects of the conventional model.

B. The Crystallographic Model for Copper/Zinc SOD

A popular presentation of the conventional model has been set forth.[313,314] The model is an attempt to describe the functional anatomy of the enzyme in terms of the published data, including X-ray structure,[315] redox and catalytic reactions,[16,43,44,316-323] anion-binding pattern,[324-330] and effects of covalent modification of positively charged amino acid side chains.[301,302,331-333] In particular, the electrostatic recognition data are extensions of previous calculations of the dipole and electrostatic potentials of the enzyme by Koppenol.[334] The essential features of the refined model are the following and can be defined as the product of extensive application of computer graphics to incorporate all the available experimental data.

First, the copper(II) and zinc(II) are bridged by His77 (bovine sequence 61). Copper(II) is coordinated also to His48, 50, and 135 (bovine sequence 44, 46, and 118) in a square planar geometry with tetrahedral distortion. The geometry of zinc ligands (His77, 86, and 95 and Asp98) which correspond to bovine sequence His61, 69, 78 and Asp81 is tetrahedral. The presence of the intermetal bridge is essential to the tetrahedral distortion of the copper(II) site and to its relatively high redox potential (+0.42 V; see References 335 and 336). The bond from His77 (bovine 61) to the copper is released upon copper reduction with concurrent protonation of the His77 (bovine 61) nitrogen not liganded to zinc (NE2). The assumption concerning the copper(I) state of the enzyme lies on the pH dependence of the redox potential[336] of the enzyme-copper(I) by O_2^-, and the only direct evidence comes from an EXAFS study[337] which suggests one less imidazole is coordinated to reduced copper as compared with oxidized copper enzyme.

Second, there is a single, highly complementary position or "pit" for O_2^- to bind to the copper(II), determined from interactive computer graphics, where substrate can be accommodated while binding to Arg158 (bovine 141) as well. The involvement of Arg158 (bovine 141) has been confirmed by chemical modification with phenylglyoxal or butanedione to be

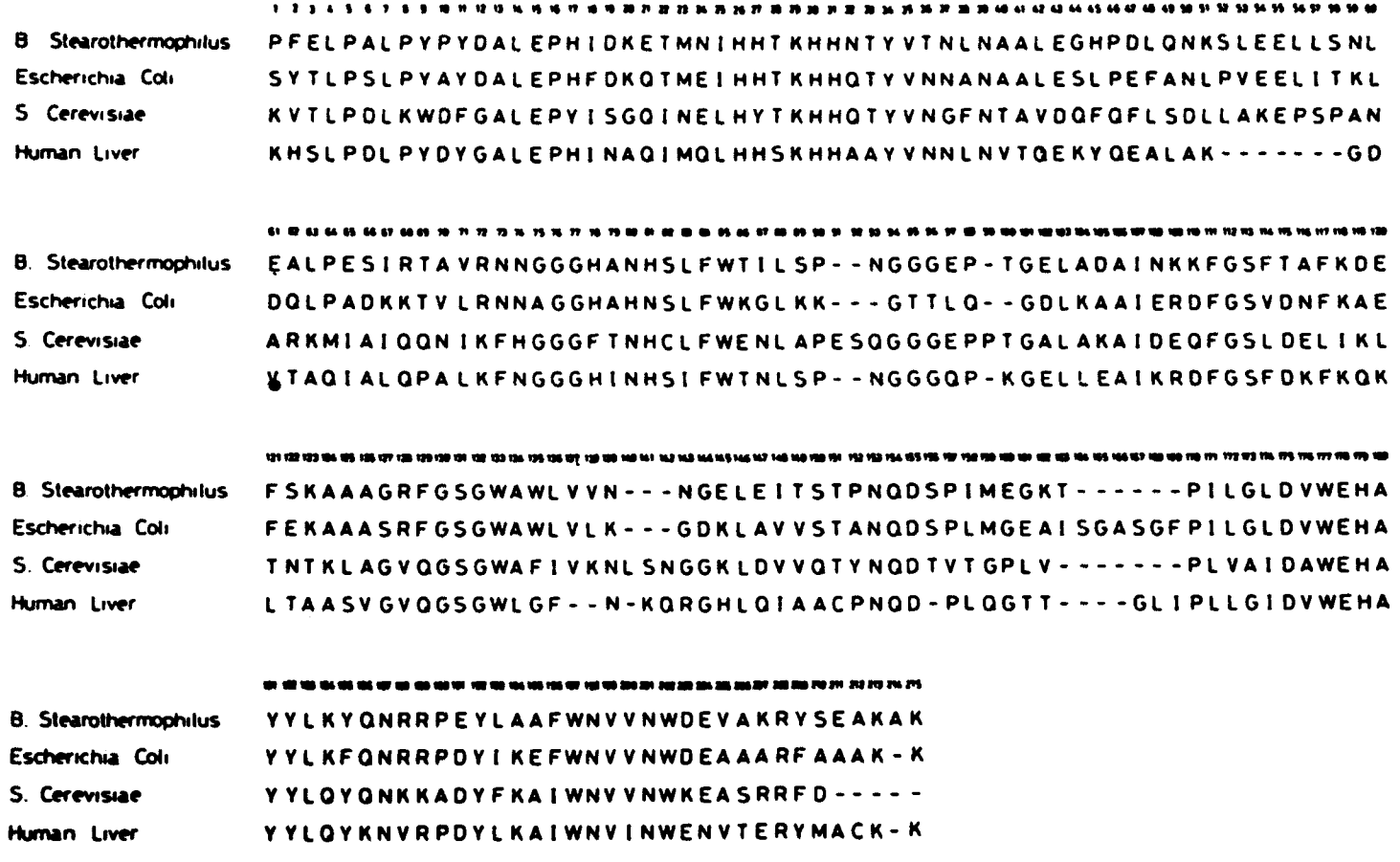

FIGURE 9. Amino acid sequences of manganese SODs.

important to the normal catalytic activity of copper/zinc SOD since the residual activity after modification varies between 2 to 15%.[301,302] The same "pit" accommodates cyanide, which is the most effective competitive inhibitor of the enzyme activity.[328]

Third, the minimal scheme for catalysis has been established by pulse radiolysis work on the copper/zinc enzyme[43,44] and then confirmed for the manganese and iron SODs.[48,50] It consists of two steps with equal rates and is referred to as the "conventional mechanism". In the case of the copper/zinc SODs, this is represented as follows:

$$\text{E Cu(II)} + O_2^- \rightarrow \text{E Cu(I)} + O_2 \quad (1)$$

$$\text{E Cu(I)} + O_2^- + 2H^+ \rightarrow \text{E Cu(II)} + H_2O_2 \quad (2)$$

The three-dimensional structure, as refined in the computer-graphics model, is compatible with the O_2^- displacing an axial water molecule and thus binding one oxygen to the copper(II) about 2 Å away, and the other oxygen, via H-bonding, to a guanidinium N of Arg158 (bovine 141) about 3 Å away. Displacement of copper-bound water by inhibitory ions and thus, presumptively, by O_2^- is indicated more or less directly by a number of spectroscopic approaches, including EPR at high pH,[324] pulsed ^{1}H NMR,[338] anion titration of water proton T_1,[325] ^{19}F NMR,[339] electron spin echo,[340] ENDOR,[341] and EXAFS.[337] No direct X-ray analysis, however, is available yet because although the CN adduct can be crystallized the crystals have been found to collapse under the electron beam.[342] The crystallographic model, in agreement with EPR data[324,343,344] which show a rhombic-axial interconversion of the EPR line shape in the presence of azide, cyanide, fluoride, and hydroxide, suggests that anion binding reorientates the coordination sphere such that one imidazole ligand is displaced from an essentially equatorial to an axial position, with the entering anion occupying the vacant equatorial site. Such a "fluxional" change is suggested to happen also with O_2^- in the computer-refined model, and His50 (bovine 46) is indicated as the axial ligand in the copper-superoxide complex. The copper is then reduced, His77 (bovine 61) NE2 becomes protonated, and O_2 is released by a reentering water molecule. Actually, fast protonation-deprotonation of the His77 (bovine 61) NE2 during catalysis has been strongly supported by elegant pulse radiolysis-rapid spectrophotometry experiments.[319] Water coordination to enzyme-copper(I) obtained by static reduction is suggested by ^{36}Cl-NMR experiments.[345] Oxygen has been shown to be kinetically unreactive with enzyme-copper(I) as compared with O_2^-.[346] The copper(I) is four-coordinated in tetrahedral geometry, and this explains the high E_0' and the very high reduction rates, which are quite unusual for a "non-blue" protein-bound copper site.[327] In fact, the crystallographic model shows that all the movements proposed by this mechanism are less than 1 Å. This means that the copper can shuttle between two proper symmetries, for the respective valence states, namely, five-coordinate enzyme-copper(II) and tetrahedral enzyme-copper(I), with minimal rearrangement of liganding nuclei.[327]

Binding of a second O_2^- to the copper(I) at the water coordination site then positions the substrate with one oxygen H-bonded to Arg158 (bovine 141), while the other oxygen H-bonds to the protonated His77 (bovine 61). At this point, the model introduces a catalytic role for the zinc since it ensures protonation of the His77 (bovine 61) NE2 throughout a wide pH range. This nitrogen is considered to be the source of the proton required to stabilize the peroxide anion produced by the second electron transfer from ECu(I) $\rightarrow O_2^-$. In fact the zinc-free protein[323,347] is fully active only between pH 5 and 7 compared with the copper/zinc enzyme which retains full activity between pH 5 and 10.[348,349] The drop below pH 5 is due to the alteration of the metal-binding sites,[350] while the alkaline drop might be due, at least in part, to deprotonation of His77 (bovine 61) NE2, which occurs at lower pH in the absence of zinc. Under steady-state conditions, hydrogen peroxide is released since it

does not compete kinetically with O_2^- for reduction of the enzyme-copper(II) at pH <10.[44,317] Only the oxidized copper enzyme, in the absence of a flux of O_2^- capable to bring the enzyme into the steady state, is reduced and then inactivated by H_2O_2 in a complex reaction. This reaction involved reduction and reoxidation of the enzyme by H_2O_2 in a catalase type of reaction, although at a rate several orders of magnitude lower than with superoxide.[351] It has been shown, however, that the reaction of the conventional mechanism can be reversed under particular conditions such as high pH and scavenging of the O_2^-.[346,351,352]

The final essential feature of the refined model is that the rate constant for superoxide dismutation by copper/zinc SODs is approximately $2 \times 10^9\ M^{-1}\ sec^{-1}$ at pH 5 to 10 and I = 0.05.[43,44] This value is very high — about 10% the maximum diffusion-controlled limit. This conflicts with the catalytic copper site being only 0.1% of the enzyme surface and having only one coordination site available to solvent exchange. Electrostatic facilitation has been proposed.[334] Tainer et al.[313,314] have calculated that the copper is at the bottom of an active site channel, forming 10% of the entire molecular surface of the enzyme and very suitable for electrostatic facilitation of the recognition between O_2^- and the enzyme. Individual residues near this channel were examined to evaluate their contribution to precollision orientation and facilitated diffusion of O_2^-. Besides Arg158 (bovine 141), a more proper candidate for short-range docking of superoxide at the copper site are the pair Lys151 (bovine 134)/Glu148 (bovine 131) and, to a lesser extent, the pair Lys137 (bovine 120)/Glu136 (bovine 119) (not always conserved) are in suitable positions to create significant electrostatic attraction toward O_2^-. This conclusion is suggested by a color computer-graphics display of the electrostatic potential and the electrostatic field vectors. Pairing with an opposite charge is essential to prevent nonproductive association of O_2^- with Lys151 (bovine 134). Neutralization or inversion of the lysine charges by extensive carbamoylation[332] or acylation[331,333] of the enzyme decreases the catalytic efficiency of the enzyme thereby giving support to this picture. It is also true that the enzyme activity is typically inhibited by high ionic strength.[326] The residual activity after enzyme modification is much less sensitive or even positively sensitive (i.e., the activity increases) to increasing salt concentration, depending on whether the charge on the lysine residues is neutralized or inverted. Elimination of the charge on the lysine residues by pH titration of the residues produced similar effects.

Although the picture worked out on the basis of the computer-graphics-refined crystallographic model relies on, and coincides with, the experimental work and conclusions, a number of ambiguities, which have given rise to various controversies, are still present.

First, is the His77 (bovine 61) copper-zinc bridge present in solution? The only possible experimental approach to this question is spectroscopic. In this regard, zinc replacement by other metal ions more suitable for this type of approach has been rewarding. The initial evidence for a bridge came from the very first of such substitutions. Calabrese et al.[353] showed that when substituting cobalt for zinc the copper(II)/cobalt(II) SOD gave samples lacking the EPR spectra for both metals, although the respective optical bands were present and indicative of paramagnetic, EPR-detectable states. Reduction of the copper led to appearance of the cobalt(II) EPR spectrum.[354] In the same publication, the magnetic interaction leading to obliteration of the EPR spectra was shown to occur over a large temperature range (in the frozen state) and therefore assigned to antiferromagnetic exchange through a common aromatic ligand. The first X-ray analysis at high resolution[335] indicated that His77 (bovine 61) might be such a ligand, and this has been shown to be the case in the refined model. It has been shown[356] that the optical and EPR spectra of cobalt in the copper(I)/cobalt(II) protein were identical to those of the cobalt(II) protein, i.e., enzyme deprived from copper but containing cobalt(II) at the native zinc place. This result, suggesting protonation of the His77 (bovine 61) N facing the copper, was the basis of the catalytic experiment[319] showing that in the copper/cobalt SOD protonation-deprotonatoin of the imidazolate bridge occurred in turnover with the same rates as the catalytic cycle. All other spectroscopic approaches to

the problem are variations of this model: ^{131}Cd NMR with cadmium in the zinc site[357] or ^{1}H NMR with the copper/cobalt protein.[358] The only work carried out with the native protein is the EXAFS study of Blackburn et al.[337] The conclusion of this work can be accepted, provided one accepts the model used for best-fitting calculation of the solution spectrum, namely, the freeze-dried enzyme. This is not completely acceptable since EPR[351] and activity[321] investigations point out that the lyophilized enzyme undergoes structural alteration. The problem is therefore twofold. First, is the copper/cobalt SOD a perfect copy of the native protein? Second, what is the proof that such a bridge can exist in solution since silent EPR is not enough to indicate that a chemical bond is actually responsible for magnetic coupling between paramagnetic centers, especially if a sufficiently large temperature range is not explored?[360] The first question lacks the confirmation of X-ray analysis of crystals; however, extensive X-ray absorption spectroscopy (XANES and EXAFS) investigations appear to indicate overlapping of structure at the metal-binding sites of native and cobalt-substituted protein.[361,362] Answering the second question requires a precise statement of the conditions (temperature, physical state of the sample) under which the appropriate measurement (EPR, magnetic susceptibility) has been carried out. The protein sample should not be subjected to prior freeze-drying and the temperature range explored should include the ambient temperature in order to be functionally significant. The EPR[354] and magnetic susceptibility studies[363] conducted over a significant temperature range were not able to measure the coupling constant; however, the upper limit of the temperature was always far below 0°C. The only magnetic susceptibility measurement available for a solution of the copper/cobalt enzyme[364] is at a single temperature (25°C). The magnetic moment measured was identical to the two independent magnetic moments of copper(II) and cobalt(II) measured in the zinc-free and cobalt(II) protein, respectively. Thus, magnetic susceptibility of the copper(II)/cobalt(II) SOD, the derivative used for mechanistic studies, is not in straightforward agreement with the refined crystallographic data.

A second ambiguity is that Arg158 (bovine 141) is conserved in all the copper/zinc SODs so far examined. All activities assayed to date in various tissues and organisms are inactivated by phenyglyoxal.[301,302,365] However, phenyglyoxal is a rather bulky reagent and may block the entrance of O_2^- to the active site channel by steric hindrance. This criticism applies even more to experiments when the modified enzyme was shown to be less susceptible to the presence of the anionic inhibitor phosphate,[366] which is clearly of a larger size than O_2^-. Such experiments cannot be definitive evidence for phosphate binding to Arg158 (bovine 141). Only the site-directed mutagenesis approach will rule out the steric hindrance criticism affecting these types of studies.

The single steps of the catalytic mechanism are also the object of much discussion. There is a general consensus that small, single-charged anions behave like superoxide in their interaction with the copper. Rigo et al.[328] showed by ^{1}H-pulsed NMR and polarographic activity assay that the water coordination to the copper was affected by chloride (3.63-Å radius) in both the copper(II) and copper(I) enzyme, Br^- (3.90 Å) only in the copper(II) enzyme, and iodide (4.32 Å) in neither copper-enzyme form. Thus, the size of the active site channel was functionally probed and the coordination of the substrate and substrate analogs to copper by displacing a water molecule was once more supported. Recently, an ^{1}H NMR study of the histidine resonances in the copper(II)/cobalt(II) SOD showed that the residues ligated to the copper can also be detected.[358] This was expected since in binuclear systems with even weak magnetic coupling the electronic relaxation time is uniquely short and involves both metals, as was already demonstrated for the copper(II)/cobalt(II) SOD in the T_1 study of Rigo et al.[367] Addition of excess azide or thiocyanate or cyanate caused large variations of the histidine proton signals, which were interpreted as signaling the removal of His48 (bovine 44) from coordination to the copper(II) by the anion ligand (not just lengthening the bond). It is unfortunate that this experiment was not performed with cyanide,

the only anion that closely fits the O_2^- site in the refined model. Cyanide binds to the enzyme with a three-order-of-magnitude higher association constant than azide, the second best inhibitor.[328] Furthermore, azide binding is significantly inhibited by doubling the concentration of the inert salt perchlorate, while cyanide inhibition is not.[328] This indicates that azide does not only probe the copper-water exchange but may bind somewhere else, especially at high concentrations. Extra-bonding may in turn affect protein conformation at the copper site. Cyanate modifies covalently all the lysine residues of the enzyme.[332] Thiocyanate has no effect on either the enzyme activity[328] or the EPR spectrum at room temperature[368] and causes gross alterations to the protein.[330]

Since cyanide is the model for studying the O_2^- enzyme (interaction) the question is does this similarity imply that an inner-sphere copper(II)-O_2^- complex is actually formed? The straightforward presentation of Tainer et al.[313] is as yet rather speculative. Kinetic saturation of the enzyme was actually observed at $[O_2^-] \geq 0.4\ \mu M$ by Rigo et al.,[318] but this result obtained with the polarographic methods of catalytic currents has not been confirmed with other methods.[369] The same caution has to be applied to the competitive pattern of inhibition shown by Rigo et al.[328] with singly charged anions, suggesting displacement of cyanide by excess O_2^-.

The formation of H_2O_2 is the most controversial point in the mechanistic scheme as far as both the weak oxidizing properties of O_2^- and the source of protons are concerned in a reaction that apparently has the same rate as the far more facile reduction of the copper(II) by the first O_2^-. This is the proper context to introduce a new mechanism, proposed on purely theoretical grounds, which should, however, be taken into consideration.[370] On the basis of quantum chemical calculations of the interaction of O_2^- with a model of the enzyme active site, this mechanism proposes that the role for Arg158 (bovine 141) is, besides positioning O_2^- in the active site, to change its redox chemistry in such a way that it forms a stable complex with the copper(II) without reducing it. Copper reduction occurs, by an outer-sphere process, when another O_2^- is oxidized by the stable intermediate

$$\text{E-Cu(II)} - H_2O\text{--}HN^+ - \text{Arg} + O_2^- \qquad \text{(I)}$$

$$\text{E-Cu(II)} - O_2^-\text{---}HN^+ - \text{Arg} + H_2O \qquad \text{(II)}$$

$$\text{E-Cu(I)} + O_2^-\text{----}HN^+ - \text{Arg} + O_2 \qquad \text{(III)}$$

The reduced form (III) "isomerizes" to a form where a proton transfer occurs to O_2^- from Arg158 (bovine 141), giving the hydroperoxyl radical, now oxidizing enough to restore the oxidized form of copper with concomitant production of the hydroperoxide anion product

$$\text{E-Cu(I)} - O_2^-\text{---}HN^+ - \text{Arg} \qquad \text{(III)}$$

$$\text{E-Cu(II)} - O_2^-H\text{---}N - \text{Arg} \qquad \text{(IV)}$$

$$H_2O + H^+\text{-E-Cu(II)} - O_2H - HN^+ - \text{Arg} - HO_2^- \qquad \text{(I)}$$

The source of proton for the outgoing peroxide is therefore Arg158 (bovine 141), and there is no need for dissociation of the copper-NE2 (His77 [bovine 61]) bond and the protonation of the resulting imidazolate anion. With some assumptions, many experimental facts can be explained by this mechanism. For instance, catalysis can start from the reduced enzyme as shown by Fielden et al.,[44] beginning with structure (III) above. More difficult is to show chemical identity between the catalytically "reduced" enzyme and the copper-free form as shown with the cobalt derivative, while this is straightforward in the conventional mechanism

of McAdam et al.[319] It should also be rationalized why the steady-state enzyme-O_2^- system is spectroscopically identical by any means to the [E Cu(I)]/[E (Cu [II])] = 1 system.

There are interesting variations also within the conventional mechanism as far as the source of proton in the second step is concerned. Fee and Ward[345] have proposed that the metal-bound peroxide is protonated by water and hydrogen-bonded to the enzyme copper (I)-O_2^- complex prior to electron transfer. Actually, no isotope effect in D_2O on K_{cat} in H_2O, which would have indicated a rate-limiting proton transfer from water, has been observed.[16] Once again, a definitive demonstration is difficult, and more experimental work is needed to elucidate the mechanism of action beyond the "conventional" scheme and the "simplistic" model.

Finally, it must be considered that lysines are certainly an appropriate site for the binding of anions. Perchlorate and cyanate,[366] which cause no perturbation to the copper(II) spectral properties, still inhibit the enzyme activity. The covalent modification experiments support this idea. Cyanate is a special case as it perturbs the copper(II) EPR spectrum and also carbamoylates lysine.[332] The carbamoylation per se has no inhibitory effects but renders the enzyme less susceptible to inhibition by perchlorate. Azide and halides are likely to bind both copper and lysine residues and therefore show complex behavior. Some halides also have different affinity towards the oxidized and reduced form of the protein. Binding of anions which do not perturb the copper site may cause conformational changes of the active site besides just ionic-strength effects. The EPR spectrum is converted to an axial line shape in the presence of perchlorate and also in the presence of Ca^{2+}, even **upon freezing** of the protein sample. All these results point to the danger of a too simplistic interpretation of the anion-binding pattern of copper/zinc SODs.

The case of phosphate is particularly instructive in this regard. Phosphate inhibits cyanide-binding[344] activity[326] and alters the optical and EPR spectrum of the cobalt when it replaces the copper[368] and appears to bind very near to the copper on the basis of ^{31}P-NMR measurements.[371] The Arg158 (bovine 141)-modified enzyme is less affected, as far as activity and anion binding are concerned, at low concentration of phosphate,[366] while at higher phosphate concentrations, it behaves like the native enzyme. A likely conclusion is that Arg158 (bovine 141) is the primary site of interaction of phosphate as well as the major source of the dependence of the activity on ionic strength at low phosphate concentrations. Lysine residues, on the other hand, become a dominant site of anion interaction at higher salt concentrations. Once again this conclusion rests heavily on the phenylglyoxal inactivation of the enzyme activity, which is not an unequivocal approach to the role of Arg158 (bovine 141).

Salt effects are strictly related to pH effects. The enzyme activity of bovine copper/zinc SOD is reversibly inhibited by [OH^-] with an apparent pK between pH 10.5 and 11.[372] Hydroxide competes with other anions for the same site[328] and, therefore, pH effects have, at least in part, the same nature as salt effects. However, the pK of the alkaline inhibition is not affected by ionic strength. More than one factor has been invoked to explain the deactivation of the enzyme at higher pH. The deprotonation of the copper-bound water may contribute to this effect,[320] but titration of the water T_1[373] has an half-transition point at approximately pH 11.5 and, rather, coincides with the titration of rhombic-axial conversion of the EPR spectrum[324] and with the residual activity of the lysine-modified enzyme.[373] Thus it appears that the pK around pH 10.5 reflects the protonic equilibrium of the activity-important lysine(s). The higher pK should be assigned to deprotonation of copper-bound water and/or Arg158 (bovine 141), which are structurally very close to each other. In fact, the EPR spectrum of the arginine-modified enzyme is axial.[374] A further activity important for ionization in the pH range may involve the NE2 of His77 (bovine 61) of the copper(I) enzyme, which in the conventional mechanism is a likely proton source for the peroxide product.[320]

All these considerations do not explain the unusual pH dependence of pig enzyme,[343] whose activity decreases almost linearly with increasing pH between pH 7.5 and 12, while EPR, T_1, and activity of lysine-modified enzyme titrate just like the bovine enzyme. The primary structure of pig copper/zinc SOD[292,293] shows conservation of the crucial residues and, in particular, of Lys151 (bovine 134) so that no obvious structure-activity relationships can be proposed on the basis of the refined crystallographic model. However, the porcine enzyme has a higher isoelectric point than the bovine enzyme,[375,376] which makes it almost neutral in contrast with the acidic bovine, yeast, or human copper/zinc SOD. Koppenol[334,377] has extended his calculations to the pig enzyme, showing a definite positive area not far from the copper due to the absence of Glu92 (bovine 75). This may compensate for less repulsion of O_2^- by negative charges on the surface of the molecule outside the area of the active site. In fact, the pig enzyme is **not** less efficient than the bovine enzyme and is even more active at low ionic strength.[376] Furthermore, the activity is affected by iodide, which is not the case with the bovine enzyme. Definitely, electrostatic potential, anion affinity, and pH dependence of activity of the pig enzyme are strong suggestions that the "simplistic" model worked out with the bovine enzyme should be accepted with some caution. As a final remark to the problem of anion binding and electrostatic interactions, it should be remembered that physiologically the SODs are immersed in a bath of chloride and phosphate, while the $[O_2^-]$ concentration is much lower. Considerations of this type may make all experiments made with the enzyme at relatively low ionic strength irrelevant to the natural situation.

C. The Dimeric Structure

The cystallographic models give no clue to structure-activity relationships regarding the role of the homodimeric structure of SODs. Native monomers have never been isolated in solution due to very tight subunit binding. The subunit association can be weakened by heating,[378] sodium lauryl sulfate,[379] or acylation,[331] but the dimer reassociates when the dissociating agents are removed. This property can be used to hybridize subunits made artifically nonequivalent[379] and to resolve a natural hybrid in artificial homodimers.[380] In one case, a succinylated copper/zinc SOD was treated under conditions likely to produce immobilized monomers,[331] thus preventing reassociation, but acylation of lysine residues and the presence of a solid matrix prevented a clear assessment of activity. The EPR spectrum was, however, of a native type, indicating that neither lysine modification nor subunit dissociation changed the conformation of the active site in a drastic way. Functional nonidentity of subunits has repeatedly been reported. Fielden et al.[44] reported that the steady state of reduction enzyme-copper(II) concentration approached 75%, instead of the expected 50%, as if one of the subunits were inactive while the other was functioning. This result was later shown to be dependent on sample history, in particular prior freeze-drying.[321,381] The phenomenon was not trivial since it was **not** associated with less-active samples, thus indicating sample-dependent alterations in subunit interaction. The occurrence of conformational communication between the two remote (34 Å) copper sites of the native dimer is in fact detectable as a break at one equivalent per mole of protein in either coulometric titrations[382] or in the process of metal reconstitution of apoprotein.[383-385]

The kinetics of such communication is not known and may be too slow to account for the cooperative effects observed in catalysis. These effects have been questioned on the basis of an experiment showing that a hybrid containing an active subunit and an H_2O_2-inactivated subunit behaved like subunits working independently.[378] An obvious question is whether an H_2O_2-inactivated copper site[317] can still "communicate" with the functioning counterpart. In fact, a "native", although "inactive", subunit can be made by selectively removing one copper ion per enzyme molecule.[386] This derivative was significantly more active than the doubly filled dimer per enzyme molecule.

D. Manganese- and Iron-Containing SODs

Less is known about the isozymes having iron or manganese as the active metal. Nevertheless, the present knowledge is enough to indicate that the general features of the mechanism are identical to the copper/zinc enzyme, although it is more difficult to identify the structural basis of the catalytic function. Structurally, manganese and iron SODs are considered to be homologous proteins[311,312] and unrelated to the copper/zinc-containing isozyme. In one case, typical (as far as activity and spectroscopy are concerned) manganese or iron SOD could naturally be made by the bacterium *P. shermanii* with the same protein moiety, depending on the selective metal supply.[118]

Iron and manganese SODs also occur as dimers (sometimes tetramers). The metal sites, however, are much nearer the dimer interfaces than in the copper/zinc enzyme (≃8.9 Å).

The monomer fold is very similar in both cases and comprises two domains, each contributing two ligands to the metals. This situation on a larger scale is reminiscent of the repeated folding pattern in the copper/zinc protein[387] where the copper site is on the dyad axis between two distinct subdomains. In the iron and manganese SODs, the metal centers are approximately 18 Å apart in the dimers, and there are several specific contacts made by amino acid residues in the environment of the metal-binding sites that could afford a pathway for interaction between the two active centers.[310,312,388,389] However, contrary to the case of the copper/zinc protein, there is no evidence for such an interaction besides some speculation on the process of metal incorporation and replacement, which is quite complicated.[390-393] The folding is made largely by α-helices, contrary to copper/zinc SOD, which is a β-structure barrel of the immunoglobulin type.[394] This makes the iron- and manganese-containing SODs more susceptible to denaturation than copper/zinc isozymes. Typically, urea facilitates metal removal and incorporation.[390]

Interchangeability of the iron and manganese[20,118] requires considerable structural homology, if not identity, of active sites. Three histidines and one aspartic acid residue have been detected as metal ligands in the X-ray structure of the manganese isozyme.[310] The iron SODs also have four protein ligands,[388,389] one of which is certainly histidine; the other three have been located in the crystal structure and recently were tentatively identified from preliminarily sequence data of the protein isolated from *P. leiognathi*.[311]

In both isozymes, these ligands form an appropriate trigonal bipyramid in which water may occupy an axial position. Both forms of the enzyme display effects on water NMR relaxation,[395,396] and metal-bound water is functionally important. This water molecule is typically embedded in a hydrophobic environment surrounding the active site,[388] which may be functionally significant in determining the actual electrostatic charge encountered by the substrate. Electrostatic facilitation of O_2^- encounter with the protein has been proposed for both isozymes, using the same residue-modification approach as used with the copper/zinc isozyme.[397] The catalytic mechanism of iron and manganese SODs follows the general scheme shown for the copper/zinc enzyme involving the iron(III)/iron(II) and manganese(III)/manganese(II) couples, respectively, also inhibition by singly charged anions and a decrease of activity with increasing ionic strength. In the case of the manganese SOD, the active site is also thought to be involved in a slow side reaction leading to a dead-end species.[45-48] The catalytic efficiency is slightly lower in the iron and manganese isozymes than in the copper/zinc SOD when a similar procedure is used to investigate the mechanism, e.g., pulse radiolysis. However, the comparison between copper and noncopper enzymes may not be straightforward because the fine details of catalysis are quite different. In contrast to the copper/zinc enzyme, the iron and manganese SODs display unquestioned substrate saturation so that K_m, V_{max}, and TN have a classical meaning in their case.[48,50,398] Moreover, both isozymes show definite inhibition at pH values above neutrality.[54] In the iron SOD, K_{cat} is pH independent between pH 6 and 10, but K_m depends on pH with a pK near 9.[398] More or less similar behavior has been observed for the manganese SOD.[50] Anion inhibitors (azide

and fluoride), which coordinate the iron, display pH dependencies of inhibition which resemble those for substrate.[128] Also in the case of iron SOD, the affinity of the enzyme for anions may be different in the oxidized and reduced form of the protein and may involve two types of sites, a primary one at the metal ion and a secondary one on the nearby protein pocket, which is better probed by metal-unreactive anions such as perchlorate and thiocyanate.

While water exchange is fast enough in the copper/zinc SOD[338,399] to account for mechanistically relevant H_2O displacement by O_2^-, this is not the case with manganese(III) or iron(III) proteins.[395,396] An outer-sphere mechanism of metal-redox reaction should rather be taken into consideration. X-ray studies concerning azide binding to the iron SOD[388] and computer-graphics analysis with manganese SOD[310] revealed a cavity centered about 3 Å from the metal which may host the substrate and the anionic inhibitors. This would make the active center able to feel anionic inhibitors and O_2^- without displacing the water molecule. Actually, at least a primary step of azide binding to either iron[400] or manganese[312] SODs does not perturb the spectral properties of the natural site.

One H^+ is bound on reduction of iron(III)[398] over a broad pH range (pH 6 to 10). If a single titratable group is responsible for this binding, its pK must shift from 6 to >9 in the iron(II) protein. This may be accounted for by the strikingly polar environment of the metal centers. There is no evidence that this group is a metal ligand as it is for the copper/zinc isozymes. Apparently, there is no special need for such a close proton source since both iron and manganese SOD[398] show an isotope effect on K_{cat} by D_2O, consistent with rate-determining proton transfer from H_2O. This may be assisted by specific residues near the active center (e.g., Tyr36 in the *T. thermophilus* manganese SOD),[310] which might be also implicated in the redox-linked pK.

VII. APPLICATIONS OF SOD

A. The Concept of Superoxide-Related Pathology

Studies on SOD in biological systems are underpinned by the hypothesis of superoxide toxicity. The evidence which supports this hypothesis has been forcefully reviewed,[401,402] while those who have thrown doubt on the hypothesis[403,404] have not produced evidence which can be considered to falsify it.[197]

The deleterious effects of O_2^--generating systems on biological macromolecules, subcellular components, cells, and tissues[405-407] suggest that O_2^- can be an initiating or contributing factor in disease.[408] Superoxide-related pathology may result either from increased production of O_2^- due to hyperoxia, activation of granulocytes and macrophages, conversion of xanthine dehydrogenase to xanthine oxidase, exposure to ionizing radiation and redox cycling of xenobiotics, or from decreased activity of SOD. In the absence of direct probe, increased production of O_2^- in vivo is currently a reasonable inference from observations in vitro. The tissue distribution of SOD shows much variation, but what is critical is not known for any tissue.[197,409-413]

Michelson et al.[414] put forward a concept of superoxide-related pathology according to which both high and low levels of SOD activity can be pathogenic, but detailed interpretations are not available. In a general way, low SOD activity can be considered to favor the formation of $OH^\cdot$ radicals from O_2^- and H_2O_2 in the presence of iron or copper complexes, as seen in vitro and discussed recently.[415] A more powerful oxidant than O_2^- is commonly invoked to explain the toxicity of O_2^--generating systems. In microenvironments of lower-than-physiological pH, this can be the conjugate acid of O_2^-, the perhydroxyl radical $HO_2^\cdot$.[416] It is less easy to see how high levels of SOD activity or low levels of O_2^- can be pathogenic. It is difficult to accept a normal role of O_2^- as a free radical-trapping agent[414,417] or a pathological role of copper/zinc SOD as a peroxidative agent.[418]

In this section, we discuss a number of areas in which the concept of superoxide-related

pathology is testable by means of measurements of SOD activity, inhibition of endogenous SOD, or administration of exogenous SOD. These include genetic aspects of SOD, the erythrocyte, inflammation, and ischemic injury. In a sense, these are areas, among others, of applications of SOD in the investigation of the pathology of disease and therapeutic possibilities.

G. Genetic Aspects of SOD

1. Introduction

The chromosomal location of the SOD genes is known for man and some other animals. The regional localization of the copper/zinc SOD gene is known in man. This gene is triplicated together with other unknown genes in (trisomy 21) Down's syndrome. The cloning of cDNA sequences complementary to human copper/zinc SOD mRNA has been achieved.[272] This has the potential to open new avenues in the understanding of Down's syndrome but does not close the old ones. The SOD genes have rare alleles which appear to be selectively neutral.

2. SOD

The gene of copper/zinc SOD is located on chromosome 21 in man,[419] on chromosome 9 in the monkey *Cebus capucinus*[420] and the domestic pig,[421] and on chromosome 16 in the mouse.[422-424] The gene for manganese SOD is found on chromosome 6 in man,[425] where it has been localized on the long arm distal to 6q16,[426] and on chromosome 17 in the mouse.[427]

The H-2^b phenotype in mice was considered to be associated with increased copper/zinc SOD activity in liver by Novak et al.,[428] but correlation between copper/zinc SOD activity and the H-2 histocompatibility phenotype was not confirmed by Bloor et al.[429] and is not expected because the H-2 complex is on chromosome 17 while the copper/zinc SOD structural gene is on chromosome 16 in the mouse.

3. Genetic Overdosage of Copper/Zinc SOD: Trisomy 21

Chromosome 21 in man is of special interest because the genes responsible for Down's syndrome, when triplicated, are located on the distal part of the long arm of this chromosome.[430] Consistent with triplication of the gene for copper/zinc SOD, an increase of about 50% in erythrocyte SOD activity[414,431-434] and immunoreactive enzyme protein[435,436] can be demonstrated in Down's syndrome. A similar increase in copper/zinc SOD activity has been observed in platelets,[437] leukocytes and fibroblasts,[438] and lymphoid cells.[439] Baret et al.[440] did not find a significant increase in immunoreactive copper/zinc SOD in trisomy 21 platelets, although a 50% increase was found in granulocytes, lymphocytes (T and non-T), and macrophages from trisomy 21 patients.[440,441]

The gene for copper/zinc SOD is probably located on the part of chromosome 21 which includes the distal part of band 21q21 and the subband 21q22.1, as indicated by the correlation between erythrocyte SOD activity and the chromosome abnormality in rare cases of partial monosomy or trisomy 21.[442-447] Leschot et al.[448] suggested that the gene for copper/zinc SOD might be localized proximal to or in band 21q21 because a patient with partial trisomy of the segment 21pter (the end of the short arm) to 21q21.4 of chromosome 21 had erythrocyte SOD activity in the range of a group of patients with regular trisomy 21. This patient had no symptoms of Down's syndrome.

Kedziora et al.[449] did not observe an increase in erythrocyte SOD activity in Down's syndrome patients with 21/D and 21/G Robertsonian translocations; they ascribed this to a position effect, but their observations have not been confirmed by others.[450-452] The copper/zinc SOD gene was inactivated as indicated by the erythrocyte SOD activity in a patient with translocation of the segment 21q11 to 21qter (the end of the long arm) of chromosome 21 to point q27 of an inactivated X chromosome. This can be explained by the spreading of the inactivation along the translocated segment of chromosome 21.[453]

The role of the copper/zinc SOD gene in the pathology of Down's syndrome is difficult to evaluate based on current evidence. A patient with partial trisomy of the subband 21q22.3 of chromosome 21 had many features of Down's syndrome without an increase in erythrocyte SOD activity.[454] Patients with mosaic trisomy 21 showing about 30% abnormal cell lines had full Down's syndrome symptomatology without an increase in erythrocyte SOD activity.[452] It is doubtful whether a 50% increase in copper/zinc SOD activity by itself is pathogenic. An accelertion of peroxidative processes in trisomy 21 has been postulated from observations on erythrocytes, which showed an increase in glutathione peroxidase and hexose monophosphate shunt activity.[418,455,456] Elevation of glutathione peroxidase activity in trisomy 21 has also been found in lymphoid cells[439] but not in cerebral cortex from fetuses. In the latter, there was a significant increase in SOD activity ($+60\%$; $p<0.001$) and a tendency to undergo lipid peroxidation compared to controls. Total polyunsaturated fatty acid content was not altered, but changes in lipid composition are not excluded.[457] Enhanced copper/zinc SOD activity might accelerate lipid peroxidation without an adaptive increase in the mechanism of H_2O_2 elimination.

The common ground sought between Alzheimer's disease and trisomy 21[458] is probably unrelated to SOD activity since both copper/zinc and manganese SODs have essentially unaltered activity in various parts of the brain in Alzheimer's disease.[459] In trisomy 21, there is a decrease in manganese SOD activity (-33%; $p<0.02$)[437] and immunoreactive manganese SOD (-49%; $p<0.001$)[440] in platelets. However, immunoreactive manganese SOD shows no significant variation in granulocytes, lymphocytes, and macrophages from trisomy 21 patients compared to controls.[440,441]

4. Genetic Polymorphism of SOD in Man

Electrophoretic variants of SOD were originally observed under the guise of tetrazolium oxidase or indophenol oxidase.[460] The population genetics of copper/zinc SOD has been amply studied. Beckman[461] proposed the existence of two allelic genes, SOD-A^1 and SOD-A^2, with the associated phenotypes SOD-A 1, SOD-A 2-1, and SOD-A 2. The rare phenotype SOD-A 2 shows a slower anodic mobility than the common phenotype SOD-A 1 in electrophoresis. It is seen only exceptionally in population studies except in Finland and northern Sweden. The SOD-A^2 allele has a relatively high frequency of 1 to 3% in Finland and northern Sweden,[462-465] the Westray Island of the Orkneys,[466] and the west coast of Newfoundland.[467] This geographic distribution is consistent with gene flow from a single Scandinavian source.[468] The occurrence of the SOD-A 2-1 phenotype in Italy[469] and Iraq,[470] where it is associated with SOD-A^2 allele frequencies of 0.1 and 0.3%, respectively, has suggested the possibility of Mediterranean-Arabian gene flow.[470] The finding of the phenotype in the Mbugu of Central Africa, where gene flow from Caucasoids can be excluded, supports the hypothesis of independent mutation in more or less recent times compared to the Scandinavian source of the mutant gene.[471]

The enzyme variant SOD-A 2 has been isolated from the erythrocytes of a homozygote and compared with SOD-A^1,[472] but the amino acid substitution has not been determined. The SOD-A 2 had the same specific activity as SOD-A 1.

Polymorphism of manganese SOD (SOD-B) has been reported from Japan[473] and Malaysia.[474] A frequency of 1.2% was found for the SOD-B^2 allele among the Senoi aborigines of Malaysia.

The variant gene appears to be harmless in populations with polymorphism for copper/zinc[475] or manganese SODs. Considering the crucial role of SOD in oxygen metabolism, it is possible that only variants with essentially normal enzymatic activity can be tolerated.[472]

C. SOD Activity in RBC

1. Introduction

Investigation of erythrocyte SOD is an active area of this research, possibly because of

the ease with which erythrocytes can be obtained. The importance of SOD as a detoxication enzyme of oxygen metabolism in RBC is related to the phenomenon of autoxidation of hemoglobin which produces O_2^-. This is one origin of oxidant stress in erythrocytes and may lead to eventual hemolysis. Several investigators have utilized SOD to study the mechanism of hemolysis. Besides measurement of SOD activity in relation to specific RBC pathology, there is also considerable interest in following erythrocyte SOD activity in relation to other diseases.

2. Superoxide Production in Erythrocytes

Erythrocytes produce O_2^- by autoxidation of hemoglobin. The molar ratio of SOD to hemoglobin in packed RBC is about 4.7×10^{-6} *M*/4.7×10^{-3} *M* or 10^{-3}.[476] Assuming that hemoglobin autoxidizes at the rate of about 3% per day,[477] equivalent to an O_2^- production of about $6.5 \times 10^{-9}\ M^{-1}\ sec^{-1}$ and a rate constant of $1.5 \times 10^9\ M^{-1}\ sec^{-1}$ for human erythrocyte SOD,[349] it can be calculated that this corresponds to a steady-state intracellular O_2^- concentration of about 1×10^{-12} *M*. This is consistent with the small oxygen consumption of erythrocyte suspensions. Generation of O_2^- in RBC suspensions has been inferred from formation of oxidized glutathione (GSSH) after inhibition of SOD by diethyldithiocarbamate. In the presence of glucose, the hexose monophosphate shunt was stimulated. Formation of methemoglobin occurred when GSSG formation reached its maximum.[478]

RBC stroma vesicles dissipate O_2^- through the anion channel into the surrounding medium.[479] RBC suspensions in glucose-containing phosphate-buffered saline liberate O_2^- at a rate of about $3 \times 10^{-9}\ M^{-1}\ sec^{-1}$ in reference to packed cells.[480] Escape of O_2^- through the anion channel may spare the RBC membrane from permeation of the bilipid layer by $HO_2^{\cdot}$, which is more reactive than O_2^- toward unsaturated fatty acids[416] and α-tocopherol.[481]

3. Autoxidation of Hemoglobin and Hemolysis

Autoxidation of hemoglobin[82] or myoglobin[83] produces O_2^-. Isolated α and β chains and unstable hemoglobins produce O_2^- at faster rates than hemoglobin A during autoxidation, and hemichrome formation occurs more rapidly.[482,483] SOD alone does not affect the autoxidation of hemoglobin.[82] It has an additional inhibitory effect of about 25% when present together with catalase.[483] SOD protects catalase from inactivation by O_2^-,[484] while catalase protects SOD against inactivation by H_2O_2 in RBC.[485] SOD inhibits Cu^{2+}-catalyzed oxidation of hemoglobin by about 35%.[483] On the other hand, it accelerates the autoxidation of oxymyoglobin.[83] Oxyhemoglobin[486] and oxymyoglobin[487] have been considered to autoxidize by a proton-catalyzed nucleophilic displacement of O_2^- from the active site. However, it appears that the deoxy rather than the oxy species is involved in the Fe^{2+} oxidation to Fe^{3+}, although the precise non-iron site for electron transfer from ferrous porphyrin to oxygen is unknown.[488]

Superoxide oxidizes oxyhemoglobin and reduces methemoglobin. The concentration of SOD in normal erythrocytes is sufficient to suppress these reactions.[489] However, H_2O_2 may compete with O_2^- for oxidation of oxyhemoglobin so that under conditions of excessive production of H_2O_2 SOD may have no effect[490] or may accelerate[491,492] the formation of methemoglobin and degradation of hemoglobin.

Oxidation of hemoglobin and peroxidation of stromal lipids are inextricable factors in hemolysis. Xanthine oxidase acting on acetaldehyde as substrate produces a slow oxidative attack on erythrocytes which causes precipitation of hemoglobin and hemolysis. SOD inhibits the hemolysis by about 50%, as measured by release of RBC catalase, but increases the precipitation of hemoglobin. In the presence of carbon monoxide, which prevents precipitation of hemoglobin, SOD inhibits the hemolysis by over 80% and a similar inhibition is observed with catalase, histidine, or mannitol.[476]

Inhibition of SOD by diethyldithiocarbamate sensitizes erythrocytes to oxidation of hemo-

globin by 1,4-naphthoquinone-2-sulfonate, which reacts with hemoglobin producing O_2^-, and hypotonic lysis occurs rapidly without lipid peroxidation.[493] Erythrocytes exposed to dihydroxyfumarate, which produces O_2^- by autoxidation, show an increase in hypotonic lysis after a brief lag during which hemoglobin is oxidized to methemoglobin and green pigment. Neither lipid peroxidation nor sulfhydryl oxidation is observed. SOD inhibits both hemoglobin degradation and hypotonic lysis. Carbon monoxide also inhibits the hemolysis.[494]

These studies adduce evidence for hemolysis either by peroxidation of stromal lipids[476] or by binding of insoluble breakdown products of hemoglobin to the cell membranes.[494] Clearly, O_2^- poses a double threat to the RBC membrane through degradation of hemoglobin and lipid peroxidation. The presence of hemoglobin modifies the reaction of O_2^- and related species with RBC membrane lipids. Lipid peroxidation of erythrocyte ghosts caused by the xanthine oxidase reaction in the presence of 2 μM hemoglobin is inhibited by SOD (or singlet oxygen scavengers) to the same extent as in white ghosts. In the presence of 10 μM hemoglobin, SOD (or singlet oxygen scavengers) have little effect, but catalase is strongly inhibitory.[495]

Hypotonic lysis of erythrocytes in the presence of paraquat is increased when intracellular SOD activity is increased as in trisomy 21 erythrocytes and is decreased when intracellular SOD activity is decreased as in erythrocytes treated with diethyldithiocarbomate. The hemolysis is inhibited by external SOD (and histidine) but not catalase.[496,497] This suggests two forms of oxidative attack on the RBC — from the inside of the cell membrane and from the outside.

Erythrocyte stroma vesicles containing xanthine oxidase lyse in the presence of acetaldehyde, as shown by release of ^{14}C-sucrose. Encapsulation of SOD inside the vesicles completely inhibits the lysis. External SOD is also effective.[498] Superoxide radicals produced in the RBC can attack the cell membrane either from the inside or the outside after traversing the anion channel.[479] Permeation of O_2^- through the anion channel appears to be a factor in methemoglobin formation and erythrocyte lysis in the presence of activated neutrophils.[499,500] Superoxide may be similarly involved in the hemolytic actions of *Streptococcus* D sp., although the bacteria also penetrate into the RBC.[501] Erythrocytes may act as scavengers of extracellular O_2^-.[479] They reduce chromosomal aberrations in human lymphoid cells caused by ionizing radiation and the cytotoxicity of enzymatically generated O_2^- toward Chinese hamster cells in vitro.[502]

Enzymatically generated O_2^- has been found to inactivate erythrocyte membrane Na^+,K^+ATPase leading to hemolysis.[503] This could be an effect of advance lipid peroxidation. Intracellular generation of O_2^- in human erythrocytes exposed to phenazine methosulfate causes an increase in passive K^+ permeability without any major effect on the Na^+,K^+ pump or the Na^+,K^+ cotransport system. This effect is markedly increased by inhibition of erythrocyte SOD by diethyldithiocarbomate and slightly increased by blockage of the anion channel. External SOD has a small protective effect. The K^+ leakage is associated with lipid peroxidation. There is also an increase of membrane-bound methemoglobin.[504]

Superoxide has been observed to increase membrane fluidity in human erythrocyte ghosts covalently labeled with a nitroxide spin trap. On the other hand, OH$^\cdot$ radicals were observed to decrease membrane fluidity as a result of lipid peroxidation.[506] Hebbel et al.[480] observed that hemichrome bound to RBC membranes facilitated the formation of OH$^\cdot$ radicals from O_2^- and H_2O_2. The resulting oxidant damage could be a determinant of RBC membrane senescence.

4. Radiation Hemolysis

Stone et al.[507] observed that preincubation of human erythrocytes with diethyldithiocarbamate without removal of the compound increased the radiosensitivity of the erythrocytes.

When the diethyldithiocarbamate is removed before irradiation is carried out, a small but significant additive effect is observed in the median glycerol hemolysis time. Residual diethyldithiocarbamate increases radiation hemolysis at a concentration of 10^{-2} *M*. Exogenous SOD plus catalase does not protect either control or diethyldithiocarbamate-pretreated cells against radiation hemolysis.[508] According to the findings of Inouye et al.,[509] exogenous SOD has, at most, only a small protective effect on the loss of intracellular K^+ prior to hemolysis in irradiated erythrocytes. Trisomy 21 erythrocytes are more resistant to hemolysis induced by photoactivated riboflavin but not more resistant to irradiation- or acetylphenylhydrazine-induced hemolysis than normal erythrocytes, and an oxygen effect is not observed in either trisomy 21 or normal erythrocytes.[510,511]

Kong and Davison[512] have delineated by means of scavengers various interactions among free radicals produced by ionizing radiation that either enhance or reduce damage in erythrocyte ghosts. In the presence of formate, which scavenged OH$^{\cdot}$, the resulting mixture of O_2^- and H_2O_2 increased membrane permeability by only 40% of the expected rate on the basis of additive effects. On the other hand, removal of either O_2^- (by SOD) or H_2O_2 (by catalase) restored the damaging potential of the remaining species. Protection against an increase in membrane permeability followed the order: formate (90%) > formate plus SOD (79%) > formate plus catalase (76%) > nitrogen plus formate (67%) > nitrogen (65%). The observed effect was therefore due to interaction of oxygen with e_{aq}^- and H$^{\cdot}$, producing O_2^-.

In irradiated rats, there is a marked decrease of SOD activity in bone marrow. However, in the surviving hemopoietic tissue, cellular SOD activity is sharply increased on the third day after irradiation. This represents a reparative process, as shown by subsequent increase of erythrocyte SOD activity together with reticulocytosis.[513,514] Treatment of mice with SOD before or after irradiation does not significantly affect the initial decrease of the RBC count but accelerates the subsequent erythropoiesis.[515]

5. Normal Erythrocyte SOD Activity

The SOD content of human erythrocytes measured as enzyme activity is about 500 μg/g Hb, with no sex or age variation, as found by Winterbourn et al.[516] and Michelson et al.[414] Saito et al.[517] found a similar value by radial immunodiffusion, although immunochemical methods and radioiummunoassay have both shown somewhat lower[518] and appreciably higher[519,520] values, respectively. It is generally considered best to express erythrocyte SOD activity per gram Hb as the reference parameter, although this is not ideal. In a normal population, the data of Michelson et al.[414] then shows microgram SOD per gram Hb = 727.7 − 20.8 × gram Hb/dℓ. SOD activity per gram Hb shows much less variation than other antioxidant enzymes in erythrocytes of different animals.[521]

6. Variation of Erythrocyte SOD Activity with Cell and Donor Age

In female B6D2F$_1$ mice, erythrocyte SOD activity per gram Hb does not change significantly with donor age.[522] Glass and Gershon[523] separated erythrocytes from WF Wistar rats into four fractions by density after removing most of the reticulocytes. Erythrocyte SOD activity decreased with increasing density in these fractions, while each fraction from old rats had less SOD activity and more catalytically inactive, antigenically reactive enzyme[524,525] than the corresponding fraction from young rats. The decrease in SOD activity with cell age in old rats was more striking when expressed on a per cell basis than per gram Hb. The decrease of mean corpuscular volume and mean corpuscular hemoglobin concentration with cell and donor age, which is found in rodents, can explain this result.[523] Erythrocytes from old rats were, on the average, younger (less dense) than those from young rats, as found by Tyan[522] in mice. This would tend to mask a decrease of SOD activity with donor age in total erythrocyte samples, if young cells have higher SOD activity than old cells.

In density-separated bovine erythrocytes, Bartosz et al.[526] observed a progressive decrease of erythrocyte SOD activity per gram Hb with cell density, down to about 65% of that of the least-dense cells. Quantitation of SOD by rocket immunoelectrophoresis showed that enzyme protein did not vary with cell density, indicating a loss of specific activity in the more dense erythrocytes.[527]

Subject to exclusion of experimental artifact, erythrocyte SOD activity does not appear to decrease with an increase of cell or donor age in humans. Michelson et al.[414] indicated that SOD activity did not vary with cell age in human erythrocytes. No variation in SOD activity per cell has been observed with human erythrocytes in the cell-age-dependent bands separable in a continuous Percoll gradient[528] and while no variation in SOD activity per gram Hb was observed in the density-related fractions of human erythrocytes separable in a discontinuous arabinogalactan gradient despite the presence of reticulocytes.[529] Furthermore, various studies have shown that erythrocyte SOD activity in humans does not change significantly with donor age from childhood to old age.[414,517,530-532]

In erythrocytes, a loss of specific activity appears to be related to the generation of one or two electromophs with raised anodic mobility.[380,533,534] A hypothesis of tissue-dependent, post-translational polymorphism of copper/zinc SOD has been put forward.[535,536]

7. Erythrocyte SOD Activity in Newborns

Various workers have reported no significant difference in erythrocyte SOD activity between maternal and cord blood. This holds when activity is expressed per gram Hb[414,537] or per milliliter blood.[538] When erythrocyte SOD activity is expressed per milligram nonhemoglobin protein, the data of Yoshioka et al.[538] show a significant decrease for erythrocytes from cord blood (-22%; $p<0.001$) compared to maternal blood. Bonta et al.[539] observed a decrease in erythrocyte SOD activity per milligram nonhemoglobin protein (-36%) in full-term compared to premature infants, implying an inverse relationship between RBC SOD activity and gestational age. Autor et al.[540] observed no significant difference in SOD activity of whole blood, expressed per mole heme, between premature and normal-term infants. On the other hand, the activity was significantly decreased with respect to infants (-27%; $p<0.001$), and in these it was significantly decreased (-15%; $p<0.001$) with respect to adults. This agrees with the observation[541] that adult erythrocyte SOD activity is reached at about 3 months postnatal age, while the activity in premature and full-term newborns can be about 33% lower than in adults. In contrast, the data of Rotilio et al.[542] and Bracci et al.[543] show no significant difference in erythrocyte SOD activity per gram Hb between premature or full-term infants (with or without hyperbilirubinemia) and adults.

Size-gestational age discrepancies may account for conflicting observations on RBC SOD activity in newborns. Saik et al.[544] have shown that RBC SOD content and activity do not differ significantly in adults and newborns of average size for gestational age, with the SOD content determined immunologically being about 700 μ/g Hb in both groups. However, erythrocyte SOD content is significantly lower (-17%; $p<0.05$) in newborns who are either small or large for gestational age with respect to average-sized newborns.

A factor which has not received detailed attention is that at birth the peripheral blood may contain a fair number of nucleated cells (and numerous reticulocytes). During differentiation of bone marrow erythroid cells in rabbits rendered anemic with phenylhydrazine, SOD activity per milligram protein increases in parallel with hemoglobin synthesis. It does not increase as proerythroblasts develop into early and intermediate normoblasts, then it increases by up to 49% in late normoblasts (orthochromatic erythroblasts). However, these cells have only 59% of the SOD activity of peripheral RBC.

The SOD activity per gram Hb in cord blood erythrocytes from normal-term infants can be slightly lower (-9%; $p<0.02$) than that of erythrocytes from normal adults, but when the activity is expressed on a per cell basis, no significant difference between the two is

found. The presence of normochromic, macrocytic erythrocytes with increased mean corpuscular hemoglobin in newborns was considered to explain this discrepancy.[546]

8. SOD Activity and Erythrocyte Copper

Copper/zinc SOD accounts for 40%,[547] if not more,[548] of the erythrocyte copper. The activity of copper/zinc SOD is sensitive to copper nutritional status in erythrocytes and in the liver of copper-deficient swine.[549] It is not sensitive to the zinc nutritional status.[550] A 50% decrease in erythrocyte SOD activity has been reported in a patient with copper deficiency,[551] but levels of human erythrocyte copper and SOD activity are not found to be significantly correlated.[552,553] In a group of patients with retinitis pigmentosa who had normal serum copper and zinc concentrations, erythrocyte SOD activity was normal.[554] A significant increase in erythrocyte SOD activity per gram Hb (+44%; $p<0.001$) has been observed in multiple sclerosis patients who may have serum zinc and copper deficiencies.[555]

Erythrocyte SOD has been thought to receive its copper from ceruloplasmin.[556,557] However, in Wilson's disease, normal erythrocyte SOD activity has been observed with a marked reduction or absence of serum ceruloplasmin (0 to 20% of normal).[558]

9. Erythrocyte SOD Activity in Various Diseases

Erythrocyte SOD activity has been measured in various specific disorders of RBC and some systemic disorders. Some results are considered below to present the scope of such measurements. The aim of most workers has been to discover oxygen free-radical pathologies via the level of activity of the enzyme in RBC. This is a valid approach and application of SOD activity measurements, but the meager and sometimes conflicting results which have been obtained suggest serious limitations, possibly imposed by the atypical nature of the mature RBC as a highly specialized terminal cell with considerable antioxidant reserves but with nonrenewable enzyme systems and one SOD isoenzyme.

a. Iron-Deficiency Anemia

In various forms of iron-deficiency anemia in infants (including prematures) and children, Panchenko et al.[559] found significant elevations of erythrocyte SOD activity per gram Hb (+43% to +67%; $p<0.001$) and on a per cell basis, which was considered to be related to an increased number of young erythrocytes in the blood. An increase of erythrocyte SOD activity in iron-deficiency anemia in children is confirmed in the data of Okahata et al.[560] on a per gram Hb basis (+29%; $p\simeq0.001$) but not on a per cell basis.

The view that reticulocytosis might be a factor in increased RBC SOD activity in peripheral blood has gained some ground. In rabbits rendered anemic by bleeding, erythrocyte SOD activity per gram Hb increases linearly with the percentage of reticulocytes. The observed regression indicates that reticulocytes have a 29% higher SOD activity than normocytes.[561] This may not apply to human reticulocytes. According to Frischer et al.,[439] human erythrocyte SOD activity per gram Hb in hemolytic anemias is not significantly altered by an average reticulocytosis of about 14%. In density-separated erythrocytes from a normal subject and a patient with marked reticulocytosis secondary to immune hemolytic anemia, SOD activity per gram Hb was about 16% higher in the least-dense than in the most-dense fraction in both cases, although reticulocytes in the least-dense fraction were 6% for the sample of normal RBC and 28% for the sample of hemolytic anemic RBC. Moreover, in favism patients in acute hemolytic crisis with about 10 to 20% reticulocytosis or higher, SOD activity per gram Hb was not correlated with the percentage of reticulocytes.[529]

b. Oxidative Hemolytic Anemia

Evidence that oxidative hemolytic anemia is a result of increased autoxidation of hemoglobin or impairment of erythrocyte SOD activity has not been readily forthcoming. The

deficiency of SOD as an inborn error of metabolism is not known. Developmental or acquired deficiency of SOD is not clearly connected with hemolytic anemia. Patients with Hodgkin's disease or myeloma receiving polychemotherapy including nitrogen-mustard derivative show a depression of SOD activity (-20%; $p<0.001$) per unit volume of RBC. If the depression is severe (-56%), it is associated with intravascular hemolysis and markedly increased sensitivity of the RBC to hemolysis by 1,4-naphthoquinone-2-sulfonate in vitro;[562] but where there is no iatrongenic factor, there is little evidence relating intravascular hemolysis to acquired decrease of erythrocyte SOD activity.

No correlation is evident between serum bilirubin concentration and erythrocyte SOD activity in neonates with hyperbilirubinemia[542,543,563] which tends to exclude erythrocyte SOD deficiency as a cause of neonatal hemolytic anemia. In a case of hemolytic-uremic syndrome in an infant, erythrocyte SOD activity per gram Hb was markedly decreased (up to -55%) with respect to an age control value.[564] Loss of erythrocyte SOD in the peripheral circulation could aggravate the microangiopathic hemolytic anemia in this condition. An increase in erythrocyte SOD activity per gram Hb ($+24\%$; $p<0.001$) has been observed in favism patients during acute hemolytic crisis. Incubation of normal RBC with divicine plus ascorbate (as a promoter of oxygen free-radical reactions) did not significantly affect their SOD activity but the cells hemolyzed when returned to homologous plasma.[529]

c. Thalassemia

Concetti et al.[565] found no significant change in SOD activity per gram Hb or per cell in erythrocytes from patients with β-thalassemia major and β-thalassemia intermedia even though such cells are stressed by the presence of free α chains, increased quantities of iron, and increased peroxidation of membrane lipids. The β-thalassemia major patients had been splenectomized and had received their last blood transfusion 14 to 96 days previously. Gerli et al.[566,567] observed no significant change in erythrocyte SOD activity in patients with β-thalassemia major and attributed this to the presence of normal RBC because of multiple transfusions. A small increase in erythrocyte SOD activity per gram Hb ($+11\%$; $p<0.02$) was observed in patients with β-thalassemia minor,[566] while the observations of Gerli et al.[567] show a small increase ($+11\%$; $p<0.05$) per unit volume of RBC. In untransfused homozygous β-thalassemic children with 6 to 8.5 g Hb/dℓ, Vanella et al.[568] observed a significant decrease (-53%; $p<0.001$) of SOD activity per milligram protein in ethanol-chloroform extracts of the erythrocytes. In patients with β^o-thalassemia/Hb E disease who had not received recent transfusions, Yenchitsomanus and Wasi[569] observed a significant increase ($+71\%$; $p<0.001$) of erythrocyte SOD activity per gram Hb, which was also significant ($+33\%$; $p<0.01$) on a per cell basis. The SOD activity in splenectomized patients did not differ from that in nonsplenectomized patients, although the former had numerous nucleated RBC. Furthermore, significantly higher ($+21\%$: $p<0.001$) SOD activity per gram Hb was associated with the more severe form of the disease. In patients with Hb H disease, with the α-thalassemia 1/α-thalassemia 2 or α-thalassemia 1/Hb Constant Spring genotype, erythrocyte SOD activity appeared to be significantly increased on a per gram Hb ($+140\%$; $p<0.001$) or per cell ($+68\%$: $p<0.001$) basis. Winterbourn et al.[483] found that isolated β chains autoxidized somewhat more rapidly than α chains. This implies a higher oxidative stress in α- than in β-thalassemic erythrocytes. This might help to explain the higher erythrocyte SOD activity observed by Yenchitsomanus and Wasi[569] in Hb H disease compared to β^0-thalassemia/Hb E disease, which was significant on a per gram Hb ($+40\%$; $p<0.001$) or per cell ($+27\%$; $p<0.02$) basis.

d. Sickle Cell Anemia

In sickle cell anemia, erythrocyte SOD activity is significantly increased on a per gram Hb ($+69\%$; $p<0.001$) or per cell ($+78\%$; $p<0.001$) basis. Sickle erythrocytes also show

decreased catalase and glutathione peroxidase activity, abnormalities of membrane lipid composition, increased lipid peroxidation, and inclusion bodies.[570] Das and Nair[570] point out that sickled erythrocytes tend to show an increased amount of copper. This can augment O_2^- production by catalyzing the autoxidation of hemoglobin[483] and by reacting with cell membrane thiol groups.[571] Chan et al.[572] have shown Cu^{2+} to be uniquely capable of catalyzing the peroxidation of rat erythrocyte membrane lipids in the presence of H_2O_2, which is likely to have an elevated concentration in sickled erythrocytes.

Beratta et al.[573] reported increased SOD activity in RBC from sickle cell-strain individuals. However, in a study done with a stringent matching of controls, Schacter et al.[574] found significantly decreased SOD activity (−11% in mild to −48% in severe cases; $p<0.001$) and an indication of essentially unchanged malonyldialdehyde formation in RBC from patients with sickle cell anemia. Furthermore, the severity of symptoms was observed to vary inversely with RBC SOD activity. This suggests increased levels of oxy radicals,[480,575] and the observation of essentially unchanged malonyldialdehyde formation in the RBC requires confirmation.

Sickled erythrocytes liberate significantly more O_2^- (+132%; $p<0.001$), H_2O_2 (+275%; $p<0.001$), and $OH^\cdot$ (+82%; $p<0.001$) radicals than normal erythrocytes when incubated in phosphate-buffered saline with glucose, and they have a marked increase in membrane-bound hemochrome (+604%), which enhances the production of $OH^\cdot$ radicals in the presence of O_2^- and H_2O_2.[480] A decreased enzymatic elimination of O_2^- is possible in sickled erythrocytes, despite an increased SOD content, because of the diffusion control of the SOD reaction and the rise in the microviscosity of the erythrocytes.

e. Malarial Parasitemia

Asexual blood forms of malaria parasites are microaerophilic and sensitive to oxidant stress, while inherited traits, such as sickle cell trait and glucose-6-phosphate dehydrogenase deficiency, and acquired cell-mediated immunity both subject malaria parasites to oxidant stress.[576] An i.v. injection of alloxan into mice infected with *Plasmodium vinckei* decreases the parasitemia. This effect is prevented by a prior injection of desferrioxamine or diethyldithiocarbamate. Alloxan also produces a transient hemolysis in malaria-infected mice which is blocked by desferrioxamine. It is conceivable that parasite death and hemolysis are mediated by $OH^\cdot$ radicals[577] or a similarly reactive metal-oxygen complex.

In erythrocytes of mice infected with *P. berghei*, showing over 40% parasitemia, there is a 48% increase in SOD activity on a per gram Hb or per cell basis, which is significant ($p=0.05$) on a per cell basis. Isolated parasites contain SOD.[577] Fairfield et al.[212] showed that the SOD of malaria parasites is derived from the host cell cytoplasm. Copper/zinc SOD activity is decreased in the liver of mice infected with *P. berghei* while the manganese SOD activity is unchanged.[579] *P. falciparum* does not multiply well in human erythrocytes bearing Hb S,[576] presumably because the intracellular environment is one of considerable oxidant stress, as already seen, even at low oxygen tension.

f. Fanconi's Anemia

Considerable attention has been given to the question of SOD activity in Fanconi's anemia since the deficiency might relate O_2^- to cytogenetic oxygen toxicity in this and other chromosome-breakage syndromes.[580] Nordenson[581] reported that exogenous SOD or catalase reduced the incidence of spontaneous chromosome breakage in cultured lymphocytes from patients with Fanconi's anemia, while Raj and Heddle[582] found that the effect of exogenous SOD in reducing the frequency of micronuclei was similar in normal and Fanconi's anemia fibroblasts for both spontaneous and mitomycin-C-induced chromosome breakage in cell culture. At mitomycin C concentrations which produce cell killing, fibroblast cell strains from patients with Fanconi's anemia or dyskeratosis congenita (a related disorder) show

increased cytotoxicity compared to normal fibroblasts, and enhancement of survival is produced by exogenous SOD in the cell cultures. This was about 15-fold in the most mitomycin-C-sensitive strain of Fanconi's anemia fibroblasts. On the other hand, SOD did not increase survival in mitomycin-C-treated normal fibroblasts at survival levels down to 1 in 1000.[583] The question of how clastogenic and cytotoxic effects,[584] presumably mediated by DNA damage, may be influenced by exogenous SOD is not clear.

It has been shown that the frequency of chromosomal aberrations in Fanconi's anemia lymphocyte cultures increases markedly with oxygen tension,[585] therefore it is meaningful to investigate SOD activity in this disorder. Most other workers have agreed that erythrocyte SOD activity can be significantly reduced in Fanconi's anemia.[560,586,587] In seven patients investigated by Joenje et al.,[586] erythrocyte SOD activity per gram Hb was decreased by 27% ($p<0.001$). In three patients of Okahata et al.,[560] mean erythrocyte SOD activity per gram Hb was decreased by 55% ($p<0.001$). The mean erythrocyte SOD activity per gram Hb in three patients studied by Mavelli et al.[587] was decreased by 22% ($p<0.01$) when expressed per gram Hb and by 37% ($p<0.02$) when expressed per unit of erythrocyte lactate dehydrogenase.

g. Muscular Dystrophy and Cystic Fibrosis

The SOD activity of rat cardiac or skeletal muscle does not change after starvation or refeeding, therefore SOD cannot be considered a marker enzyme of muscle breakdown.[588] Skeletal muscle from patients with muscular dystrophies has the same SOD activity as control specimens, although an increase of spontaneous lipid peroxidation is observed.[589] That SOD activity may increase in muscular dystrophy is reported for chickens.[590] Hunter et al.[591] found no change in erythrocyte SOD activity per gram Hb in a group of Duchenne muscular dystrophy patients 12 to 20 years of age, although a significant decrease (-19%; $p<0.01$) per milliliter packed RBC was reported by Burri et al.[592] in a group of patients 2 to 21 years of age. Matkovics et al.[593] found a large increase ($+463\%$; $p<0.001$) in the SOD activity per gram protein of ethanol-chloroform extract of erythrocytes from a group of Duchenne muscular dystrophy patients 6 to 12 years of age. The reason for these discrepancies is not clear. The SOD activity measured by Matkovics et al.[593] was determined as a percentage of inhibition of epinephrine autoxidation, as in the work of Burri et al.[592] This method has also shown an increase in erythrocyte SOD activity ($+43\%$; $p<0.001$) in children with cystic fibrosis.[594] However, determination of erythrocyte SOD activity per gram Hb in cystic fibrosis children as a percentage of inhibition of nitroblue tetrazolium reduction by photochemically produced O_2^- showed no significant difference ($+6\%$; $p<0.05$) with respect to controls.[595]

h. Metabolic Insults

Erythrocyte SOD activity per gram Hb is increased in uremia associated with acute ($+62\%$; $p<0.05$) or chronic renal failure ($+75\%$; $p<0.05$) and returns to normal after adequate dialysis or renal transplantation. This was interpreted as an adaptive increase to the metabolic insult of uremia.[596] This is an interesting concept, although it also seems that augmented erythrocyte SOD activity can occur in normal adults, possibly as a result of increased environmental or nutritional oxidative stress or both, and that the response may shown ethnic differences.[414]

A significant increase of erythrocyte SOD content per milligram Hb ($+18\%$; $p<0.001$), determined by radioimmunoassay, has been observed in black compared to white alcoholics.[597] Erythrocyte SOD activity was on the lower side of the normal range in rats ingesting ethanol, and the RBC showed a substantially increased amount of lipid hydroperoxides.[598]

In the liver of chronically ethanol-treated rats, there appears to be an increased microsomal production of O_2 which is not compensated for by an increase of cytosolic SOD.[599] There

is, however, an increase of manganese SOD activity in the liver and kidneys of rats after chronic ingestion of ethanol.[600]

Erythrocyte SOD activity per gram Hb is significantly increased (+25%; $p<0.01$) in acatalasemia. Erythrocyte glutathione peroxidase activity is not significantly elevated. However, acatalasemia is asymptomatic, except for a tendency to develop oral ulcerations in certain cases.[601]

In patients with chronic hypoxia and polycythemia (chronic cor pulmonale), Gerli et al.[567] observed a tendency to an elevation of SOD activity per unit volume of RBC (+13%; $p\leq0.05$). This was interpreted as a response to low arterial oxygen tension and acidosis. Erythrocyte SOD activity per gram Hb increases in polycythemia vera (+32%).[414]

Although Michelson et al.[414] observed normal erythrocyte SOD activity in two cases of porphyria of unspecified type, the data of Medeiros et al.[602] show a 33% increase of erythrocyte SOD activity per gram Hb ($p<0.001$) in asymptomatic intermittent acute porphyria carriers, with a further increase (+50%; $p<0.001$) in patients undergoing acute crisis.

Hagglof et al.[603] found a small but significant decrease in erythrocyte SOD activity per gram Hb (−11%; $p<0.001$) in controlled juvenile diabetics. This finding requires further exploration.

D. Superoxide and SOD in Inflammatory Disease

1. Introduction

Production of O_2^- by granulocytes and macrophages is well established. Activated phagocytes release O_2^- into phagosomes and also into the surrounding medium. Release of O_2^- into the extracellular fluid is potentially deleterious to host tissue, particularly since higher organisms have evolved with little SOD in their extracellular fluids,[240] whereas the intracellular environment is amply provided with SOD. Intraphagosomal release of O_2^- is regarded as a component of the mechanism of killing ingested bacteria by polymorphonuclear neutrophils. Extracellular release of O_2^- is seen as a component of the defense mechanism against foreign cells which are killed without engulfment and also as a mechanism for generation of mediators of the inflammatory process. Release of O_2^- probably also initiates the destruction of the phagocytes themselves. In chronic phagocyte-mediated inflammatory processes, O_2^- and related species probably damage host cells, connective tissues, and ground substance. Furthermore, promotion of formation of aggregates of IgG with properties of immune complexes by these radicals may provide a pathway to autoimmune disease. Several reviews of the mechanism of O_2^- production by phagocytic cells and its consequences are available.[604-609]

2. Antiinflammatory Activity of SOD

Antiinflammatory activity of bovine copper/zinc SOD (protein) was discovered without the knowledge of its enzymatic activity. The effect has been observed in several animal models of inflammation.[610] Multiple i.v. injections of SOD inhibit the prostaglandin phase of carrageenan-induced foot edema in normal rats, in which neutrophils comprise about 70% of inflammatory cells at the site of carrageenan injection, and in agranulocytic rats, in which the inflammatory cells are almost entirely mononuclear phagocytes.[611] McCord et al.[612] obtained inhibition of carrageenan-induced edema and the reverse passive Arthus reaction, which is mediated by neutrophils, with i.v. Ficoll-coupled SOD but not with underivatized SOD. According to Huber et al.,[613] underivatized SOD is effective in the carrageenan and Arthus models of inflammation by any parenteral route of administration and has long-lasting antiinflammatory efficacy observable when the serum level has fallen to 10 ng/mℓ (0.3 nM) after administration of the enzyme. It is possible that the carrageenan model is sensitive to nonspecific effects difficult to control. Hirschelmann and Bekemeier[614] observed inhibition of carrageenan-induced edema with native or heat-treated (10 min at 100°C) SOD injected

intravenously. Native SOD injected locally with the carrageenan did not significantly inhibit edema formation. Granuloma formation in rats, induced by subdermal implantation of carrageenan-soaked sponges and measured after 7 days, was not inhibited by local injection of SOD at the moment of sponge implantation. However, SOD potentiated an inhibitory effect of catalase.[615]

Exogenous bovine copper/zinc SOD is removed from circulation in the renal cortex and its plasma half-life is of the order of a few minutes.[610] The enzyme has been conjugated with Ficoll,[616] polyethylene glycols (PEG),[617-620] or homologous albumin[621] to delay its plasma clearance and reduce its immunogenicity and antigenicity.[621,622] I.v. or i.p. PEG-SOD appeared to be superior to underivatized SOD in reducing granuloma formation due to implantation of cotton twine in rats,[617] while i.v. albumin-SOD appeared to be superior to native SOD or Ficoll-SOD in the carrageenan model, and an increase in radioactivity in inflamed paws, compared to controls, after i.v. injection of ^{125}I-labeled albumin-SOD was demonstrated.[621] Bovine serum albumin,[611] human serum albumin,[614] and homologous albumin-albumin conjugates[621] are not antiinflammatory in the carrageenan model, although Cleland et al.[623] have maintained that homologous albumin-albumin and albumin-SOD conjugates have equipotent antiinflammatory activities in this model.

Antiinflammatory activity of copper/zinc SOD is not specific to bovine enzyme. Guinea pig copper/zinc SOD is as active against the reverse passive Arthus reaction in guinea pigs as bovine copper/zinc SOD.[624] Baret et al.[625] have shown that human copper/zinc SOD injected intraperitoneally is antiinflammatory in the carrageenan model in the rat, acting during both the serotonin and prostaglandin phases of edema formation. On the other hand, human manganese SOD suppressed only the serotonin phase and high doses briefly augmented the prostaglandin phase of edema formation. These effects were observed with plasma levels of exogeneous copper/zinc SOD below the endogeneous copper/zinc SOD level of 248 ng/mℓ (7.5 n*M*) and plasma levels of exogeneous manganese SOD in the range 1 to 10 μg/mℓ (10 to 100 n*M*), with endogenous manganese SOD being undetectable in rat plasma.

Pharmacological observations and clinical studies suggest that the antiinflammatory efficacy of copper/zinc SOD does not depend on the bioavailability of native enzyme after systemic administration. The persistent effect observed with antiinflammatory doses after the enzyme has been eliminated from the circulation in animal models of inflammation and in double-blind, placebo-controlled trials in patients with rheumatoid arthritis of active ostroarthritis[424] has no obvious explanation. An i.p. injection of SOD produces in rats a neutrophil leukocytosis with a time course of 24 hr, with an increase in immature forms indicating mobilization of neutrophils from the bone marrow into the circulation,[610,624] but the significance of this observation is not clear. SOD is not chemotactic in vitro.[610] There are no observations on in vitro responses of neutrophils from experimental animals or patients receiving SOD.

Production of O_2^- by activated neutrophils from patients with rheumatoid arthritis receiving steroids or nonsteroidal antiinflammatory drugs (although not SOD) was not significantly depressed, but significant depression was observed in patients with Felty's syndrome of chronic arthritis and splenic neutropenia.[626] SOD activity is similar in neutrophils from normal infants, their mothers, and controls.[627] Neutrophils from children with rheumatoid arthritis show significantly decreased SOD activity. On activation, they show significantly increased production of O_2^-.[628,629] Exogenous SOD maintains the viability of phagocytosing neutrophils incubated with *E. coli*.[630]

SOD appears to inhibit the migration of neutrophils to sites of inflammatory challenge. Activation of a plasma component by O_2^- appears to generate a neutrophil chemotactic factor, probably by reduction of a preexisting hydroperoxy fatty acid bound to serum albumin.[616] All potent leukotaxins, including *N*-formyl-methionyl-leucyl-phenylalanine (FMLP), the complement component C5a, and leukotriene B_4, cause both leukotaxis and O_2^- pro-

duction and release by polymorphonuclear neutrophils. Leukotriene-B_4-induced leukotaxis is inhibited by SOD or catalase. The generation of oxy radicals appears to be a necessary but not a sufficient prerequisite of leukotaxis as not all compounds triggering the respiratory burst of polymorphonuclear neutrophils are also leukotactic.[631,632] SOD inhibits prostaglandin production by phagocytosing neutrophils in a dose-dependent manner. The inhibitory effect is reversed by arachidonic acid, suggesting that the antiinflammatory properties of SOD may depend to some extent on inhibition of phospholipase activation.[633]

A s.c. injection of SOD inhibits paw edema in mice produced by injection of serotonin, histamine, or kinins. This action of SOD is antagonized by inhibitors of protein synthesis. It is similar to the action of glucocorticoids (dexamethasone), but it is not blocked by progesterone.[634] Increase of serotonin-induced paw edema by diethyldithiocarbamate is antagonized by SOD or dexamethasone. It seems that SOD protects a protein which regulates vascular permeability (vasoregulin) from inactivation by O_2^-, while glucocorticoids stimulate the synthesis of this protein.[635,636]

An increase in vascular permeability is an early event in inflammation. Del Maestro et al.[637,638] have shown that oxy radicals increase capillary permeability, possibly by interaction of $OH^\cdot$ with plasmalemmal lipids. Superoxide may generate a lipid hydroperoxide responsible for alterations of leukocyte behavior, leading to an increased adhesion to the endothelium.[639,640] Sustained vasodilation in rat intestinal mesentery, produced by the xanthine oxidase reaction, was reversed by SOD at pH 7.4 and by SOD plus mannitol at pH 6.6, so that it was attributed to O_2^- at physiological pH and to $OH^\cdot$ derived from O_2^- at low pH.[641] Postburn edema in rat hind paws is significantly reduced by pretreatment with PEG-SOD, PEG-catalase, or PEG-albumin, although PEG-SOD was shown to more effective than PEG-albumin.[642]

In the reverse passive Arthus reaction, local injection of SOD suppresses the early phase of vascular injury in skin, as quantitated by leakage of ^{125}I-homologous albumin. The enzyme markedly suppresses the influx of neutrophils but does not appear to block either the deposition of immune complexes or their ability to fix complement at the reaction site. Similar results are obtained with an i.p. injection of SOD in rat lung undergoing acute immune-complex-induced alveolitis. The inability of SOD to suppress lysosomal enzyme release in tripeptide-activated neutrophils, except at very high doses, suggests that exogenous SOD acts by intercepting O_2^-.[643] That complement-stimulated granulocytes may damage endothelial cells as a result of release of O_2^- was suggested by Sacks et al.[644] The resulting exposure of the basement membrane may activate Hagemen factor with diverse effects.[643]

3. Rheumatoid Arthritis as an Example of Superoxide-Related Pathology

Rheumatoid arthritis is the clinical model, *par excellence*, of chronic phagocyte-mediated inflammation, with cellular immune reactions in synovial tissue and a continuous interaction of granulocytes with immune complexes in synovial fluid. It is reasonable to implicate O_2^- in the tissue injury. Depolymerization of the synovial fluid hyaluronic acid by the products of the xanthine oxidase reaction is well established. It is inhibited by either SOD or catalase, being probably due to $OH^\cdot$ radicals derived from O_2^- and H_2O_2 in the presence of metal catalysts.[645-647] Hyaluronic acid becomes more susceptible to further degradation by β-*N*-acetylglucosaminidase A after attack by oxy radicals produced by activated neutrophils or the xanthine oxidase reaction.[645] McCord[645] postulated that O_2^- produced by invading neutrophils could account for the observed deterioration of synovial fluid in inflammatory arthritis. An oxy radical rather than an enzymic mechanism is supported by the observation that synovial fluid samples from patients with various inflammatory arthritides do not alter the viscosity of pure hyaluronic acid.[649]

This reasoning can be extended to degradation of cartilage since O_2^- attacks collagen and proteoglycans.[650,651] Furthermore, O_2^- inhibits antiproteases,[652] while cartilaginous wear par-

ticles cause the release of neutral proteinases from mononuclear phagocytes and synovial cells active against collagen and other substrates.[653]

Blake et al.[654] did not find increased SOD activity in synovial fluid from patients with rheumatoid arthritis. Igari et al.[655] found a large increase of SOD activity per milligram protein (+444%; $p<0.01$) with respect to normal serum, but this requires confirmation. Serum SOD activity itself was only slightly increased. The synovial fluid SOD activity tended to increase with the severity of the rheumatoid arthritis. Banford et al.[553] found that erythrocyte SOD activity showed a low but significant negative correlation with the articular index in rheumatoid arthritis and appeared to be an indicator of disease response. Intra-articular injection of SOD is as clinically effective in active rheumatoid arthritis[656] as it is in active osteoarthritis.[657] However, proof or disproof of the hypothesis of superoxide-related pathology in rheumatoid arthritis awaits a means of measuring the O_2^- radicals in vivo.

E. Superoxide and SOD in Ischemic Injury

1. Introduction

Ischemic injury in the small intestine has been shown to be due to reperfusion injury associated with conversion of xanthine dehydrogenase to xanthine oxidase during the period of ischemia and production of O_2^- during the period of reperfusion. This concept of superoxide-related pathology may apply to other tissues.

2. Ischemic Injury in the Small Intestine

The vascular endothelial and mucosal cell damage which occurs on reperfusion of ischemic small intestine is due to liberation of O_2^-. This is suggested by the protective effect of exogenous SOD and the rapid, irreversible conversion of xanthine dehydrogenase to xanthine oxidase in ischemic intestine. Allopurinol, which inhibits xanthine oxidase, is as effective as SOD in preventing an increase in capillary permeability and mucosal lesions.[658-660] Tissue damage on reperfusion is enhanced by aminophylline, a xanthine derivative that might generate O_2^-.[661] Intraluminal perfusion of the intestine with hypoxanthine plus xanthine oxidase to generate O_2^- increases the permeability of the mucosa to albumin. This is prevented by SOD.[662] Local intra-arterial infusion of hypoxanthine produces an increase in capillary permeability in small intestine, which is prevented by SOD.[663] There is no change of endogenous SOD activity in ischemic intestine. Hypoxanthine accumulates as a result of ATP catabolism.[664]

The administration of SOD prevents sinking of mean arterial pressure on reperfusion of ischemic small intestine in the cat. In dogs subjected to hemorrhagic shock treatment, SOD prevents intestinal shunting and hemorrhagic lesions in the gut on restoration of the blood volume but does not affect survival rate.[664]

3. Ischemic Injury in Other Tissues

Xanthine dehydrogenase is converted to xanthine oxidase in other ischemic tissues except skeletal muscle. However, the rate of conversion is not as fast as in intestine and may be reversible.[660] Although most tissues contain xanthine dehydrogenase to a lesser extent than intestinal mucosa, they can in principle suffer the same type of O_2^--dependent postischemic tissue damage as intestine.

Superoxide may be involved in ischemic damage to the myocardium. This is a developing concept.[663,665,666] Hypoxic cardiac perfusion decreases myocardial SOD activity, and the loss continues acutely during oxygen readmission.[667] Supplementation of hypothermic cardioplegia solution with SOD plus catalase significantly enhances the protection of the globally ischemic, reperfused heart, as shown by various indexes of myocardial metabolism and contractility.[668]

ADDENDUM

Since the submission of this review, manganese SOD has been demonstrated to be absent from plant chloroplasts.[669] A complete amino acid sequence has been published for the iron SOD from *P. leiognathi*.[670]

REFERENCES

1. **Mann, T. and Keilin, D.,** Haemocuprein and hepatocuprein, copper-protein compounds of blood and liver in mammals, *Proc. R. Soc. London Ser. B*, 128, 303, 1939.
2. **McCord, J. M. and Fridovich, I.,** Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein), *J. Biol. Chem.*, 244, 6049, 1969.
3. **Fridovich, I.,** The discovery of superoxide dismutases: a history, in *Superoxide Dismutase*, Vol. I, Oberley, L. W., Ed., CRC Press, Boca Raton, Fla., 1983, 2.
4. **Porter H. and Folch, J.,** Cerebrocuprein. I. A copper-containing protein isolated from brain, *J. Neurochem.*, 1, 260, 1957.
5. **Porter H. and Ainsworth, S.,** The isolation of the copper-containing protein cerebrocuprein I from normal human brain, *J. Neurochem.*, 5, 91, 1959.
6. **Porter, H., Sweeney, M., and Porter, E. M.,** Human hepatocuprein. Isolation of a copper protein from the subcellular soluble fraction of adult human liver, *Arch. Biochem. Biophys.*, 105, 319, 1964.
7. **Markowitz, H., Cartwright, G. E., and Wintrobe, M. M.,** Studies on copper metabolism. XXVII. The isolation and properties of an erythrocyte cuproprotein (erythrocuprein), *J. Biol. Chem.*, 234, 40, 1959.
8. **Carrico, R. J. and Deutsch, H. F.,** Isolation of human hepatocuprein and cerebrocuprein. Their identity with erythrocuprein, *J. Biol. Chem.*, 244, 6087, 1969.
9. **Carrico, R. J. and Deutsch, H. F.,** The presence of zinc in human cytocuprein and some properties of the apoprotein, *J. Biol. Chem.*, 245, 723, 1970.
10. **Keele, B. B., Jr., McCord, J. M., and Fridovich, I.,** Superoxide dismutase from *Escherichia coli* B. A new manganese-containing enzyme, *J. Biol. Chem.*, 245, 6176, 1970.
11. **Yost, F. J., Jr. and Fridovich, I.,** An iron-containing superoxide dismutase from *Escherichia coli*, *J. Biol. Chem.*, 248, 4905, 1973.
12. **Weisiger, R. A. and Fridovich, I.,** Superoxide dimutase. Organelle specificity, *J. Biol. Chem.*, 248, 3582, 1973.
13. **Fridovich, I.,** Superoxide dismutases, *Adv. Enzymol.*, 41, 35, 1974.
14. **Symonyan, M. A. and Nalbandyan, R. M.,** Interaction of hydrogen peroxide with superoxide dismutase from erythrocytes, *FEBS Lett.*, 28, 22, 1972.
15. **Bray, R. C., Cockle, S. A., Fielden, E. M., Roberts, P. B., Rotilio, G., and Calabrese, L.,** Reduction and inactivation of superoxide dismutase by hydrogen peroxide, *Biochem. J.*, 139, 43, 1974.
16. **Hodgson, E. K. and Fridovich, I.,** The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of the enzyme, *Biochemistry*, 14, 5294, 1975.
17. **Misra, H. P. and Fridovich, I.,** Inhibition of superoxide dismutase by azide, *Arch. Biochem. Biophys.*, 189, 317, 1978.
18. **Asada, K., Kanematsu, S., Okada, S., and Hayakawa, T.,** Phylogenetic distribution of three types of superoxide dismutase in organisms and in cell organelles, in *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase*, Dev. Biochem., 11A, Bannister, J. V. and Hill, H. A. O., Eds., Elsevier, New York, 1980, 136.
19. **Kirby, T., Blum, J., Kahane, I., and Fridovich, I.,** Distinguishing between Mn containing and Fe containing superoxide dismutases in crude extracts of cells, *Arch. Biochem. Biophys.*, 201, 551, 1980.
20. **Gregory, E. M. and Dapper, C. H.,** Isolation of iron-containing superoxide dismutase from *Bacteriodes fragilis:* reconstitution as a Mn-containing enzyme, *Arch. Biochem. Biophys.*, 220, 293, 1983.
21. **Geller, B. L. and Winge, D. R.,** A method for distinguishing Cu, Zn- and Mn- containing superoxide dismutases, *Anal. Biochem.*, 128, 86, 1985.
22. **Michelson, A. M., McCord, J. M., and Fridovich, I., Eds.,** *Superoxide and Superoxide Dismutases*, Academic Press, New York, 1977, 569.
23. **Hayaishi, O. and Asada, K., Eds.,** *Biochemical and Medical Aspects of Active Oxygen*, Japan Scientific Societies Press, Tokyo, 1977, 200.
24. **Bannister, J. V. and Hill, H. A. O., Eds.,** *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase*, Dev. Biochem., 11A, Elsevier, New York, 1980, 411.

25. **Bannister, W. H. and Bannister, J. V., Eds.,** *Biological and Clinical Aspects of Superoxide and Superoxide Dismutase,* Dev. Biochem., 11B, Elsevier, New York, 1980.
26. **Rodgers, M. A. J. and Powers, E. L., Eds.,** *Oxygen and Oxy-Radicals in Chemistry and Biology,* Academic Press, New York, 1981, 808.
27. **Oberley, L. W., Ed.,** *Superoxide Dismutase,* Vol. I, CRC Press, Boca Raton, Fla., 1983, 152.
28. **Oberley, L. W., Ed.,** *Superoxide Dismutase,* Vol. II, CRC Press, Boca Raton, Fla., 1983, 177.
29. **Cohen, G. and Greenwald, R. A., Eds.,** *Oxy-Radicals and Their Scavenging Systems, Molecular Aspects,* Vol. I, Elsevier, New York, 1983, 399.
30. **Greenwald, R. A. and Cohen, G., Eds.,** *Oxy-Radicals and Their Scavenging Systems, Cellular and Medical Aspects,* Vol. II, Elsevier, New York, 1983, 420.
31. **Rotilio, G. and Bannister, J. V., Eds.,** Oxidative damage and related enzymes, *Life Chem. Rep.,* Suppl. 2, 436, 1984.
32. **Bors, W., Saran, M., and Tait, D., Eds.,** *Oxygen Radicals in Chemistry and Biology,* William de Gruyter, Berlin, 1984, 1029.
33. **Bannister, J. V. and Bannister, W. H., Eds.,** *The Biology and Chemistry of Active Oxygen,* Dev. Biochem., 26, Elsevier, New York, 1984, 262.
34. **Halliwell, B., O'Neill, P., and Bannister, J. W., Eds.,** Free radicals in biology and medicine, *Life Chem. Rep.,* 3, 1, 1985.
35. **Halliwell, B. and Gutteridge, J. M. C.,** *Free Radicals in Biology and Medicine,* Clarendon Press, Oxford, 1985, 346.
36. **Rotilio, G.,** *Superoxide and Superoxide Dismutase in Chemistry, Biology, and Medicine,* Elsevier, Amsterdam, 1986, 688.
37. **Greenwald, R.,** *Handbook of Methods for Oxygen Radical Research,* CRC Press, Boca Raton, Fla, 1985, 447.
38. **Knowles, P. F., Gibson, J. F., Pick, F. M., and Bray, R. C.,** Electron-spin-resonance evidence for enzymic reduction of oxygen to a free radical, the superoxide ion, *Biochem. J.,* 11, 53, 1969.
39. **Ballou, D., Palmer, G., and Massey, V.,** Direct demonstration of superoxide anion production during the oxidation of reduced flavin and of its catalytic decomposition by erythrocuprein, *Biochem. Biophys. Res. Commun.,* 36, 898, 1969.
40. **Marklund, S.,** Spectrophotometric study of spontaneous disproportionation of superoxide anion radical and sensitive direct assay for superoxide dismutase, *J. Biol. Chem.,* 251, 7504, 1976.
41. **McClune, G. J. and Fee, J. A.,** Stopped flow spectrophotometric observation of superoxide dismutation in aqueous solution, *FEBS Lett.,* 67, 294, 1976.
42. **Takahashi, M. and Asada, K.,** A flash photometric method for determination of reactivity of superoxide: application to superoxide dismutase assay, *J. Biochem.,* 91, 889, 1982.
43. **Klug-Roth, D., Fridovich, I., and Rabani, J.,** Pulse radiolytic investigations of superoxide catalyzed disproportionation. Mechanism for bovine superoxide dismutase, *J. Am. Chem. Soc.,* 95, 2786, 1973.
44. **Fielden, E. M., Roberts, P. B., Bray, R. C., Lowe, D. J., Mautner, G. N., Rotilio, G., and Calabrese, L.,** The mechanism of action of superoxide dismutase from pulse radiolysis and electron paramagnetic resonance, *Biochem. J.,* 139, 499, 1974.
45. **Pick, M., Rabani, J., Yost, F., and Fridovich, I.,** The catalytic mechanism of the manganese-containing superoxide dismutase of *Escherichia coli* studied by pulse radiolysis, *J. Am. Chem. Soc.,* 96, 7329, 1974.
46. **McAdam, M. E., Fox, R. A., Lavelle, F., and Fielden, E. M.,** A pulse radiolysis study of the manganese-containing superoxide dismutase from *Bacillus stearothermophilus.* A kinetic model for the enzyme action, *Biochem. J.,* 165, 71, 1977.
47. **McAdam, M. E., Lavelle, F., Fox, R. A., and Fielden, E. M.,** A pulse radiolysis study of the manganese-containing superoxide dismutase from *Bacillus stearothermophilus.* Further studies on the properties of the enzyme, *Biochem. J.,* 165, 81, 1977.
48. **Lavelle, F., McAdam, M. E., Fielden, M. E., Roberts, P. B., Puget, K., and Michelson, A. M.,** A pulse radiolysis study of the catalytic mechanism of the iron-containing superoxide dismutase from *Photobacterium leiognathi, Biochem. J.,* 161, 3, 1977.
49. **Fee, J. A., McClune, G. J., O'Neill, P., and Fielden, E. M.,** Saturation behaviour of superoxide dismutation catalyzed by the iron containing superoxide dismutase of *E. coli, Biochem. Biophys. Res. Commun.,* 100, 377, 1981.
50. **Terech, A., Pucheault, J., and Ferradini, C.,** Saturation behaviour of the manganese-containing superoxide dismutase from *Paracococcus denitrificans, Biochem. Biophys. Res. Commun.,* 113, 1114, 1983.
51. **Kenne, J. P.,** The technique of pulse radiolysis by optical absorption, in *Pulse Radiolysis,* Ebest, M., Keene, J. P., Swallow, A. J., and Baxendale, J. H., Eds., Academic Press, London, 1965, 1.
52. **Asmus, K.-D.,** Pulse radiolysis methodology, *Methods Enzymol.,* 105, 167, 1984.
53. **Rigo, A., Viglino, P., and Rotilio, G.,** Polarographic determination of superoxide dismutase, *Anal. Biochem.,* 68, 1, 1975.

54. **Argese, E., DeCarli, B., Orsega, E., Rigo, A., and Rotilio, G.,** A rotating disk electrode for kinetic studies of superoxide dismutases. Applicability in a wide pH range and for continuous monitoring of enzyme inactivation, *Anal. Biochem.*, 132, 110, 1983.
55. **Argese, E., Rigo, A., Viglino, P., Orsega, E., Marmocchi, F., Cocco, D., and Rotilio, G.,** A study of the pH dependence of the activity of porcine Cu,Zn superoxide dismutase, *Biochim. Biophys. Acta*, 787, 205, 1984.
56. **Misra, H. P. and Fridovich, I.,** The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, *J. Biol. Chem.*, 247, 3170, 1972.
57. **Sun, M. and Zigman, S.,** An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation, *Anal. Biochem.*, 90, 81, 1978.
58. **Elstner, E. F. and Heupel, A.,** Inhibition of nitrite formation from hydroxylammonium chloride: a simple assay for superoxide dismutase, *Anal. Biochem.*, 70, 616, 1976.
59. **Kono, Y.,** Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase, *Arch. Biochem. Biophys.*, 186, 189, 1978.
60. **Marklund, S. and Marklund, G.,** Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, *Eur. J. Biochem.*, 47, 469, 1974.
61. **Heikkila, R. E. and Cabbat, F.,** A sensitive assay for superoxide dismutase based on the autoxidation of 6-hydroxydopamine, *Anal. Biochem.*, 75, 356, 1976.
62. **Beauchamp, C. and Fridovich, I.,** Superoxide dismutase: improved assays and assay applicable to acrylamide gels, *Anal. Biochem.*, 44, 276, 1971.
63. **Nishikimi, M., Rao, N. A., and Yagi, K.,** The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, *Biochem. Biophys. Res. Commun.*, 46, 849, 1972.
64. **Rapp, U., Adams, W. C., and Miller, R. W.,** Purification of superoxide dismutase from fungi and characterisation of the reaction of the enzyme with cathecols by electron spin resonance spectroscopy, *Can. J. Biochem.*, 51, 158, 1973.
65. **Nishikimi, M.,** The generation of superoxide anion in the reaction of tetrohydropteridines with molecular oxygen, *Arch. Biochem. Biophys.*, 166, 273, 1975.
66. **Auclair, C., Torres, M., and Hakim, J.,** Superoxide anion involvement in the NBT reduction catalyzed by NADPH-cytochrome P-450 reductase: a pitfall, *FEBS Lett.*, 89, 26, 1978.
67. **Fridovich, I.,** Measuring the activity of superoxide dismutases: an embarrassment of riches, in *Superoxide Dismutase*, Vol. I, Oberley. L. W., Ed., CRC Press, Boca Raton, Fla., 1983, 69.
68. **Azzi, A. C., Montecucco, C., and Richter, C.,** The use of acetylated ferricytochrome c for the detection of superoxide radicals produced in biological membranes, *Biochem. Biophys. Res. Commun.*, 65, 597, 1975.
69. **Buchanan, A. G. and Lees, H.,** The effects of pH and temperature on the assay of superoxide dismutase, *Can. J. Microbiol.*, 22, 1643, 1976.
70. **Kuthan, H., Ullrich, V., and Estabrook, R. W.,** A quantitative test for superoxide radicals produced in biological systems, *Biochem. J.*, 203, 551, 1982.
71. **Misra, H. P. and Fridovich, I.,** Superoxide dismutase: a photochemical augmentation assay, *Arch. Biochem. Biophys.*, 181, 308, 1977.
72. **Misra, H. P. and Fridovich, I.,** Superoxide dismutase: "postive" spectrophotometric assays, *Anal. Biochem.*, 79, 553, 1977.
73. **Misra, H. P. and Fridovich, I.,** Superoxide dismutase and peroxidase: a positive activity stain applicable to polyacrylamide gel electrophoresis, *Arch. Biochem. Biophys.*, 183, 511, 1977.
74. **Bannister, J. V., Anastasi, A., and Bannister, W. H.,** Active subunits from swordfish liver superoxide dismutase, *Biochem. Biophys. Res. Commun.*, 18, 469, 1978.
75. **Thornalley, P. J. and Bannister, J. W.,** Spin trapping of superoxide, in *Handbook of Oxy-Radical Research*, Greenwald, R. A., Ed., CRC Press, Boca Raton, Fla., 1985, 133.
76. **Rosen, G. M., Finkelstein, E., and Rauchman, E. R.,** A method for the detection of superoxide in biological systems, *Arch. Biochem. Biophys.*, 215, 367, 1982.
77. **Misra, H. P. and Fridovich, I.,** The univalent reduction of oxygen by reduced flavins and quinones, *J. Biol. Chem.*, 247, 188, 1972.
78. **Cohen, G. and Heikkila, R. E.,** The generation of hydrogen peroxide superoxide radical and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents, *J. Biol. Chem.*, 249, 2447, 1974.
79. **Misra, H. P.,** Generation of superoxide free radicals during the autoxidation of thiols, *J. Biol. Chem.*, 249, 2151, 1974.
80. **Saez, G., Thornalley, P. J., Hill, H. A. O., Hems, R. A., and Bannister, J. V.,** The production of free radicals during cysteine autoxidation and their effect on rat liver hepatocytes, *Biochim. Biophys. Acta*, 719, 24, 1982.
81. **Fisher, O. B. and Kaufman, S.,** Tetrahydropterin oxidation without hydroxylation catalyzed by rat liver phenylalanine hydroxylase, *J. Biol. Chem.*, 248, 4300, 1973.

82. **Misra, H. P. and Fridovich, I.,** The generation of superoxide radical during the autoxidation of hemoglobin, *J. Biol. Chem.*, 247, 6960, 1972.
83. **Gotoh, T. and Shikama, K.,** Generation of superoxide radical during autoxidaton of oxymyoglobin, *J. Biochem.*, 80, 397, 1976.
84. **Misra, H. P. and Fridovich, I.,** The generation of superoxide radical during the autoxidation of ferredoxins, *J. Biol. Chem.*, 246, 6886, 1971.
85. **Halliwell, B.,** Hydroxylation of p-coumaric acid by illuminated chloroplasts. The role of superoxide, *Eur. J. Biochem.*, 55, 355, 1975.
86. **Loschen, G., Azzi, A., Richter, C., and Flohe, L.,** Superoxide radicals as precursors of mitochondrial hydrogen peroxide, *FEBS Lett.*, 42, 68, 1974.
87. **Fridovich, I.,** Superoxide dismutases, *Annu. Rev. Biochem.*, 44, 147, 1975.
88. **Hamilton, G. A., Libby, R. D., and Hartzell, C. R.,** The valence of copper and the role of superoxide in the D-galactose oxidase catalysed reaction, *Biochem. Biophys. Res. Commun.*, 55, 333, 1973.
89. **Hirata, F. and Hayaishi, O.,** Studies on indoleamine 2,3-dioxygenase. I. Superoxide anion as substrate, *J. Biol. Chem.*, 250, 5960, 1975.
90. **Kido, T., Soda, K., Suzuki, T., and Asada, K.,** A new oxygenase, 2-nitropropane dioxygenase of *Hanserula amrakii*, *J. Biol. Chem.*, 251, 6994, 1976.
91. **Kido, T. and Soda, K.,** Oxidation of anionic nitroalkenes by flavoenzymes, and participation of superoxide anion in the catalysis, *Arch. Biochem. Biophys.*, 234, 468, 1984.
92. **Bannister, J. V. and Bannister, W. H.,** The production of oxygen-centred radicals by neutrophils and macrophages as studied by electron spin resonance (esr), *Environ. Health Perspect.*, 104, 37, 1985.
93. **Halliwell, B.,** Superoxide dismutase and the superoxide theory of oxygen toxicity. A critical appraisal, in *Copper Proteins*, Vol. II, Lontie, R., Ed., CRC Press, Boca Raton, Fla., 1984, 63.
94. **McCord, J. M., Keele, B. B., Jr., and Fridovich, I.,** An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase, *Proc. Natl. Acad. Sci. U.S.A.*, 68, 1024, 1981.
95. **Loesche, W. J.,** Oxygen sensitivity of various anaerobic bacteria, *Appl. Microbiol.*, 18, 723, 1969.
96. **Bell, G. R.,** Studies on Electron Transfer System in *Desulfovibrio:* Purification of Hydrogenase, Catalase and Superoxide Dismutase, Ph.D. thesis, University of Georgia, Athens, 1973, 206.
97. **Norrod, P. and Morse, S. A.,** Absence of superoxide dismutase in some strains of *Neisseria gonorrhoeae*, *Biochem. Biophys. Res. Commun.*, 90, 1287, 1979.
98. **Lynch, R. E. and Cole, B. C.,** *Mycoplasma pneumoniae:* a prokaryote which consumes oxygen and generates superoxide but which lacks superoxide dismutase, *Biochem. Biophys. Res. Commun.*, 96, 98, 1980.
99. **Austin, F. E., Barbieri, J. T., Corin, R. E., Grigas, K. E., and Cox, C. D.,** Distribution of superoxide dismutase, catalase, and peroxidase activities among *Trephnema palidum* and other spirochetes, *Infect. Immun.*, 33, 372, 1981.
100. **Norrod, E. P., Bartenstein, L., and Morse, S. A.,** Comparison of the superoxide dismutases of *Branhamella catarrhalis*, *Neisseria ovis*, and *Neisseria caviae*, *Int. J. Syst. Bacteriol.*, 31, 392, 1981.
101. **Gregory, E. M. and Fridovich, I.,** Oxygen metabolism in *Lactobacillus plantarum*, *J. Bacteriol.*, 117, 166, 1974.
102. **Archibald, F. S. and Fridovich, I.,** Defenses against oxygen toxicity in *Lactobililus plantarum*, *J. Bacteriol.*, 145, 442, 1981.
102a. **Carlioz, A. and Touati, D.,** Isolation of superoxide dismutase mutants in *Escherichia coli:* is superoxide dismutase necessary for anaerobic life?, *EMBO J.*, 5, 623, 1986.
102b. **Van Loon, A. P. E. M., Pesold-Hart, B., and Schatz, G.,** A yeast mutant lacking mitochondria manganese superoxide dismutase is hypersensitive to oxygen, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 3920, 1986.
102c. **Elroy-Stein, O., Bernstein, Y., and Groner, Y.,** Over production of human copper/zinc superoxide dismutase in transfected cells: extenuation of paraquat mediated cytotoxicity and enhancement of lipid peroxidation, *EMBO J.*, 5, 615, 1986.
103. **Hewitt, J. and Morris, J. G.,** Superoxide dismutase in some obligately anaerobic bacteria, *FEBS Lett.*, 50, 315, 1975.
104. **Lumsden, J. and Hall, D. O.,** Superoxide dismutase in photosynthetic organisms provides an evolutionary hypothesis, *Nature (London)*, 257, 670, 1975.
105. **Asada, K., Kanematsu, S., Takahashi, M., and Kono, Y.,** Superoxide dismutases in photosynthetic organisms, in *Iron and Copper Proteins*, Vol. 74, Yasonobu, K. T., Mower, H. F., and Hayaishi, O., Eds., Plenum Press, New York, 1976, 551.
106. **Asada, K., Kanematsu, S., and Uchida, K.,** Superoxide dismutases in photosynthetic organisms: absence of the cuprozinc enzyme in eukaryotic algae, *Arch. Biochem. Biophys.*, 179, 243, 1977.
107. **Gregory, E. M., Kowalski, J. B., and Holdeman, L. V.,** Production and some properties of catalase and superoxide dismutase from the anaerobe *Bacteriodes distasonis*, *J. Bacteriol.*, 129, 1298, 1977.
108. **Carlsson, J., Wrethen, J., and Beckman, G.,** Superoxide dismutase in *Bacteriodes fragilis* and related *Bacteriodes* species, *J. Clin. Microbiol.*, 6, 280, 1977.

109. **Tally, F. P., Goldin, H. R., Jacobus, N. V., and Gorbach, S. L.,** Superoxide dismutase in anaerobic bacteria of clinical significance, *Infect. Immun.*, 16, 20, 1977.
110. **Gregory, E. M., Moore, W. E. C., and Holdeman, L. V.,** Superoxide dismutase in anaerobes: survey, *Appl. Environ. Microbiol.*, 35, 988, 1978.
111. **Daily, O. P., Debell, R. M., and Joseph, S. M.,** Superoxide dismutase and catalase levels in halophilic vibrios, *J. Bacteriol.*, 134, 375, 1978.
112. **Fulghum, R. S. and Worthington, J. M.,** Superoxide dismutase in ruminal bacteria, *Appl. Environ. Microbiol.*, 48, 675, 1984.
113. **Hatchikan, E. C. and Henry, Y. A.,** An iron-containing superoxide dismutase from the strict anaerobe *Desulfovibrio desulfuricans* (Norway 4), *Biochemie*, 59, 153, 1977.
114. **Kanematsu, S. and Asada, K.,** Superoxide dismutase from anaerobic photosynthetic bacterium, *Chromatium vinosum*, *Arch. Biochem. Biophys.*, 185, 473, 1978.
115. **Kanematsu, S. and Asada, K.,** Crystalline ferric superoxide dismutase from an anaerobic green sulfur bacterium, *Chlorobium thiosulfatophilum*, *FEBS Lett.*, 91, 94, 1978.
116. **Meier, B. and Schwartz, A. C.,** Properties of the iron-containing superoxide dismutase from *Propionibacterium shermanii*, in *Chemical and Biochemical Aspects of Superoxide Dismutase*, Dev. Biochem., 11A, Bannister, J. V. and Hill, H. A. O., Eds., Elsevier, New York, 1980, 160.
117. **Kirby, T. W., Lancaster, J. R., Jr., and Fridovich, I.,** Isolation and characterisation of the iron-containing superoxide dismutase of *Methanobacterium bryantii*, *Arch. Biochem. Biophys.*, 210, 140, 1981.
118. **Meier, B., Barra, D., Bossa, I. F., Calabrese, L., and Rotilio, G.,** Synthesis of either Fe- or Mn-superoxide dismutase with an apparently identical protein moiety by an anaerobic bacterium dependent on the metal supplied, *J. Biol. Chem.*, 257, 13977, 1982.
119. **Gregory, E. M.,** Characterisation of the O_2-induced manganese-containing superoxide dismutase from *Bacterioides fragilis*, *Arch. Biochem. Biophys.*, 238, 83, 1985.
120. **Dougherty, H. W., Sadowski, S. J., and Baker, E. E.,** A new iron-containing superoxide dismutase from *Escherichia coli*, *J. Biol. Chem.*, 253, 5220, 1978.
121. **Stowers, M. D. and Elkan, G. H.,** An inducible iron-containing superoxide dismutase in *Rhizobium japonicum*, *Can. J. Microbiol.*, 27, 1202, 1981.
122. **Clara, R. W. and Knowles, R.,** Superoxide dismutase, catalase, and peroxidase in ammonium-grown and nitrogen fixing *Azospirillum brasilense*, *Can. J. Microbiol.*, 30, 1222, 1984.
123. **Dimitroijovic, L., Puppo, A., and Rigaud, J.,** Superoxide dismutase activities in *Rhizobium phaseoli* bacteria and bacteroids, *Arch. Microbiol.*, 139, 174, 1984.
124. **Buchanan, A. G. and Lees, H.,** Superoxide dismutase from nitrogen-fixing *Azobacter chrococcum:* purification, characterizaton and intracellular location, *Can. J. Microbiol.*, 26, 441, 1980.
125. **Moore, E. R. B., Norrod, E. P., and Jurtshuk, P., Jr.,** Superoxide dismutases of *Azobacter vinelandii* and other aerobic, free-living nitrogen-fixing bacteria, *FEMS Microbiol. Lett.*, 24, 261, 1984.
126. **Eady, R. R. and Smith, B. E.,** Physico-chemical properties of nitrogenase and its components, in *A Treatise on Nitrogen Fixation*, Hardy, R. W. F., Bottomley, F., and Burns, R. C., Eds., John Wiley & Sons, New York, 1979, 399.
127. **Yates, M. G.,** Physiological aspects of nitrogen fixation, in *Recent Developments in Nitrogen Fixation*, Newton, W., Postgate, J. R., and Rodriquez-Barrueco, C., Eds., Academic Press, New York, 1977, 218.
128. **Slykhouse, T. O. and Fee, J. A.,** Physical and chemical studies on bacterial superoxide dismutases. Purification and some anion binding properties of the iron-containing protein of *Escherichia coli B*, *J. Biol. Chem.*, 251, 5472, 1976.
129. **Kusunose, M., Noda, Y., Ichihara, K., and Kusunose, E.,** Superoxide dismutase from *Mycobacterium* species, strain Takeo, *Arch. Microbiol.*, 108, 65, 1976.
130. **Kusunose, M., Ichihara, K., Noda, Y., and Kusunose, M.,** Superoxide dismutase from *Mycobacterium* tuberculosis, *J. Biochem.*, 80, 1343, 1976.
131. **Yamakura, F.,** Purification, crystallization and properties of iron-containing superoxide dismutase from *Pseudomonas ovalis*, *Biochim. Biophys. Acta*, 422, 280, 1976.
132. **Anastasi, A., Bannister, J. V., and Bannister, W. H.,** Isolation and characterization of iron superoxide dismutase from *Bacillus megaterium*, *Int. J. Biochem.*, 7, 541, 1976.
133. **Puget, K. and Michelson, A. M.,** Iron containing superoxide dismutases from luminous bacteria, *Biochemie*, 56, 1255, 1974.
134. **Greenow, M., Fritzsche, F., Ozierenski, B., and Schopp, W.,** Reinigang und kristallisation einer suproxid dismutase aus *pseudomonas patida*, *Acta Biol. Med. Ger.*, 38, 1643, 1979.
135. **Baldensperger, T. B.,** An iron-containing superoxide dismutase from the chemolithotrophic *Thiobacillus denitrificans* "RT" strain, *Arch. Microbiol.*, 119, 237, 1978.
136. **Vance, P. G., Keele, B. B. Jr., Rajakopalan, K. V., and Fridovich, I.,** Superoxide dismutase from *Streptococcus mutans*. Isolation and characterization of two forms of the enzyme, *J. Biol. Chem.*, 247, 4782, 1972.

137. **Chickata, Y., Kusunose, E., Ichihara, K., and Kusunose, M.,** Purification of superoxide dismutase from *Mycobacterium phlei, Osaka City Med. J.*, 21, 127, 1975.
138. **Lumsden, J., Cammack, R., and Hall, D. O.,** Purification and physicochemical properties of superoxide dismutase from two photosynthetic microorganisms, *Biochim. Biophys. Acta*, 438, 380, 1976.
139. **Ichihara, K., Kusunose, E., Kusunose, M., and Mori, T.,** Superoxide dismutase from *Mycobacterium lepraemurium, J. Biochem.*, 81, 1427, 1977.
140. **Harris, J. I.,** Superoxide dismutase from *Bacillus stearothermophilus*, in *Superoxide and Superoxide Dismutases*, Michelson, A. M., McCord, J. M., and Fridovich, I., Eds., Academic Press, New York, 1977, 151.
141. **Sato, S. and Harris, J. I.,** Superoxide dismutase from *Thermus aquaticus, Eur. J. Biochem.*, 73, 373, 1977.
142. **Britton, L., Malinowski, D. P., and Fridovich, I.,** Superoxide dismutase and oxygen metabolism in *Streptococcus facealis* and comparisons with other organisms, *J. Bacteriol.*, 134, 229, 1978.
143. **Sato, S. and Nakazawa, K.,** Purification and properties of superoxide dismutase from *Thermus thermophilus* HB8, *J. Biochem.*, 83, 1165, 1978.
144. **Terech, A. and Vugnais, P. V.,** A manganese containing syperoxide dismutase from *Paracoccus denitrificans, Biochim. Biophys. Acta*, 57, 411, 1981.
145. **Tsukuda, K., Kido, T., Shimasne, Y., and Soda, K.,** Isolation of manganese-containing superoxide dismutase from *Bacillus subtilis, Agric. Biol. Chem.*, 47, 2865, 1983.
146. **Maejima, K., Miyata, K., and Tomoda, K.,** A manganese superoxide dismutase from *Serratia marcescens. Agric. Biol. Chem.*, 47, 1527, 1983.
147. **Asada, K. and Kanematsu, S.,** Distribution of cuprozinc, manganic and ferric superoxide dismutases in plants and fungi: an evolutionary aspect, in *Evolution of Protein Molecules*, Matsubara, H. and Yamanka, T., Eds., Japan Scientific Societies Press, Tokyo, 1978, 361.
148. **Misra, H. P. and Keele, B. B., Jr.,** The purification and properties of superoxide dismutase from a blue-green alge, *Biochim. Biophys. Acta*, 379, 418, 1975.
149. **Asada, K., Yoshikawa, K., Takahashi, M., Maeda, Y., and Emmanji, K.,** Superoxide dismutases from a blue-green algae, *Plectonema boryanum, J. Biol Chem.*, 250, 2801, 1975.
150. **Cseke, C., Horvath, L. I., Simon, O., Borbely, G., Keszthelyi, L., and Farkas, G. L.,** An iron-containing superoxide dismutase from *Anacystis nidulans, J. Biochem.*, 85, 1397, 1979.
151. **Misra, H. P. and Fridovich, I.,** Purification and properties of superoxide dismutases from a red algae, *Porphyridium cruentum, J. Biol. Chem.*, 252, 6421, 1977.
152. **Kanematsu, S. and Asada, K.,** Ferric and manganic superoxide dismutases in *Euglena gracilis, Arch. Biochem. Biophys.*, 195, 535, 1979.
153. **Trant, N. L., Meshnick, S. R., Kitchener, K., Eaton, J. W., and Cerami, A.,** Iron-containing superoxide dismutase from *Crithidia fasiculata*. Purification, chracterization, and similarity to leishmanial and trypanasomal enzymes, *J. Biol. Chem.*, 258, 125, 1983.
154. **Lindmark, D. K. and Muller, M.,** Superoxide dismutase in the anaerobic flagellates, *Tritrichomonas foetus* and *Monocercomones sp., J. Biol. Chem.*, 249, 4634, 1974.
155. **Ravindranath, S. D. and Fridovich, I.,** Isolation and characterisation of a manganese-containing superoxide dismutase from yeast, *J. Biol. Chem.*, 250, 6107, 1975.
156. **Lavelle, F. and Michelson, A. M.,** Purification et etude des deux superoxyde dismutases du champignon *Pleurotus olearius, Biochimie*, 57, 375, 1975.
157. **Goscin, S. A. and Fridovich, I.,** The purification and properties of superoxide dismutase from *Saccharomyces cerevisiae, Biochim. Biophys. Acta*, 289, 276, 1972.
158. **Weser, U., Fritzdorff, A., and Prinz, R.,** Superoxide dismutase in baker's yeast, *FEBS Lett.*, 27, 267, 1972.
159. **Misra, H. P. and Fridovich, I.,** The purification and properties of superoxide dismutase from *Neurospora crassa, J. Biol. Chem.*, 241, 3410, 1972.
160. **Shatzman, A. R. and Kosman, D. J.,** Biosynthesis and cellular distribution of the two superoxide dismutases of *Dactylium dendroides, J. Bacteriol.*, 137, 313, 1979.
161. **Sawada, Y., Ohyama, T., and Yamazaki, I.,** Preparation and physio-chemical properties of green pea superoxide dismutase, *Biochim. Biophys. Acta*, 268, 305, 1972.
162. **Duke, M. V. and Salin, M. L.,** Isoenzymes of cuprozinc superoxide dismutase from *Pisum sativum, Phytochemistry*, 22, 2369, 1983.
163. **Beauchamp, C. O. and Fridovich, I.,** Isozymes of superoxide dimutase from wheat germ, *Biochim. Biophys. Acta*, 317, 50, 1973.
164. **Asada, K., Urano, M., and Takahashi, M.,** Subcellular location of superoxide dismutase in spinach leaves and preparation and properties of crystalline spinach superoxide dismutase, *Eur. J. Bichem.*, 36, 257, 1973.
165. **Kono, Y., Takakashi, M., and Asada, K.,** Superoxide dismutases from kidney bean leaves, *Plant Cell Physiol.*, 20, 1229, 1979.

166. **Baker, J. E.,** Superoxide dismutase in ripening fruit, *Plant Physiol.*, 58, 644, 1976.
167. **Baum, J. A. and Scandalios, J. G.,** Isolation and characterization of the cytosolic and mitochondrial superoxide dismutases of maize, *Arch. Biochem. Biophys.*, 206, 249, 1981.
168. **Baum, J. A., Chandler, J. M., and Scandalios, J. G.,** Purification and partial characterization of a genetically defined superoxide dismutase (SOD-1) associated with maize chloroplasts, *Plant Physiol.*, 73, 31, 1983.
169. **Giannopolitis, C. N. and Ries, S. K.,** Superoxide dismutases. I. Occurrence in higher plants, *Plant Physiol.*, 59, 309, 1977.
170. **Giannopolitis, C. N. and Ries, S. K.,** Superoxide dismutases. II. Purification and quantitative relationship with water-soluble protein in seedlings, *Plant Physiol.*, 59, 315, 1977.
171. **Del Rio, L. A., Sevilla, F., Gomez, M., Yanez, J., and Lopez-Gorge, J.,** Superoxide dismutase: an enzyme system for the study of micronutrient interactions in plants, *Planta*, 140, 221, 1978.
172. **Bridges, S. M. and Salin, M. L.,** Distribution of iron-containing superoxide dismutase in vascular plants, *Plant Physiol.*, 68, 275, 1981.
173. **Sevilla, F., Lopez-Gorge, J., and Del Rio, L. A.,** Manganese superoxide dismutase from a high plant. Purification of a new Mn-containing enzyme, *Planta*, 150, 153, 1980.
174. **Sevilla, F., Lopez-Gorge, J., and Del Rio, L. A.,** Characterization of a manganese superoxide dismutase from the higher plant *Pisum sativum*, *Plant Physiol.*, 70, 1321, 1982.
175. **Hakamada, K., Kanematsu, S., and Asada, K.,** Superoxide dismutase in tea leaf, *Study of Tea*, 54, 6, 1978.
176. **Jackson, C., Dench, J., Moore, A. L., Halliwell, B., Foyer, C. H., and Hall, D. O.,** Subcellular localisation and identification of superoxide dismutase in the leaves of higher plants, *Eur. J. Biochem.*, 91, 339, 1978.
177. **Arron, G. P., Henry, L., Palmer, J. M., and Hall, D. O.,** Superoxide dismutases in mitochondria from *Helianthus tuberosus* and *Neurospora crassa*, *Biochem. Soc. Trans.*, 4, 618, 1976.
178. **Gerasimov, A. M., Kasatkina, N. V., and Darmova, E. N.,** Presence of anti-oxidant defense enzymes in the ascarid *Ascaris lumbricoides*, *J. Evol. Biochem. Physiol.*, 15, 126, 1979.
179. **Paul, J. M. and Barret, J.,** Peroxide metabolism in the cestodes *Hymenolepis diminuta* and *Moniezia expansa*, *Int. J. Parasitol.*, 10, 121, 1980.
180. **Manwell, C.,** Superoxide dismutase and NADH diaphorase in haemerythrocytes of sipuriculans, *Comp. Biochem. Physiol.*, 58B, 331, 1977.
181. **Lin, A. L.,** Isozymes of tropical sea anemones, *Comp. Biochem. Physiol.*, 62B, 425, 1979.
182. **Bannister, J. V.,** unpublished observations.
183. **Fernandez-Sousa, J. M. and Michelson, A. M.,** Variation of superoxide dismutases during the development of the fruit fly *Ceratitis capitata L.*, *Biochem. Biophys. Res. Commun.*, 73, 217, 1976.
184. **Massie, H. R., Aiello, V. R., and Williams, T. R.,** Changes in superoxide dismutase activity and copper during development and ageing in the fruit fly *Drosophila melanogaster*, *Mech. Ageing Dev.*, 12, 279, 1980.
185. **Massie, H. R., Williams, T. R., and Aiello, V. R.,** Superoxide dismutase activity in two different wild-type strains of *Drosophila melanogaster*, *Gerontology*, 27, 205, 1981.
186. **Rhoads, M. L.,** *Trichinella spiralis:* identification and purification of superoxide dismutase, *Exp. Parasitol.*, 56, 41, 1983.
187. **Tallandini, L., Cassini, A., Ricchelli, F., and Filippi, B.,** Chemical and spectroscopic characterization of a copper-zinc superoxide dismutase from a marine bivalve mollusc *(Myttilus galloprovincialis)* in *Oxy-Radicals and Their Scavenger Systems, Molecular Aspects*, Vol. 1, Cohen, K. and Greenwald, R. A., Eds., Elsevier, Amsterdam, 1983, 324.
188. **Lee, Y. M., Ayala, F. J., and Misra, H. P.,** Purification and properties of superoxide dismutase from *Drosophila melanogaster*, *J. Biol. Chem.*, 256, 8506, 1981.
189. **Lee, Y. M., Misra, H. P., and Ayala, F. J.,** Superoxide dismutase in *Drosophila melanogaster:* biochemical and structural characterisation of allozyme variants, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 7052, 1981.
190. **Bannister, J. V., Anastasi, A., and Bannister, W. H.,** Cytosol superoxide dismutase from swordfish *(Xiphias gladius)* liver, *Comp. Biochem. Physiol.*, 56B, 235, 1977.
191. **Martin, J. P. and Fridovich, I.,** Evidence for a natural gene transfer from the ponyfish to its bioluminescent bacterial symbiont *Photobacterium leiognathi*, *J. Biol. Chem.*, 256, 6080, 1981.
192. **Movis, S. M. and Albright, J. T.,** Superoxide dismutase, catalase and glutathione peroxide in the swim bladder of the physochlistous fish *Opsanus tau* L., *Cell Tissue Res.*, 220, 739, 1981.
193. **Mazeaud, F., Maral, J., and Michelson, A. M.,** Distribution of superoxide dismutase and glutathione peroxidase in the carp: erythrocyte manganese SOD, *Biochem. Biophys. Res. Commun.*, 86, 1161, 1979.
194. **Healy, J. A. and Mulcahy, M. F.,** Polymorphic tetrameric superoxide dismutase in the pike *Esox lucius L.* (Pisces; Esocidae), *Comp. Biochem. Physiol.*, 62B, 563, 1979.

195. **Aksnes, A. and Njaa, L. R.,** Catalase, glutathione peroxidase and superoxide dismutase in different fish species, *Comp. Biochem. Physiol.*, 69B, 893, 1981.
196. **Peeters-Joris, C., Vandevoorde, A. M., and Baudhuin, P.,** Subcellular localization of superoxide dismutase in rat liver, *Biochem. J.*, 150, 31, 1975.
197. **Bannister, W. H. and Bannister, J. V.,** Adipocytes do not falsify the superoxide theory of oxygen toxicity, *FEBS Lett.*, 142, 42, 1982.
198. **Bannister, J. V., Bannister, W. H., and Wood, E. J.,** Bovine erythrocyte cupro-zinc protein. I. Isolation and characterisation, *Eur. J. Biochem.*, 18, 178, 1971.
199. **Bannister, W. H., Dalgleish, D. E., Bannister, J. V., and Wood, E. J.,** Physicochemical and spectroscopic properties of human enthrocyte cupro-zinc protein (enthrocuprein), *Int. J. Biochem.*, 3, 560, 1972.
200. **Bannister, W. H., Anastasi, A., and Bannister, J. V.,** Human superoxide dismutase (enthrocuprein), in *Superoxide and Superoxide Dismutases*, Michelson, A. M., McCord, J. M., and Fridovich, I., Eds., Academic Press, New York, 1977, 107.
201. **Albergoni, V. and Cassini, A.,** A cupro-zinc protein with superoxide dismutase activity from horse liver. Isolation and properties, *Comp. Biochem. Physiol.*, 4TB, 767, 1974.
202. **Salin, M. L. and Wilson, W. W.,** Porcine superoxide dismutase isolation and characterisation of a relatively basic cuprozinc enzyme, *Mol. Cell. Biochem.*, 36, 157, 1981.
203. **Bartkowiak, A., Leyko, W., and Fried, R.,** A comparative charaterization of cystolic superoxide dismutase from hog liver and erythrocytis, *Comp. Biochem. Physiol.*, 62B, 61, 1979.
204. **Bensinger, R. E., Crabb, J. W., and Johnson, C. M.,** Purification and properties of superoxide dismutase from bovine retina, *Exp. Eye Res.*, 34, 623, 1982.
205. **Abe, Y., Okazaki, T., Shukuya, R., and Furuta, H.,** Copper and zinc superoxide dismutase from the liver of bullfrog *Rana catesbeiana*, *Comp. Biochem. Physiol.*, 77B, 125, 1984.
206. **McCord, J. M., Boyle, J. A., Day, E. D., Jr., Rizzolo, L. J., and Salin, M. L.,** A manganese-containing superoxide dimustase from human liver, in *Superoxide and Superoxide Dismutases*, Michelson, A. M., McCord, J. M., and Fridovich, I., Eds., Academic Press, New York, 1977, 129.
207. **Salin, M. L., Day, E. D., Jr., and Crapo, J. D.,** Isolation and characterisation of a manganese-containing superoxide dismutase from rat liver, *Arch. Biochem. Biophys.*, 187, 223, 1978.
208. **Puget, K. and Michelson, A. M.,** Isolation of a new copper-containing superoxide dismutase. Bacteriocuprein, *Biochem. Biophys. Res. Commun.*, 58, 830, 1974.
209. **Steinman, H. M.,** Copper-zinc superoxide dismutase from *Caulobacter crescentus* CB15. A novel bacteriocuprein form of the enzyme, *J. Biol. Chem.*, 257, 10283, 1982.
210. **Steinman, H. M.,** Bacteriocuprein superoxide dismutases in Pseudomonads, *J. Bacteriol.*, 162, 1255, 1985.
211. **Bannister, J. V. and Parker, M. W.,** The presence of a Cu/Zn superoxide dismutase in the symbiant *Photobacterium leiognathi:* a likely case for gene transfer from eukaryotes to prokaryotes, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 149, 1985.
212. **Fairfield, A. S., Mesknick, S. R., and Eaton, J. W.,** Malaria parasites adopt rust cell superoxide dismutase, *Science*, 221, 764, 1983.
213. **Vignais, P. M., Henry, M.-F., Terech, A., and Chabert, J.,** Production of superoxide anion and superoxide dismutases in *Paracoccus denitrificans*, in *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase*, Dev. Biochem., 11A, Bannister, J. V. and Hill, H. A. O., Eds., Elsevier, Amsterdam, 1980, 154.
214. **Vignais, P. M., Terech, A., Meyer, C. M., and Henry, M.-F.,** Isolation and characterisation of a protein with cyanide-sensitive superoxide dismutase activity from the prokaryote, *Paracoccus denitrificans*, *Biochim. Biophys. Acta*, 701, 305, 1982.
215. **Sevilla, F., Del Rio, L. A., and Hellin, E.,** Superoxide dismutases from a *Citrus* plant: presence of two iron-containing isoenzymes in leaves of lemon trees (*Citrus lemonum* L.), *J. Plant. Physiol.*, 116, 381, 1984.
216. **Salin, M. L. and Bridges, S. M.,** Isolation and characterization of an iron-containing superoxide dismutase from a eukaryote *Brassica campestris*, *Arch. Biochem. Biophys.*, 201, 369, 1980.
217. **Salin, M. L. and Bridges, S. M.,** Isolation and characterization of an iron-containing superoxide dismutase from water lily, *Nuphar luteum*, *Plant Physiol.*, 69, 161, 1982.
218. **Kwiatowski, J. K., Saflanowska, A., and Kaniuga, Z.,** Isolation and characterization of an iron-containing superoxide dismutase from tomato leaves, *Lycopersicon esculentum*, *Eur. J. Biochem.*, 146, 459, 1985.
219. **Duke, M. V. and Salin, M. L.,** Purification and characterization of an iron-containing superoxide dismutase from a eukaryote, *Ginkyo biloba*, *Arch. Biochem. Biophys.*, 243, 305, 1985.
220. **Rotilio, G., Calabrese, L., Finazzi-Agro, A., Argento-Ceru, P., Autuori, F., and Mondovi, B.,** Intracellular localization of superoxide dismutase and its relation to the distribution and mechanism of hydrogen peroxide-producing enzymes, *Biochim. Biophys. Acta*, 98, 102, 1973.
221. **Weisiger, R. A., and Fridovich, I.,** Mitochondrial superoxide dismutase. Site of synthesis and intramitochondrial localization, *J. Biol. Chem.*, 248, 4793, 1983.

222. **Tyler, D. D.,** Polarographic assay and intracellular distribution of superoxide dismutase in rat liver, *Biochem. J.*, 147, 493, 1975.
223. **Panchenko, L. F., Brusor, O. S., Gerasimov, A. M., and Liktaeva, T. D.,** Intramitochondrial localization and release of rat liver superoxide dismutase, *FEBS Lett.*, 55, 84, 1975.
224. **Auclair, C., Hakim, J., and Boicin, P.,** Subcellular superoxide dismutase activity in phagocytozing human blood polymorphonuclear leucocytes, *FEBS Lett.*, 79, 390, 1977.
225. **Rest, R. F. and Spitznagel, J. K.,** Subcellular distribution of superoxide dismutases in human neutrophils. Influence of myeloperoxidase on the measurement of superoxide dismutase activity, *Biochem. J.*, 166, 145, 1977.
226. **Henry, L. E. A., Cammack, R., Schwitzguebel, J., Palmer, J. M., and Hall, D. O.,** Intracellular localization, isolation and characterization of two distinct varieties of superoxide dismutase from *Neurospora crassa, Biochem. J.*, 187, 321, 1980.
227. **Geller, B. L. and Winger, D. R.,** Rat liver Cu,Zn-superoxide dismutase. Subcellular location in lysosomes, *J. Biol. Chem.*, 257, 8945, 1982.
228. **McCord, J. M.,** Superoxide dismutases: occurrence, function and evolution, *Curr. Top. Biol. Med. Res.*, 3, 1, 1979.
229. **Baum, J. A. and Scandalios, J. G.,** Developmental expression and intra-cellular localization of superoxide dismutases in maize, *Differentiation*, 13, 133, 1979.
230. **Foster, J. K. and Edwards, G. E.,** Localization of superoxide dismutase in leaves of C_3 and C_4 plants, *Plant Cell Physiol.*, 21, 895, 1980.
231. **Salin, M. L. and Bridges, S. M.,** Localization of superoxide dismutases in chloroplasts from *Brassica campestris, Z. Pflanzenphysiol.*, 99, 37, 1980.
232. **Salin, M. L. and Bridges, S. M.,** Absence of the iron-containing superoxide dismutase in mitochondria from mustard *(Brassica campestris), Biochem. J.*, 195, 229, 1981.
233. **Del Rio, L. A., Lyon, D. S., Olah, I., Glick, B., and Salin, M. L.,** Immunocytochemical evidence for a peroxisomal localizaiton of manganese superoxide dismutase in leaf protoplasts from a higher plant, *Planta*, 158, 216, 1983.
234. **Lumsden, J. and Hall, D. O.,** Soluble and membrane-bound superoxide dismutases in a blue-green alga *(Spirulina)* and spinach, *Biochem. Biophys. Res. Commun.*, 58, 35, 1974.
235. **Hayakawa, T., Kanematsu, S., and Asada, K.,** Occurrence of Cn,Zn-superoxide dismutase in the intrathylakoid space of spinach chloroplasts, *Plant Cell Physiol.*, 25, 883, 1984.
236. **Elstner, E. F. and Heupel, A.,** Lamellar superoxide dismutase of isolated chlorplasts, *Planta*, 123, 145, 1975.
237. **Okada, S., Kanematsu, S., and Asada, K.,** Intracellular distribution of manganic and ferric superoxide dismutases in blue-green algae, *FEBS Lett.*, 103, 106, 1979.
238. **Gregory, E. M., Yost, F. J., and Fridovich, I.,** Superoxide dismutases of *Escherichia coli:* intracellular localization and function, *J. Bacteriol.*, 115, 987, 1973.
239. **Britton, L. and Fridovich, I.,** Intracellular localization of the superoxide dismutases of *Escherica coli:* a reevaluation, *J. Bacteriol.*, 131, 815, 1977.
240. **Marklund, S. L., Holme, E., and Hellner, L.,** Superoxide dismutase in extracellular fluids, *Clin. Chim. Acta*, 126, 41, 1982.
241. **Marklund, S. L.,** Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species, *Biochem. J.*, 222, 649, 1984.
242. **Beaman, B. L., Scates, S. M., Moring, S. E., Deem, R., and Misra, H. P.,** Purification and properties of a unique superoxide dismutase from *Nocardia asteroides, J. Biol. Chem.*, 258, 91, 1983.
243. **Marklund, S. L.,** Human copper-containing superoxide dismutase of high molecular weight, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 1634, 1982.
244. **Marklund, S. L.,** Properties of extracellular superoxide dismutase from human lung, *Biochem. J.*, 220, 269, 1984.
245. **Gregory, E. M. and Fridovich, I.,** toxicity amel superoxide dismutase, Induction of superoxide dismutase by molecular oxygen, *J. Bacteriol.*, 14, 543, 1978.
246. **Gregory, E. M. and Fridovich, I.,** Oxygen toxicity and superoxide dismutase, *J. Bacteriol.*, ll4, 1193, 1973.
247. **Hassam, H. M. and Fridovich, I.,** Enzymatic defenses against the toxicity of oxygen and of streptonigrin in *Escherichia coli, J. Bacteriol.*, 129, 1574, 1977.
248. **Hansson, L. and Haggstron, M. H.,** Effects of growth conditions on superoxide dismutase and catalase activities in *Saccharomyces cerevisiae* var. *ellipsoidens, Curr. Microbiol.*, 9, 19, 1983.
249. **Crapo, J. D. and Tierney, D. F.,** Superoxide dismutase and pulmonary oxygen toxicity, *Am. J. Physiol.*, 226, 1401, 1974.
250. **Autor, A. P., Frank, L., and Roberts, R. J.,** Developmental characteristics of pulmonary superoxide dismutase: relationship of idiopathic respiratory distress syndrome, *Pediatr. Res.*, 10, 154, 1976.

251. **Rister, M. and Baehner, R. L.,** The alteration of superoxide dismutase, catalase, glutathione peroxidase, and NAD(P)H cytochrome c reductase in guinea pig polymorphonuclear leucocytes and alveolar macrophages during hyperoxia, *J. Clin. Invest.*, 58, 1174, 1976.
252. **Privalle, C. T. and Gregory, E. M.,** Superoxide dismutase and O_2 lethality in *Bacteriodes fragilis*, *J. Bacteriol.*, 138, 139, 1979.
253. **Abeliovich, A. D., Kellenbug, D., and Shilo, M.,** Effect of photo-oxidative conditions on levels of superoxide dismutase in *Anacystis nidulans*, *Photochem. Photobiol.*, 19, 379, 1974.
254. **Pueich, W. M.,** Resistance to high oxygen tension, streptionigrin and ultraviolet irradiation in the green alga *Chlorella sorokiniana* strain ORS, *J. Cell Biol.*, p. 62904, 1974.
255. **Yano, K. and Nishie, H.,** Superoxide dismutase in facultatively anaerobic bacteria: enzyme levels in relation to growth conditions, *J. Gen. Appl. Microbiol.*, 24, 33, 1978.
256. **Foster, J. G. and Hess, J. L.,** Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen, *Plant Physiol.*, 66, 482, 1980.
257. **Dykens, J. A. and Shick, J. M.,** Oxygen production by endosymbiotic algae controls superoxide dismutase activity in their animal host, *Nature (London)*, 297, 579, 1982.
258. **Clare, D. A., Blum, J., and Fridovich, I.,** A hybrid superoxide dismutase containing both functional iron and manganese, *J. Biol. Chem.*, 259, 5932, 1984.
259. **Hassan, H. M. and Fridovich, I.,** Regulation of the synthesis of superoxide dismutase in *Escherichia coli*, *J. Biol. Chem.*, 252, 7667, 1977.
260. **Hassan, H. M. and Fridovich, I.,** Paraquat and *Escherichia coli*. Mechanism of production of extracellular superoxide radical, *J. Biol. Chem.*, 254, 10864, 1979.
261. **Von Stein, R. S., Barber, L. E., and Hassan, H. M.,** Biosynthesis of oxygen-detoxifying enzymes in *Bdellovibrio stolpii*, *J. Bacteriol.*, 152, 792, 1982.
262. **Kao, S. M. and Hassan, H. M.,** Isolation of paraquat-resistant mutants of *Escherichia coli:* lack of correlation between resistance and the activity of superoxide dismutase, *FEMS Microbiol. Lett.*, 28, 93, 1985.
263. **Shatzman, A. R. and Kosman, D. J.,** The utilization of copper and its role in the biosynthesis of copper-containing proteins in the fungus, *Dactylum dendroides*, *Biochim. Biophys. Acta*, 544, 163, 1478.
264. **Ljutakova, S. G., Russanov, E. M., and Liochev, S. I.,** Copper increases superoxide dismutase activity in rat liver, *Arch. Biochem. Biophys.*, 235, 636, 1984.
265. **Martin, M. E., Strachan, R. G., Aranha, H., Evans, S. L., Salin, M. L., Welch, B., Arceneaux, J. E. L., and Byers, B. L.,** Oxygen toxicity in *Streptococcus mutans:* manganese, iron and superoxide dismutase, *J. Bacteriol.*, 159, 745, 1984.
266. **Moody, C. S. and Hassan, H. M.,** Anaerobic biosynthesis of the manganese-containing superoxide dismutase in *Escherichia coli*, *J. Biol. Chem.*, 259, 12821, 1984.
267. **Pugh, S. Y. R. and Fridovich, I.,** Induction of superoxide dismutases in *Escherichia coli B* by metal chelators, *J. Bacteriol.*, 162, 196, 1985.
268. **Hassan, H. M. and Fridovich, I.,** Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds, *Arch. Biochem. Biophys.*, 196, 385, 1979.
269. **Hassan, H. M. and Moody, C. S.,** Induction of the manganese-containing superoxide dismutase in *Escherichia coli* by nalidixic acid and by iron chelators, *FEMS Microbiol. Lett.*, 25, 233, 1984.
270. **Bannister, J. V., Hill, H. A. O., and Bannister, W. H.,** Cell-free synthesis of human Cu/Zn superoxide dismutase, *FEBS Lett.*, 121, 215, 1980.
271. **Lieman-Hurwitz, J., Wolf, D., Goldman, D., and Groner, Y.,** Isolation and cell-free translation of human mRNA coding for cytoplasmic super-oxide dismutase (SOD-1), *Biochem. Int.*, 3, 107, 1981.
272. **Lieman-Hurwitz, J., Dafni, N., Lavie, V., and Groner, Y.,** Human cytoplasmic superoxide dismutase cDNA clone: a probe for studying the molecular biology of Downs syndrome, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 2808, 1982.
273. **Sherman, L., Lieman-Hurwitz, J., and Groner, Y.,** Nucleotide sequence and expression of human chromosome 21-encoded superoxide dismutase mRNA, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 5465, 1983.
274. **Barra, D., Martini, F., Bannister, J. V., Shinina, M. E., Rotilio, G., Bannister, W. H., and Bossa, F.,** The complete amino acid sequence of human CuZn superoxide dismutase, *FEBS Lett.*, 120, 53, 1980.
275. **Jabusch, J. R., Farb, D. L., Kerschensteiner, D. A., and Deutsch, H. F.,** Some sulfhydryl properties and primary structure of human erythrocyte superoxide dismutase, *Biochemistry*, 19, 2310, 1980.
276. **Sherman, L., Levanon, D., Lieman-Hurwitz, J., Dafni, N., and Groner, Y.,** Human Cu/Zn superoxide dismutase gene: molecular characterization of its two mRNA species, *Nucleic Acids Res.*, 12, 9349, 1984.
277. **Levanon, D., Lieman-Hurwitz, J., Dafni, N., Wigderson, M., Sherman, L., Banstein, Y., Laver-Rudich, Z., Daniger, E., Stein, O., and Groner, Y.,** Architecture and anatomy of the chromosomal locus in human chromosome 21 encoding the Cu/Zn superoxide dismutase, *EMBO J.*, 4, 77, 1985.

278. **Marres, C. A. M., Van Loon, A. P. G. M., Oudshoorn, P., Van Steeg, H., Grivell, L. A., and Slater, E. C.,** Nucleotide sequence analysis of the nuclear gene coding for manganese superoxide dismutase of yeast mitochondria, a gene previously assumed to code for the Rieske iron-sulphur protein, *Eur. J. Biochem.*, 147, 153, 1985.
279. **Ditlow, C., Johansen, J. T., Martin, B. M., and Svendsen, I. B.,** The complete amino acid sequence of manganese-superoxide dismutase from *Saccharomyces cerevisiae, Carlsberg Res. Commun.*, 47, 81, 1984.
280. **Autor, A. P.,** Biosynthesis of mitochondrial manganese superoxide dismutase in *Saccharomyces cerevisiae.* Precursor form of mitochondrial superoxide dismutase made in the cytoplasm, *J. Biol. Chem.*, 257, 2713, 1982.
281. **Sakamoto, H. and Touati, D.,** Cloning of the iron superoxide dismutase gene (sodB) in *Escherichia coli* K-12, *J. Bacteriol.*, 159, 418, 1984.
282. **Touati, D.,** Cloning and mapping of the manganese superoxide dismutase gene (sodA) of *Escherichia coli* K-12, *J. Bacteriol.* 155, 1078, 1983.
283. **Nettleton, C. J., Bull, C., Baldwin, T. O., and Fee, J. A.,** Isolation of the *Escherichia coli* iron superoxide dismutase gene: evidence that intracellular superoxide concentration does not regulate oxygen-dependent synthesis of the manganese superoxide dismutase, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 4970, 1984.
284. **Bannister, J. V. and Bannister, W. H.,** Isolation and characterization of superoxide dismutase, *Methods Enzymol.*, 105, 88, 1984.
285. **Bannister, J. V. and Bannister, W. H.,** Purification of copper/zinc superoxide dismutase, in *Handbook of Methods for Oxygen Radical Research,* Greenwald, R. A., Ed., CRC Press, Boca Raton, Fla., 1985, 5.
286. **Salin, M. L.,** Preparation of iron-containing superoxide dismutases from eukaryotic organisms, in *Handbook of Methods for Oxygen Radical Research,* Greenwald, R. A., Ed., CRC Press, Boca Raton, Fla., 1985, 9.
287. **Gregory, E. M. and Pennington, C. D.,** Isolation of manganese-containing superoxide dismutases, in *Handbook of Methods for Oxygen Radical Research,* Greenwald, R. A., Ed., CRC Press, Boca Raton, Fla., 1985, 15.
288. **Keele, B. B., Jr., McCord, J. M., and Fridovich, I.,** Further characterization of bovine superoxide dismutase and its isolation from bovine heart, *J. Biol. Chem.*, 246, 2875, 1971.
289. **Hartz, J. W. and Deutsch, H. F.,** Subunit structure of human superoxide dismutase, *J. Biol. Chem.*, 247, 7043, 1972.
290. **Steinman, H. M., Naik, U. R., Abernethy, J. L., and Hill, R. A.,** Bovine erythrocyte superoxide dismutase. Complete amino acid sequence, *J. Biol. Chem.*, 249, 7326, 1974.
291. **Lerch, K. and Ammer, D.,** Amino acid sequence of copper-zinc superoxide dismutase from horse liver, *J. Biol. Chem.*, 256, 11545, 1981.
292. **Schinina, M. E., Barra, D., Simmaco, M., Bossa, F., and Rotilio, G.,** Primary structure of porcine Cu,Zn superoxide dismutase, *FEBS Lett.*, 186, 267, 1985.
293. **Hering, K., Kim, S.-M. A., Michelson, A. M., Otting, F., Puget, K., Steffens, E. J., and Flohe, L.,** The primary structure of porcine Cu-Zn superoxide dismutase. Evidence for allotypes of superoxide dismutase in pigs., *Biol. Chem. Hoppe-Seyler*, 366, 435, 1985.
294. **Flohe, L. and Michelson, A. M.,** in *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine,* Rotilio, G., Ed., Elsevier, Amsterdam, in press.
295. **Rocha, H. A., Bannister, W. H., and Bannister, J. V.,** The amino-acid sequence of copper/zinc superoxide dismutase from swordfish liver. Comparison of copper/zinc superoxide dismutase sequences, *Eur. J. Biochem.*, 145, 477, 1984.
296. **Lee, Y. M., Friedman, D. J., and Ayala, F. J.,** Superoxide dismutase: an evolutionary puzzle, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 824, 1985.
297. **Johansen, J. T., Overballe-Petersen, C., Martin, B., Hasemann, V., and Svendsen, I.,** The complete amino acid sequence of copper, zinc superoxide dismutase from *Saccharomyces cerevisiae, Carlsberg Res. Commun.*, 44, 201, 1979.
298. **Steinman, H. M.,** The amino acid sequence of copper-zinc superoxide dismutase from bakers' yeast, *J. Biol. Chem.*, 255, 6758, 1980.
299. **Lerch, K. and Schent, E.,** Primary structure of copper-zinc superoxide dimutase from *Neurospora crassa, J. Biol. Chem.*, 260, 9559, 1985.
300. **Steffens, G. J., Bannister, J. V., Bannister, W. H., Flohe, L., Gunzler, W. A., Kim, S.-M. A., and Otting, F.,** The primary structure of Cu-Zn superoxide dismutase from *Photobacterium leiognathi:* evidence for a separate evolution of Cu-Zn superoxide dismutase in bacteria, *Hoppe-Seyler's Z. Physiol. Chem.*, 364, 675, 1983.
301. **Malinowski, D. P. and Fridovich, I.,** Chemical modification of arginine at the active site of the bovine erythrocyte superoxide dismutase, *Biochemistry*, 18, 5909, 1979.

302. **Borders, C. L., Jr., Saunders, J. E., Blech, D. M., and Fridovich, I.,** Essentiality of the active-site arginine residue for the normal catalytic activity of Cu,Zn superoxide dismutase, *Biochem. J.*, 230, 771, 1985.
303. **McLachlan, A. D.,** Tests for comparing related amino-acid sequences. Cytochrome c and cytochrome c_{551} *J. Mol. Biol.*, 61, 408, 1971.
304. **Parker, M. W., Schinina, M. E., Bossa, F., and Bannister, J. V.,** Chemical aspects of the structure, function and evolution of superoxide dismutases, *Inorg. Chim. Acta*, 91, 307, 1984.
305. **Ringe, D., Petsko, G. A., Yamakura, F., Suzuki, K., and Ohmori, D.,** The iron content of iron superoxide dismutase: determination by anomalous scattering, *Proc. R. Soc. London*, B218, 119, 1983.
306. **Brock, C. J. and Walker, J. E.,** Superoxide dismutase from *Bacillus stearothermophilus*. Complete amino acid sequence of a manganese enzyme, *Biochemistry*, 19, 2873, 1980.
307. **Steinman, H. M.,** The amino acid sequence of manganese superoxide dismutase from *Escherichia coli* B, *J. Biol. Chem.*, 253, 8708, 1978.
308. **Brock, C. J. and Walker, J. E.,** Superoxide dismutase from *Bacillus stearothermophilus:* metal binding and complete amino acid sequence, in *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase*, Dev. Biochem., 11A, Bannister, J. V. and Hill, H. A. O., Eds., Elsevier, Amsterdam, 1980, 227.
309. **Barra, D., Schinina, M. E., Simmaco, M., Bannister, J. V., Bannister, W. H., Rotilio, G., and Bossa, F.,** The primary structure of human liver manganese superoxide dismutase, *J. Biol. Chem.*, 259, 12595, 1984.
310. **Stallings, W. C., Pattridge, K. A., Strong, R. K., and Ludwig, M. L.,** The structure of manganese superoxide dismutase from *Thermus thermophilus* HB8 at 2.4 Å resolution, *J. Biol. Chem.*, 260, 16424, 1985.
311. **Barra, D., Schinina, M. E., Bossa, F., and Bannister, J. V.,** Identity of the metal ligands in the manganese- and iron-containing superoxide dismutases, *FEBS Lett.*, 179, 329, 1985.
312. **Stallings, W. C., Pattridge, K. A., Strong, R. K., and Ludwig, M. L.,** Manganese and iron superoxide dismutases are structural homologs, *J. Biol. Chem.*, 259, 10695, 1984.
313. **Tainer, J. A., Getzoff, E. D., Richardson, J. S., and Richardson, D. E.,** Structure and mechanism of copper, zinc superoxide dismutase, *Nature (London)*, 306, 284, 1983.
314. **Getzoff, E. D., Tainer, J. A., Weiner, P. K., Kollman, P. A., Richardson, J. S., and Richardson, D. C.,** Electrostatic recognition between superoxide and copper, zinc superoxide dismutase, *Nature (London)*, 306, 287, 1983.
315. **Tainer, J. A., Getzoff, E. D., Beam, K. M., Richardson, J. S., and Richardson, D. C.,** Determination and analysis of the 2 Å structure of copper, zinc superoxide dismutase, *J. Mol. Biol.*, 160, 181, 1982.
316. **Rotilio, G., Morpargo, L., Calabrese, L., and Mondovi, B.,** On the mechanism of superoxide dismutase. Reaction of the bovine enzyme with hydrogen peroxide and ferricyanide, *Biochim. Biophys. Acta*, 302, 229, 1973.
317. **Bray, R. C., Cockle, S. A., Fielden, E. M., Roberts, P. B., Rotilio, G., and Calabrese, L.,** Reduction and inactivation of superoxide dismutase by hydrogen peroxide, *Biochem. J.*, 139, 43, 1974.
318. **Rigo, A., Viglino, P., and Rotilio, E.,** Kinetic study of O_2^- dismutation by bovine superoxide dismutase. Evidence for saturation of the catalytic sites by O_2^-, *Biochem. Biophys. Res. Commun.*, 63, 1013, 1975.
319. **McAdam, M. E., Fielden, E. M., Lavelle, F., Calabrese, L., Cocco, D., and Rotilio, G.,** The involvement of the bridging imidazolate in the catalytic mechanism of action of bovine superoxide dismutase, *Biochem. J.*, 167, 271, 1977.
320. **Rotilio, G., Rigo, A., and Calabrese, K.,** Recent developments on the active-site structure and mechanism of bovine copper- and zinc-containing superoxide dismutase, in *Frontiers on Physicochemical Biology*, Pullman, B., Ed., Academic Press, New York, 1978, 357.
321. **Viglino, P., Rigo, A., Argese, E., Calabrese, L., Cocco, D., and Rotilio, G.,** ^{19}F Relaxation as a probe of the oxidation state of Cu,Zn superoxide dismutase. Studies of the enzyme in steady-state turnover, *Biochem. Biophys. Res. Commun.*, 100, 125, 1981.
322. **O'Neill, P., Fielden, E. M., Cocco, D., Rotilio, G., and Calabrese, L.,** Evidence for catalytic dismutation of superoxide by cobalt(II) derivatives of bovine superoxide dismutase in aqueous solution as studied by pulse radiolysis, *Biochem. J.*, 205, 181, 1982.
323. **O'Neill, P., Fielden, E. M., Cocco, D., Calabrese, L., and Rotilio, G.,** Mechanistic study of superoxide dismutation by Zn-free bovine superoxide dismutase, in *Oxy Radicals and Their Scavenger Systems, Molecular Aspects*, Cohen, G. and Greenwald, R. A., Eds., Elsevier, New York, 1983, 316.
324. **Rotilio, G., Finazzi-Agro, A., Calabrese, L., Bossa, F., Guerrieri, P., and Mondovi, B.,** Studies of the metal sites of copper proteins. Ligands of copper in hemocuprein, *Biochemistry*, 10, 616, 1971.
325. **Fee, J. A. and Gaber, B. P.,** Anion binding to bovine erythrocyte superoxide dismutase. Evidence for multiple binding sites with qualitatively different properties, *J. Biol. Chem.*, 247, 60, 1972.
326. **Rigo, A., Viglino, P., Rotilio, G., and Tomat, R.,** Effect of ionic strength on the activity of bovine superoxide dismutase, *FEBS Lett.*, 50, 86, 1975.

327. **Rotilio, G., Morpurgo, L., Calabrese, L., Finazzi-Agro, A., and Mondovi, B.,** Metal-ligand interactions in Cu-proteins, in *Metal Ligands Interactions in Organic Chemistry and Biochemistry*, part 1, Pullman, B. and Goldblum, N., Eds., D. Reidel, Dordrecht, 1977, 243.
328. **Rigo, A., Stevanato, R., Viglino, P., and Rotilio, G.,** Competitive inhibition of Cu,Zn superoxide dismutase by monovalent anions, *Biochem. Biophys. Res. Commun.*, 79, 776, 1977.
329. **Bertini, I., Luchinat, C., and Scozzafava, A.,** Superoxide dismutase-thiocyanate: a study of the binding sites of anions on copper(II) in superoxide dismutase, *J. Am. Chem. Soc.*, 102, 7349, 1980.
330. **Strothkamp, K. G. and Lippard, S. J.,** Anion binding to the ferri-copper form of bovine erythrocyte superoxide dismutase; mechanistic implications, *Biochemistry*, 20, 7488, 1981.
331. **Marmocchi, F., Mavelli, I., Rigo, A., Stevanato, R., Bossa, F., and Rotilio, G.,** Succinylated copper, zinc superoxide dismutase. A novel approach to the problem of active subunits, *Biochemistry*, 21, 2853, 1982.
332. **Cocco, D., Rossi, L., Barra, D., Bossa, F., and Rotilio, G.,** Carbomaylation of Cu,Zn-superoxide dismutase by cyanate. Role of lysines in the enzyme action, *FEBS Lett.*, 150, 303, 1982.
333. **Cudd, A. and Fridovich, I.,** Electrostatic interactions in the reaction mechanism of bovine erythrocyte superoxide dismutase, *J. Biol. Chem.*, 257, 11443, 1982.
334. **Koppenol, W. H.,** The physiological role of the charge distribution of superoxide dismutase, in *Oxygen and Oxy-Radicals in Chemistry and Biology*, Rodgers, M. A. J. and Powers, E. L., Eds., Academic Press, New York, 1981, 671.
335. **Rotilio, G., Morpurgo, L., Calabrese, L., and Mondovi, B.,** On the mechanism of superoxide dismutase. Reaction of the bovine enzyme with hydrogen peroxide and ferrocyanide, *Biochim. Biophys. Acta*, 302, 229, 1973.
336. **Fee, J. A. and Dicorleto, P. E.,** Observations on the oxidation-reducton properties of bovine erythrocyte superoxide dismutase, *Biochemistry*, 12, 4893, 1973.
337. **Blackburn, N. J., Hasnain, S. S., Binsted, N., Diakun, G. P., Garner, C. D., and Knowles, P. F.,** An extended-X-ray-absorption-fine-structure study of bovine erythrocyte superoxide dismutase in aqueous solution. Direct evidence for three-co-ordinate Cu(I) in reduced enzyme, *Biochem. J.*, 219, 985, 1984.
338. **Terenzi, M., Rigo, A., Franconi, C., Mondovi, B., Calabrese, L., and Rotilio, G.,** pH Dependence of the nuclear magnetic relaxation rate of solvent water protein in solutions of bovine superoxide dismutase, *Biochim. Biophys. Acta*, 351, 230, 1974.
339. **Rigo, A., Viglino, P., Argese, E., Terenzi, M., and Rotilio, G.,** Nuclear magnetic relaxation of ^{19}F as a novel assay method of superoxide dismutase, *J. Biol. Chem.*, 254, 1759, 1979.
340. **Fee, J. A., Peisach, J., and Mims, W. B.,** Superoxide dismutase. Examination of the metal binding sites by electron spin echo spectroscopy, *J. Biol. Chem.*, 256, 1910, 1981.
341. **Van Camp, H. L., Sands, R. H., and Fee, J. A.,** An examination of the cyanide derivative of bovine superoxide dismutase with electron-nuclear double resonance, *Biochim. Biophys. Acta*, 704, 75, 1982.
342. **Richardson, D. C.,** personal communication,
343. **Rotilio, G., Calabrese, L., Bossa, F., Barra, D., Finazzi-Agro, A., and Mondovi, B.,** Properties of the apoprotein and role of copper and zinc in protein conformation and enzyme activity of bovine superoxide dismutase, *Biochemistry*, 11, 2182, 1972.
344. **Rotilio, G., Morpurgo, L., Giovagnoli, C., Calabrese, L., and Mondovi, B.,** Studies of the metal sites of copper proteins. Symmetry of copper in bovine superoxide dismutase and its functional significance, *Biochemistry*, 11, 2187, 1972.
345. **Fee, J. A. and Ward, R. L.,** Evidence for a coordination position available to solute molecules on one of the metals at the active center of reduced bovine superoxide dismutase, *Biochem. Biophys. Res. Commun.*, 71, 427, 1976.
346. **Viglino, P., Scarpa, M., Coin, F., Rotilio, G., and Rigo, A.,** Oxidation of reduced Cu, Zn superoxide dismutase by molecular oxygen. A kinetic study, *Biochem J.*, 237, 305, 1986.
347. **Pantoliano, M. W., Valentine, J. S., Burger, A. R., and Lippard, S. J.,** A pH-dependent superoxide dismutase activity for zinc-free bovine erythrocuprein. Reexamination of the role of zinc in the holoprotein, *J. Inorg. Biochem.*, 17, 325, 1982.
348. **Rotilio, G., Bray, R. C., and Fielden, E. M.,** A pulse radiolysis study of superoxide dismutase, *Biochim. Biophys. Acta*, 268, 605, 1972.
349. **Bannister, J. V., Bannister, W. H., Bray, R. C., Fielden, E. M., Roberts, P. B., and Rotilio, G.,** The superoxide dismutase activity of human erythrocuprien, *FEBS Lett.*, 32, 303, 1973.
350. **Calabrese, L., Cocco, D., Morpurgo, L., Mordovi, B., and Rotilio, G.,** Reversible uncoupling of the copper and cobalt spin systems in cobalt bovine superoxide dismutase at low pH, *FEBS Lett.*, 59, 29, 1975.
351. **Rigo, A., Viglino, P., and Rotilio, G.,** Reduction and oxidation of bovine superoxide dismutase by H_2O_2, in *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Rotilio, G., Ed., Elsevier, Amsterdam, 1986, 184.
352. **Hodgson, E. K. and Fridovich, I.,** Reversal of the superoxide dismutase reaction, *Biochem. Biophys. Res. Commun.*, 54, 270, 1973.

353. **Calabrese, L., Rotilio, G., and Mondovi, B.,** Cobalt erythrocuprein: preparation and properties, *Biochim. Biophys. Acta,* 263, 827, 1972.
354. **Rotilio, G., Calabrese, L., Mondovi, B., and Blumberg, W. E.,** Electron paramagnetic resonance studies of cobalt-copper bovine superoxide dismutase, *J. Biol. Chem.*, 249, 3157, 1974.
355. **Richardson, J. S., Thomas, K. A., Rubin, B. H., and Richardson, D. C.,** Crystal structure of bovine Cu,Zn superoxide dismutase at 3 Å resolution; chain tracing and metal ligands, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 1349, 1975.
356. **Calabrese, L., Cocco, D., Morpurgo, L., Mondovi, B., and Rotilio, G.,** Cobalt bovine superoxide dismutase. Reactivity of the cobalt chromosphore in the copper-containing and in the copper-free enzyme, *Eur. J. Biochem.*, 64, 465, 1976.
357. **Bailey, D. B., Ellis, P. D., and Fee, J. A.,** Cadmium-113 nuclear magnetic resonance studies of cadmium substituted derivatives of bovine superoxide dismutase, *Biochemistry*, 19, 591, 1980.
358. **Bertini, I., Lanini, G., Luchinat, C., Messori, L., Monnanni, R., and Scozzafava, A.,** Investigation of Cu_2Co_2SOD and its anion derivatives. ^1H NMR and electronic spectra, *J. Am. Chem. Soc.*, 105, 4391, 1985.
359. **Strothkamp, K. G. and Lippard, S. J.,** Lyophilization results of cleavage of the active-site histidine bridge in the four-copper form of bovine erythrocyte superoxide dismutase, *J. Am. Chem. Soc.*, 104, 852, 1982.
360. **Calabrese, L. and Rotilio, G.,** Electron paramagnetic resonance in the study of binuclear centres of copper protein, in *Structure and Function of Hemocyanin*, Bannister, J. V., Ed., Springer-Verlag, Berlin, 1977, 180.
361. **Desideri, A., Coin, F., Morpurgo, L., Cocco, D., Calabrese, L., Mondovi, B., Maret, W., and Rotilio, G.,** X-ray absorption edge spectroscopy of Co(II)-binding sites of copper- and zinc-containing proteins, *Biochim. Biophys. Acta,* 670, 312, 1981.
362. **Desideri, A., Morante, S., Cocco, D., and Rotilio, G.,** EXAFS study at the copper K edge of the derivative of bovine superoxide dismutase modified at the zinc site, in *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Rotilio, G., Ed., Elsevier, Amsterdam, 1986, 161.
363. **Moss, T. H. and Fee, J. A.,** On the magnetic properties of cobalt substituted bovine superoxide dismutase derivatives, *Biochem. Biophys. Res. Commun.*, 66, 799, 1975.
364. **Desideri, A., Morpurgo, L., Cocco, D., and Rotilio, G.,** A room temperature magnetic susceptibility study on the cobalt derivative of cuprozinc superoxide dismutase, *J. Inorg. Biochem.*, 26, 149, 1986.
365. **Borders, C. L., Jr. and Fridovich, I.,** A comparison of the effects of cyanide, hydrogen peroxide and phenylglyoxal in eucaryotic and procaryotic Cu,Zn superoxide dismutase, *Arch. Biochem. Biophys.*, 241, 472, 1985.
366. **Mota de Freitas, D. and Valentine, J. S.,** Phosphate is an inhibitor of copper-zinc superoxide dismutase, *Biochemistry*, 23, 2079, 1984.
367. **Rigo, A., Terenzi, M., Franconi, C., Mondovi, B., Calabrese, L., and Rotilio, G.,** A nuclear magnetic relaxation study of Co(II) bovine superoxide dismutase. Evidence that the Co(II) site is exchange-coupled to the copper and not accessible to the solvent, *FEBS Lett.*, 39, 154, 1974.
368. **Calabrese, L., Cocco, D., and Rotilio, G.,** Physico-chemical studies of Cu-Zn superoxide dismtase, in *Oxy-Radicals and Their Scavenger Systems, Molecular Aspects*, Vol. I, Cohen, G. and Greenwald, R. A., Eds., Elsevier, Amsterdam, 1983, 179.
369. **Bull, C. and Fee, J. A.,** Steady-state kinetic studies of superoxide dismutases: properties of the iron containing protein from *Escherichia coli*, *J. Am. Chem. Soc.*, 107, 3295, 1985.
370. **Osman, R. and Basch, H.,** On the mechanism of action of superoxide dismutase: theoretical study, *J. Am. Chem. Soc.*, 106, 5710, 1984.
371. **Valentine, J. S. and De Freitas, D. M.,** NMR studies of the anion binding sites of oxidized and reduced bovine copper-zinc superoxide dismutase, in *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Rotilio, G., Ed., Elsevier, Amsterdam, 1986, 149.
372. **Roberts, P. B., Fielden, E. M., Rotilio, E., Calabrese, L., Bannister, J. V., and Bannister, W. H.,** Superoxide dismutase inactivation by radiation-induced radicals: evidence for histidine residues in the active site, *Radiat. Res.*, 60, 441, 1974.
373. **Argese, E., Rigo, A., Viglino, P., Orsega, E., Marmocchi, F., Cocco, D., and Rotilio, G.,** A study of the pH dependence of the activity of porcine Cu,Zn superoxide dismutase, *Biochim. Biophys. Acta*, 787, 205, 1984.
374. **Bermingham-McDonogh, O., Mota de Freitas, D., Kunamoto, A., Saunders, J. E., Blech, D. M., Borders, C. L., Jr., and Valentine, J. S.,** Reduced anion-binding affinity of Cu,Zn superoxide dismutases chemically modified at arginine, *Biochem. Biophys. Res. Commun.*, 108, 1376, 1982.
375. **Salin, M. L. and Wilson, W. W.,** Porcine superoxide dismutase. Isolation and characterization of a relatively basic cuprozinc enzyme, *Mol. Cell. Biochem.*, 36, 157, 1981.
376. **Marmocchi, F., Argese, E., Rigo, A., Mavelli, I., Rossi, L., and Rotilio, G.,** A comparative study of bovine, porcine and yeast super-oxide dismutases, *Mol. Cell. Biochem.*, 51, 161, 1983.

377. **Koppenol, W. H.,** personal communication.
378. **Malinowski, D. P. and Fridovich, I.,** Bovine erythrocyte superoxide dismutase: diazo coupling, subunit interactions, and electrophoretic variants, *Biochemistry*, 18, 237, 1979.
379. **Rigo, A., Marmocchi, F., Cocco, D., Viglino, P., and Rotilio, G.,** On the quaternary structures of copper-zinc superoxide dismutases. Reversible dissociation into protomers of the isozyme I from wheat germ, *Biochemistry*, 17, 534, 1978.
380. **Civalleri, L., Pini, C., Rigo, A., Federico, R., Calabrese, L., and Rotilio, G.,** Isolation and preliminary characterization of electrophoretic variants of copper, zinc superoxide dismutase, *Mol. Cell. Biochem.*, 47, 3, 1982.
381. **Cockle, S. A. and Bray, R. C.,** Do all the copper atoms in bovine superoxide dismutase function in catalysis?, in *Superoxide and Superoxide Dismutases*, Michelson, A. M., McCord, J. M., and Fridovich, I., Eds. Academic Press, London, 1977, 215.
382. **Lawrence, G. D. and Sawyer, D. T.,** Potentiometric titrations and oxidation-reduction potentials of manganese and copper-zinc super-oxide dismutases, *Biochemistry*, 18, 3045, 1979.
383. **Rigo, A., Viglino, P., Calabrese, L., Cocco, D., and Rotilio, G.,** The binding of copper ions to copper-free bovine superoxide dismutase. Copper distribution in protein samples recombined with less than stoichometric copper ion/protein ratios, *Biochem. J.*, 161, 27, 1977.
384. **Rigo, A., Terenzi, M., Viglino, P., Calabrese, L., Cocco, D., and Rotilio, G.,** The binding of copper ions to copper-free bovine superoxide dismutase. Properties of the protein recombined with increasing amounts of copper ions, *Biochem. J.*, 161, 31, 1977.
385. **Rigo, A., Viglino, P., Bonori, M., Cocco, D., Calabrese, L., and Rotilio, G.,** The binding of copper ions to copper-free bovine super-oxide dismutase. Kinetic aspects, *Biochem. J.*, 169, 277, 1978.
386. **Cocco, D., Calabrese, L., Rigo, A., Marmocchi, F., and Rotilio, G.,** Preparation of selectively metal-free and metal-substituted derivatives by reaction of Cu-Zn superoxide dismutase by diethyldithiocarbonate, *Biochem. J.*, 199, 675, 1981.
387. **McLachlan, A. D.,** Repeated folding patterns in copper-zinc superoxide dismutase, *Nature (London)*, 285, 267, 1980.
388. **Stallings, W. C., Powers, T. B., Pattridge, K. A., Fee, J. A., and Ludwig, M. L.,** Iron superoxide dismutase from *Escherichi coli* at 3.1-Å resolution: a structure unlike that of copper/zinc protein at both monomer and dimer levels, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 3884, 1983.
389. **Ringe, D., Petsko, G. A., Yamakura, F., Suzuki, K., and Ohmori, D.,** Structure of iron superoxide dismutase from *Pseudomonas ovalis*, at 2.9-Å resolution, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 3879, 1983.
390. **Brock, C. J., Harris, J. I., and Sato, S.,** Superoxide dismutase from *Bacillus stearothermophilus*. Preparation of stable apoprotein and reconstitution of fully active Mn enzyme, *J. Mol. Biol.*, 107, 175, 1976.
391. **Ose, D. E. and Fridovich, I.,** Superoxide dismutase reversible removal of manganese and its substitution by cobalt, nickel or zinc, *J. Biol. Chem.*, 251, 1217, 1976.
392. **Ose, D. E. and Fridovich, I.,** Manganese-containing superoxide dismutase from *Escherichia coli:* reversible resolution and metal replacements, *Arch. Biochem. Biophys.*, 194, 360, 1979.
393. **Yamakura, F. and Suzuki, K.,** Cadmium, chromium, and manganese replacement for iron in iron-superoxide dismutase from *Pseudomonas ovalis*, *J. Biochem.*, 988, 191, 1980.
394. **Richardson, J. S., Richardson, D. C., Thomas, K. A., Silverton, E. W., and Davies, D. R.,** Similarity of three-dimensional structure between the immunoglobin domain and the copper-zinc superoxide dismutase subunit, *J. Mol. Biol.*, 102, 221, 1976.
395. **Villafranca, J. J., Yost, F. J., and Fridovich, I.,** Magnetic resonance studies of manganese(III) and iron(III) superoxide dismutase, *J. Biol. Chem.*, 249, 3532, 1974.
396. **Villafranca, J. J.,** EPR spectra of Fe(III)-superoxide dismutase with special reference to the electron spin relaxation time of Fe(III), *FEBS Lett.*, 62, 230, 1976.
397. **Benovic, J., Tillman, T., Cudd, A., and Fridovich, I.,** Electrostatic facilitation of the reaction catalysed by the manganese-containing and the iron-containing superoxide dismutases, *Arch. Biochem. Biophys.*, 221, 321, 1983.
398. **Bull, C. and Fee, J. A.,** Steady-state kinetic studies of superoxide dismutase: properties of the iron containing protein from *Escherichia coli*, *J. Am. Chem. Soc.*, 107, 3295, 1985.
399. **Terenzi, M., Rigo, A., Franconi, C., Mondovi, B., Calabrese, L., and Rotilio, G.,** pH Dependence of the nuclear magnetic resonance relaxation rate of solvent water protons in solutions of bovine superoxide dismutase, *Biochim. Biophys. Acta*, 351, 230, 1974.
400. **Fee, J. A., McClune, G. J., Lees, A. C., Zidovetzki, R., and Pecht, I.,** The pH dependence of the spectral and anion binding properties of iron containing superoxide dismutase from *E. coli* B: an explanation for the azide inhibition of dismutase activity, *Isr. J. Chem.*, 21, 54, 1981.
401. **Fridovich, I.,** The biology of oxygen radicals, *Science*, 201, 875, 1978.
402. **Fridovich, I.,** Superoxide radical: an endogenous toxicant, *Annu. Rev. Pharmacol. Toxicol.*, 23, 239, 1983.

403. **Sawyer, D. T. and Valentine, J. S.,** How super is superoxide?, *Acc. Chem. Res.*, 14, 393, 1981.
404. **Fee, J. A.,** Is superoxide important in oxygen poisoning?, *Trends Biochem. Sci.*, March, 84, 1982.
405. **Del Maestro, R. F., Thaw, H. H., Bjork, J., Planker, M., and Arfors, K.-E.,** Free radicals as mediators of tissue injury, *Acta Physiol. Scand.*, 492, 43, 1980.
406. **Halliwell, B.,** Superoxide and superoxide-dependent formation of hydroxyl radicals are important in oxygen toxicity, *Trends Biochem. Sci.*, August, 270, 1982.
407. **Freeman, B. A. and Crapo, J. D.,** Free radicals and tissue injury, *Lab. Invest.*, 47, 412, 1982.
408. **Bannister, W. H.,** Superoxide dismutase and disease, *Dev. Biochem.*, 26, 208, 1984.
409. **Hartz, J. W., Funakoshi, S., and Deutsch, H. F.,** The levels of superoxide dismutase and catalase in human tissues as determined immunochemically, *Clin. Chim. Acta*, 46, 125, 1973.
410. **Matkovics, B., Novak, R., Hanh, H. D., Szabo, L., and Varga, S. I.,** A comparative study of some more important experimental animal peroxide metabolism enzymes, *Comp. Biochem. Physiol.*, 56B, 397, 1977.
411. **Grankvist, K., Marklund, S. L., and Taljedal, I. B.,** CuZn superoxide dismutase, Mn superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse, *Biochem. J.*, 199, 393, 1981.
412. **Westman, N. G. and Marklund, S. L.,** Copper- and zinc-containing superoxide dismutase and manganese-containing superoxide dismutase in human tissues and human malignant tumours, *Cancer Res.*, 41, 2962, 1981.
413. **Marklund, S. L., Westman, N. G., Lundgren, E., and Roos, G.,** Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues, *Cancer Res.*, 42, 1955, 1982.
414. **Michelson, A. M., Puget, K., Durosay, P., and Bonneau, J. C.,** Clinical aspects of the dosage of erythrocuprein, in *Superoxide and Superoxide Dismutases*, Michelson, A. M., McCord, J. M., and Fridovich, I., Eds., Academic Press, London, 1977, 417.
415. **Halliwell, B. and Gutteridge, J. M. C.,** Oxygen toxicity, oxygen radicals, transition metals and disease, *Biochem. J.*, 219, 1, 1984.
416. **Bielski, B. H. J., Arudi, R. L., and Sutherland, M. W.,** A study of the reactivity of $HO_2^{\cdot}/O_2^{-}$ with unsaturated fatty acids, *J. Biol. Chem.*, 258, 4758, 1983.
417. **Michelson, A. M.,** Superoxide dismutases, in *Metalloprotein, Structure, Molecular Function and Clinical Aspects*, Weser, U., Ed., Georg Theime, Stuttgart, 1979, 88.
418. **Sinet, P. M.,** Metabolism of oxygen derivatives in Down's syndrome, *Ann. N.Y. Acad. Sci.*, 3965, 83, 1982.
419. **Tan, Y. H., Tischfield, J., and Ruddle, F. H.,** The linkage of genes for the human interferon-induced antiviral protein and indophenol oxidase B traits to chromosome-21, *J. Exp. Med.*, 137, 317, 1973.
420. **Creau-Goldberg, N., Cochet, C., Turleau, C., Finaz, C., and De Grouchy, J.,** Comparative gene mapping of man and *Cebus capucinus* for PGD, ENO 1, PGM 2, and SOD 1, *Cytogenet. Cell Genet.*, 28, 140, 1980.
421. **Leong, M. M. L., Lin, C. C., and Ruth, R. F.,** Assignment of superoxide dismutase (SOD-1) gene to chromosome no. 9 of domestic pig, *Can. J. Genet. Cytol.*, 25, 233, 1983.
422. **Francke, U. and Taggart, R. T.,** Assignment of the gene for cytoplasmic superoxide dismutase *(Sod-1)* to a region of chromsome 16 and of *Hprt* to a region of the X chromosome in the mouse, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 5230, 1979.
423. **Lin, P. F., Slate, D. L., Lawyer, F. C., and Ruddle, F. H.,** Assignment of the mouse interferon sensitivity and cytoplasmic superoxide dismutase genes to chromosome 16, *Science*, 209, 285, 1980.
424. **Cox, D. R., Epstein, L. B., and Epstein, C. J.,** Genes coding for sensitivity to interferon *(Ifrec)* and soluble superoxide dismutase *(SOD-1)* are linked in mouse and man and map to mouse chromosome 16, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 2168, 1980.
425. **Creagan, R., Tischfield, J., Ricciuti, F., and Ruddle, F. H.,** Chromosome assignments of genes in man using mouse-human somatic cell hybrids: mitochondrial superoxide dismutase (indophenol oxidase-B, tetrameric) to chromosome 6, *Humangenetik*, 20, 203, 1973.
426. **Smith, M., Turner, B. M., Tanigaki, N., and Hirschhorn, K.,** Regional localization of HLA, ME_S and SOD_M on chromosome 6, *Cytogenet. Cell Genet.*, 22, 428, 1978.
427. **Szymura, J. M., Wabl, M. R., and Klein, J.,** Mouse mitochondrial superoxide dismutase locus is on chromosome 17, *Immunogenetics*. 14, 231, 1981.
428. **Novak, R., Bosze, Z., Matkovics, B., and Fachet, J.,** Gene affecting superoxide dismutase activity linked to the histocompatibility complex in H-2 congenic mice, *Science*, 207, 86, 1980.
429. **Bloor, J. H., Holtz, D., Kaars, J., and Kosman, D. J.,** Characterization of superoxide dismutase (SOD-1 and SOD-2) activities in inbred mice: evidence for quantitative variability and possible nonallelic SOD-1 polymorphism, *Biochem. Genet.*, 21, 349, 1983.
430. **Hagemeijer, A. and Smit, E. M. E.,** Partial trisomy 21: further evidence that trisomy of band 21q22 is essential for Down's phenotype, *Hum. Genet.*, 38, 15, 1977.

431. **Sichitiu, S., Sinet, P. M., Lejeune, J., and Frezal, J.,** Overactivity of the indophenol oxidase (form dimeric) in trisomy 21, secondary to triplicated gene, *Humangenetik*, 23, 65, 1974.
432. **Sinet, P. M., Allard, D., Lejeune, J., and Jerome, H.,** Increased erythrocyte superoxide dismutase activity in trisomy for chromosome 21, *C.R. Acad. Sci. Ser. D.*, 278, 3267, 1974.
433. **Crosti, N., Serra, A., Rigo, A., and Viglino, P.,** Dosage effect of SOD-A gene in 21-trisomic cells, *Hum. Genet.*, 31, 197, 1976.
434. **Gilles, L., Ferradini, C., Foos, J., Pucheault, J., Allard, D., Sinet, P. M., and Jerome, H.,** The estimation of red cell superoxide dismutase activity by pulse radiolysis in normal and trisomic 21 subject, *FEBS Lett.*, 69, 55, 1976.
435. **Frants, R. R., Eriksson, A. W., Jongbloet, P. H., and Hamers, A. J.,** Superoxide dismutase in Down's syndrome, *Lancet*, 2, 42, 1975.
436. **Mattei, J. F., Baeteman, M. A., Baret, A., Ardissone, J. P., Rebuffel, P., and Giraud, F.,** Erythrocyte superoxide dismutase and redox enzymes in trisomy 21, *Acta Paediatr. Scand.*, 71, 589, 1982.
437. **Sinet, P. M., Lavelle, F., Michelson, A. M., and Jerome, H.,** Superoxide dismutase activities of blood platelets in trisomy 21, *Biochem. Biophys. Res. Commun.*, 67, 904, 1975.
438. **Feaster, W. W., Kwok, L. W., and Epstein, C. J.,** Dosage effects for superoxide dismutase-1 in nucleated cells aneuploid for chromosome 21, *Am. J. Hum. Genet.*, 29, 563, 1977.
439. **Frischer, H., Chu, L. K., Ahmad, T., Justice, P., and Smith, G. F.,** Superoxide dismutase and glutathione peroxidase abnormalities in erythrocytes and lymphoid cells in Down's syndrome, *Prog. Clin. Biol. Res.*, 55, 269, 1981.
440. **Baret, A., Baeteman, M. A., Mattei, J. F., Michel, P., Brousolle, B., and Giraud, F.,** Immunoreactive CuSOD and MnSOD in the circulating blood cells from normal and trisomy 21 subjects, *Biochem. Biophys. Res. Commun.*, 98, 1035, 1981.
441. **Baeteman, M. A., Baret, A., Courtiere, A., Rebuffel, P., and Mattei, J. F.,** Immunoractive Cu-SOD and Mn-SOD in lymphocyte sub-populations from normal and trisomy 21 subjects according to age, *Life Sci.*, 32, 895, 1983.
442. **Sinet, P. M., Couturier, J., Dutrillaux, B., Poisonnier, M., Raoul, O., Rethore, M.-O., Allard, D., Lejeune, J., and Jerome, H.,** Trisomy 21 and superoxide dismutase-1 (IPO-A indophenol oxidase A). Attempt to localize the 21q22.1 subband, *Exp. Cell Res.*, 97, 47, 1976.
443. **Philip, T., Fraisse, J., Sinet, P. M., Lauras, B., Robert, J. M., and Freycon, F.,** Confirmation of the assignment of the human SOD, gene to chromosome 21q22, *Cytogenet. Cell Genet.*, 22, 521, 1978.
444. **Yamamoto, Y., Ogasawara, N., Gotoh, A., Komiya, H., Nakai, H., and Kuroki, Y.,** A case of 21q-syndrome with normal SOD-1 activity, *Hum. Genet.*, 48, 321, 1979.
445. **Habedank, M. and Rodewald, A.,** Moderate Down's syndrome in three siblings having partial trisomy 21q22.2 to qter and therefore no SOD-1 excess, *Hum. Genet.*, 60, 74, 1982.
446. **Wulfsberg, E. A., Carrell, R. E., Klisak, I. J., O'Brien, T. J., Sykes, J. A., and Sparkes, R. S.,** Normal superoxide dismutase-1 (SOD-1) activity with deletion of chromosome band 21q21 supports localization of SOD-1 locus to 21q22, *Hum. Genet.*, 64, 271, 1983.
447. **Yoshimitsu, K., Hatano, S., Kobayashi, Y., Takeoka, Y., Hayashidani, M., Ueda, K., Numura, K., Ohama, K., and Usui, T.,** A case of 21q-syndrome with half normal SOD-1 activity, *Hum. Genet.*, 64, 200, 1983.
448. **Leschot, N. J., Slater, R. M., Joenje, H., Becker-Bloemkolk, M. J., and De Nef, J. J.,** Sod-A and chromosome 21. Conflicting findings in a familial translocation (9p24;21q214), *Hum. Genet.*, 57, 220, 1981.
449. **Kedziora, J., Bartosz, G., Leyko, W., and Rozynkowa, D.,** Dismutase activity in translocation trisomy, *Lancet*, 1, 105, 1979.
450. **Garber, P., Sinet, P. M., Jerome, H., and Lejeune, J.,** Copper/zinc superoxide dismutase activity in trisomy 21 by translocation, *Lancet*, 2, 914, 1979.
451. **Crosti, N., Rigo, A., Stevanato, R., Bajer, J., Neri, G., Bova, R., and Serra, A.,** Lack of position effect on the activity of the $SOD^{Cu/Zn}$ gene in subjects with 21/D and 21/G Robertsonian translocations, *Hum. Genet.*, 57, 203, 1981.
452. **Jeziorowska, A., Jakubowski, L., Armatys, A., and Kaluzewski, B.,** Copper/zinc superoxide dismutase (SOD 1) activity in regular trisomy 21, trisomy 21 by translocation and mosaic trisomy 21, *Clin. Genet.*, 22, 160, 1982.
453. **Couturier, J., Dutrillaux, B., Garber, P., Raoul, O., Croquette, M.-F., Fourlinnie, J. C., and Maillard, E.,** Evidence for a correlation between late replication and autosomal gene inactivation in a familiar translocation t(X;21), *Hum. Genet.*, 49, 319, 1979.
454. **Mattei, J. F., Mattei, M. G., Baeteman, M. A., and Giraud, F.,** Trisomy 21 for the region 21 q 223; identification by high resolution R-banding patterns, *Hum. Genet.*, 56, 409, 1981.
455. **Sinet, P. M., Michelson, A. M., Bazin, A., Lejeune, J., and Jerome, H.,** Increase in glutathione peroxidase activity in erythrocytes from trisomy 21 patients, *Biochem. Biophys. Res. Commun.*, 67, 910, 1975.

456. **Sinet, P. M., Lejeune, J., and Jerome, H.,** Trisomy 21 (Down's syndrome). Glutathione peroxidase, hexose monophosphate shunt and I.Q., *Life Sci.*, 24, 29, 1979.
457. **Brooksbank, B. W. L. and Balazs, R.,** Superoxide dismutase and lipoperoxidation in Down's syndrome fetal brain, *Lancet*, 1, 881, 1983.
458. **Heston, L. L.,** Alzheimer's disease, trisomy 21 and myeloproliferative disorders: associations suggesting a genetic diathesis, *Science*, 196, 322, 1977.
459. **Marklund, S. L.,** Superoxide dismutase, catalase and glutathione peroxidase in degenerative diseases, *Clin. Resp. Physiol.*, 17 (Suppl.), 259, 1981.
460. **Brewer, G. J.,** Achromatic regions of tetrazolium stained starch gels: inherited electrophoretic variation, *Am. J. Hum. Genet.*, 19, 674, 1967.
461. **Beckman, G.,** Population studies in northern Sweden. VI. Polymorphism of superoxide dismutase, *Hereditas*, 73, 305, 1973.
462. **Kirjarinta, M., Fellman, J., Gustafson, C., Keisala, E., and Eriksson, A. W.,** Two rare electrophoretic variants of erythrocyte enzymes in Finland, *Scand. J. Clin. Lab. Invest.*, 22(Suppl. 108), 46, 1969.
463. **Beckman, G., Beckman, L., and Nilsson, L.-O.,** A rare homozygous phenotype of superoxide dismutase, SOD 2, *Hereditas*, 75, 138, 1973.
464. **Beckman, G. and Pakarinen, A.,** Superoxide dismutase. A population study, *Hum. Hered.*, 23, 346, 1973.
465. **Eriksson, A. W.,** Genetic polymorphism in Finno-Ugrian populations. Finns, Lapps and Maris, *Isr. J. Med. Sci.*, 9, 1156, 1973.
466. **Welch, S. G. and Mears, G. W.,** Genetic variants of human indophenol oxidase in the Westray Island of the Orkenys, *Hum. Hered.*, 22, 38, 1972.
467. **Carter, N. D., Auton, J. A., Welch, S. G., Marshall, W. H., and Fraser, G. R.,** Superoxide dismutase variants in Newfoundland — a gene from Scandinavia?, *Hum. Hered.*, 26, 4, 1976.
468. **Weissman, J., Vollmer, M., and Pribilla, O.,** Survey of the distribution of adenosine deaminase and superoxide dismutase markers in different populations, *Hum. Hered.*, 35, 344, 1982.
469. **Crosti, N., Serra, A., Cagiano-Malvezzi, D., and Tagliaferri, I.,** The rare allele SOD-A^2 in the Italian population, *Ann. Hum. Biol.*, 3, 343, 1976.
470. **Papiha, S. S. and Al-Agidi, S. K.,** Esterase D and superoxide dismutase polymorphism in Iraq, *Hum. Hered.*, 26, 394, 1976.
471. **Spedini, G., Capucci, E., Crosti, N., Danubio, M. E., and Romagnoli, S.,** Erythrocyte glyoxalase I and superoxide dismutase polymorphism in the Mbugu and some other populations of the Central African Republic, *Hum. Hered.*, 32, 253, 1982.
472. **Marklund, S., Beckman, G., and Stigbrand, T.,** A comparison between the common type and a rare genetic variant of human cupro-zinc superoxide dismutase, *Eur. J. Biochem.*, 65, 415, 1976.
473. **Shinoda, T., Matsunaga, E., and Koshinaga, J.,** Polymorphism at a second structural locus for tetrazolium oxidase in Japanese, *Jpn. J. Hum. Genet.*, 19, 243, 1974.
474. **Tan, S. G. and Teng, Y. S.,** Saliva acid phosphatase and amylase in Senoi and aboriginal Malays and superoxide dismutase in various racial groups of peninsular Malaysia, *Jpn. J. Hum. Genet.*, 23, 133, 1978.
475. **Beckman, G., Beckman, L., and Nilsson, L.-O.,** Genetics of human superoxide dismutase, *Hereditas*, 79, 43, 1975.
476. **Kellogg, E. W., III and Fridovich, I.,** Liposome oxidation and erythrocyte lysis by enzymically generated superoxide and hydrogen peroxide, *J. Biol. Chem.*, 252, 6721, 1977.
477. **Carrell, R. W., Winterbourn, C. C., and Rachmilewitz, E. A.,** Activated oxygen and haemolysis, *Br. J. Haematol.*, 30, 259, 1975.
478. **Muller, M., Dumdey, R., and Rapoport, S.,** Superoxide radicals in the metabolism of the red cell, *Biomed. Biochim. Acta*, 42(11—12 Suppl.), 297, 1983.
479. **Lynch, R. E. and Fridovich, I.,** Permeation of erythrocyte stroma by superoxide radical, *J. Biol. Chem.*, 253, 4697, 1978.
480. **Hebbel, R. P., Eaton, J. W., Balasingam, M., and Steinberg, M. H.,** Spontaneous oxygen radical generation by sickle erythrocytes, *J. Clin. Invest.*, 70, 1253, 1982.
481. **Fuzukawa, K. and Gebicki, J. M.,** Oxidation of α-tocopherol in micelles and liposomes by the hydroxyl, perhydroxyl and superoxide free radicals, *Arch. Biochem. Biophys.*, 226, 242, 1983.
482. **Brunori, M., Falcioni, G., Fioretti, E., Giardina, B., and Rotilio, G.,** Formation of superoxide in the autoxidation of the isolated α and β chains of human hemoglobin and its involvement in hemichrome precipitation, *Eur. J. Biochem.*, 53, 99, 1975.
483. **Winterbourn, C. C., McGrath, B. M., and Carrell, R. W.,** Reactions involving superoxide and normal and unstable haemoglobins, *Biochem. J.*, 155, 493, 1976.
484. **Kono, Y. and Fridovich, I.,** Superoxide radical inhibits catalase, *J. Biol. Chem.*, 257, 5751, 1982.
485. **McMahon, S. and Stern, A.,** The interrelationship of superoxide dismutase and peroxidatic enzymes in the red cell, *Biochim. Biophys. Acta*, 566, 253, 1979.

486. **Wallace, W. J., Maxwell, J. C., and Caughey, W. S.,** The mechanisms of hemoglobin autoxidation. Evidence for proton-assisted nucleophilic displacement of superoxide by anions, *Biochem. Biophys. Res. Commun.*, 57, 1104, 1974.
487. **Sugawara, Y. and Shikama, K.,** Autoxidation of native oxymyoglobin. Thermodynamic analysis of the pH profile, *Eur. J. Biochem.*, 110, 241, 1980.
488. **Wallace, W. J., Houtchens, R. A., Maxwell, J. C., and Caughey, W. S.,** Mechanisms of autoxidation for hemoglobins and myoglobins. Promotion of superoxide production by protons and anions, *J. Biol. Chem.*, 257, 4966, 1982.
489. **Sutton, H. C., Roberts, P. B., and Winterbourn, C. C.,** The rate of reaction of superoxide radical with oxyhaemoglobin and methaemoglobin, *Biochem. J.*, 155, 503, 1976.
490. **Lynch, F. E., Thomas, J. E., and Lee, G. R.,** Inhibition of methemolgobin formation from purified oxyhemoglobin by superoxide dismutase, *Biochemistry*, 16, 4563, 1977.
491. **Miura, T., Ogawa, N., and Ogiso, T.,** Involvement of superoxide radicals in the formation of methemoglobin from oxyhemoglobin: inhibition of superoxide dismutase by diethyldithiocarbamate, *Chem. Pharm. Bull.*, 26, 1261, 1978.
492. **Miura, T. and Ogiso, T.,** Effect of 3-amino-1,2,4-triazole on catalase and formation of methemoglobin from oxyhemoglobin in erythrocyte by superoxide radicals, *Chem. Pharm. Bull.*, 26, 3450, 1978.
493. **Goldberg, B. and Stern, A.,** Superoxide anion as a mediator of drug-induced oxdiative hemolysis, *J. Biol. Chem.*, 251, 6468, 1976.
494. **Goldberg, B. and Stern, A.,** The role of the superoxide anion as a toxic species in the erythrocyte, *Arch. Biochem. Biophys.*, 178, 218, 1977.
495. **Miura, T. and Ogiso, T.,** Lipid peroxidation of erythrocyte membrane induced by xanthine oxidase system: modification of superoxide dismutase effect by hemoglobin. *Chem. Pharm. Bull.*, 30, 3662, 1982.
496. **Kobayashi, Y., Okahata, S., and Usui, T.,** Hemolysis of human erythrocytes by paraquat in relation to superoxide dismutase activity, *Biochem. Biophys. Res. Commun.*, 91, 1288, 1979.
497. **Okahata, S.,** Mechanism of methylviologen induced hemolysis, *Hiroshima J. Med Sci.*, 29, 49, 1980.
498. **Lynch, R. E. and Fridovich, I.,** Effects of superoxide on the erythrocyte membrane, *J. Biol. Chem.*, 253, 1838, 1978.
499. **Weiss, S. J.,** The role of superoxide in the destruction of erythrocyte targets by human neutrophils, *J. Biol. Chem.*, 255, 9912, 1980.
500. **Weiss, S. J.,** Neutrophil-mediated methemoglobin formation in the erythrocyte: the role of superoxide and hydrogen peroxide, *J. Biol. Chem.*, 257, 2497, 1982.
501. **Falcioni, G. C., Coderoni, S., Tedeschi, G. G., Brunori, M., and Rotilio, G.,** Red cell lysis by microorganisms as a case of superoxide- and hydrogen peroxide-dependent hemolysis mediated by oxyhemoglobin, *Biochim. Biophys. Acta*, 678, 437, 1981.
502. **Lin, P. S., Quamo, S., and Ho, K. C.,** Erythrocyte as scavenger, in *Oxy-Radicals and Their Scavenger Systems, Molecular Aspects*, Vol. I, Cohen, G. and Greenwald, R. A., Eds., Elsevier, New York, 1983, 246.
503. **Bartosz, G., Fried, R., Grzelinska, E., and Leyko, W.,** Effect of hyperoxide radicals on bovine erythrocyte membrane, *Eur. J. Biochem.*, 73, 261, 1977.
504. **Maridonneau, I., Braquet, P., and Garay, R. P.,** Sodium and potassium transport damage induced by oxygen free radicals in human red cell membranes, *J. Biol. Chem.*, 258, 3107, 1983.
505. **Girotti, A. W. and Thomas, J. P.,** Damaging effects of oxygen radicals on resealed erythrocyte ghosts, *J. Biol. Chem.*, 259, 1744, 1984.
506. **Rosen, G. M., Barber, M. J., and Rauckman, E. J.,** Disruption of erythrocyte membrane organization bv superoxide, *J. Biol. Chem.*, 258, 2225, 1983.
507. **Stone, D., Lin, P.-S., and Kwock, L.,** Radiosensitization of human erythrocytes by diethyldithiocarbamate, *Int. J. Radiat. Biol.*, 33, 393, 1978.
508. **Westman, N. G. and Marklund, S. L.,** Diethyldithiocarbamate inhibition of CuZn superoxide dismutase in human erythrocytes: no increase in radiation haemolysis, *Int. J. Radiat. Biol.*, 43, 103, 1983.
509. **Inouye, B., Aono, K., Iida, S., and Utsumi, K.,** Influence of superoxide generating system, vitamin E and superoxide dismutase on radiation consequences, *Physiol. Chem. Phys.*, 11, 151, 1978.
510. **Bartosz, G., Leyko, W., Kedziora, J., and Jeske, J.,** Radiation sensitivity of erythrocytes with an increased level of superoxide dismutase, *Int. J. Radiat. Biol.*, 31, 197, 1977.
511. **Bartosz, G., Leyko, Kedziora, J., and Jeske, J.,** Superoxide dismutase and radiation-induced haemolysis: no benefit of its increased content in red cells, *Int. J. Radiat. Biol.*, 38, 187, 1980.
512. **Kong, S. and Davison, A. J.,** The role of interactions between O_2, H_2O_2, $OH^{\cdot}$, e^-, and O_2^- in free radical damage to biological systems, *Arch. Biochem. Biophys.*, 204, 18, 1980.
513. **Krizala, J. and Ledvina, M.,** The activity of superoxide dismutase in the liver and bone marrow of γ-irradiated rats, *Int. J. Radiat. Biol.*, 37, 459, 1980.
514. **Krizala, J., Kovarova, H., Stoklasova, A., and Ledvina, M.,** Superoxide dismutase levels in various radioresistant and radiosensitive tissue of irradiated rats, *Physiol. Bohemoslov.*, 31, 151, 1982.

515. **Pektau, A., Kelly, K., Chelack, W. S., and Barefoot, C.,** Protective effect of superoxide dismutase on erythrocytes of X-irradiated mice, *Biochem. Biophys. Res. Commun.*, 70, 452, 1976.
516. **Winterbourn, C. C., Hawkins, R. E., Brian, M., and Carrell, R. W.,** The estimation of red cell superoxide dismutase activity, *J. Lab. Clin. Med.*, 85, 337, 1975.
517. **Saito, T., Ito, K., Kurasaki, M., Fujimoto, S., Kaji, H., and Saito, K.,** Determination of true specific activity of superoxide dismutase in human erythrocytes, *Clin. Sci.*, 63, 251, 1982.
518. **Baret, A., Michel, P., Imbert, M. R., Morcellet, J. L., and Michelson, A. M.,** A radioimmunoassay for copper containing superoxide dismutase, *Biochem. Biophys. Res. Commun.*, 88, 337, 1979.
519. **Stansell, M. J. and Deutsch, H. F.,** The levels of catalase and erythrocuprein in human erythrocytes, *Clin. Chim. Acta*, 14, 598, 1966.
520. **Del Villano, B. C. and Tischfield, J. A.,** A radioimmunoassay for human cupro-zonc superoxide dismutase and its application to erythrocytes, *J. Immunol. Methods*, 29, 253, 1979.
521. **Maral, J., Puget, K., and Michelson, A. M.,** Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals, *Biochem. Biophys. Res. Commun.*, 77, 1525, 1977.
522. **Tyan, M. L.,** Age-related increase in erythrocyte oxidant sensitivity, *Mech. Ageing Dev.*, 20, 25, 1982.
523. **Glass, G. A. and Gershon, D.,** Enzymatic changes in rat erythrocytes with increasing cell donor age: loss of superoxide dismutase activity associated with increases in catalytically defective forms, *Biochem. Biophys. Res. Commun.*, 103, 1245, 1981.
524. **Reiss, U. and Gershon, D.,** Rat-liver superoxide dismutase. Purification and age-related modification, *Eur. J. Biochem.*, 63, 617, 1976.
525. **Glass, G. A., Lavie, L., Dovrat, A., Shpund, S., and Gershon, D.,** Further studies on SOD function and properties in different tissues of animals of different ages, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. 2, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 1.
526. **Bartosz, G., Tannert, C., Fried, R., and Leyko, W.,** Superoxide dismutase activity decreases during erythrocyte ageing, *Experientia*, 34, 1464, 1978.
527. **Bartosz, G., Soszynski, M., and Retelewska, W.,** Ageing of the erythrocyte. X. Immunoelectrophoretic studies on the denaturation of superoxide dismutase, *Mech. Ageing Dev.*, 17, 237, 1981.
528. **Vanella, A., Geremia, E., Tiriolo, P., Monachino, M. E., Vanella, G., Marino, M., and Pinturo, R.,** Superoxide dismutase activity and reduced glutathoine content in red blood cells as a function of age, *IRCS Med. Sci.*, 10, 235, 1982.
529. **Mavelli, I., Ciriolo, M. R., Rossi, L., Meloni, T., Forteleoni, G., De Flora, A., Benatti, U., Morelli, A., and Rotilio, G.,** Favism: a hemolytic disease associated with increased superoxide dismutase and decreased glutathione peroxidase activities in red blood cells, *Eur. J. Biochem.*, 139, 13, 1984.
530. **Stevens, C., Goldblatt, M. J., and Freedman, J. C.,** Lack of erythrocyte superoxide dismutase change during human senescence, *Mech. Ageing Dev.*, 4, 415, 1975.
531. **Ueda, K. and Ogata, M.,** Levels of erythrocyte superoxide dismutase activity in Japanese people, *Acta Med. Okayama*, 32, 393, 1978.
532. **Joenje, H., Frants, R. R., Arwert, F., and Eriksson, A. W.,** Specific activity of human erythrocyte superoxide dismutase as a function of donor age. A brief note, *Mech. Ageing Dev.*, 8, 265, 1978.
533. **Bartkowiak, A., Grzelinska, E., and Bartosz, G.,** Ageing of the erythrocyte. XVII. Changes in the properties of superoxide dismutase, *Int. J. Biochem.*, 15, 763, 1983.
534. **Mavelli, I., Ciriolo, M. R., and Rotilio, G.,** Multiple electrophoretic variants of copper, zinc superoxide dismutase as an expression of enzyme ageing. Effects of hydrogen peroxide, ascorbate and metal ions, *Biochem. Biophys. Res. Commmun.*, 117, 677, 1983.
535. **Crosti, N. and Sausa, P.,** Evidence for tissue-specific electromorphs of Cu/Zn SOD unrelated to genetic control, *Biochem. Genet.*, 18, 693, 1980.
536. **Crosti, N.,** Post-translational polymorphism of copper/zinc SOD: hypothesis of a biological role, *Acta Med. Rom.*, 20, 178, 1983.
537. **Kobayashi, Y., Ishigame, K., Ishigame, Y., and Usui, T.,** Superoxide dismutase activity of human blood cells, in *Biochemical and Medical Aspects of Active Oxygen*, Hayaishi, O. and Asada, K., Eds., University of Tokyo Press, Tokyo, 1977, 261.
538. **Yoshioka, T., Suguie, A., Shimada, T., and Utsumi, K.,** Superoxide dismutase activity in the maternal and cord blood, *Biol. Neonat.*, 36, 173, 1979.
539. **Bonta, B. W., Gawron, E. R., and Warshaw, J. B.,** Neonatal red cell superoxide dismutase enzyme levels: possible role as a cellular defence mechanism against pulmonary oxygen toxicity, *Paediatr. Res.*, 11, 754, 1977.
540. **Autor, A. P., Frank, L., and Roberts, R. J.,** Developmental characteristics of pulmonary superoxide dismutase: relationship to idiopathic respiratory distress syndrome, *Paediatr. Res.*, 10, 154, 1976.
541. **Legge, M., Brian, M., Winterbourn, C. C., and Carrell, R.,** Red cell superoxide dismutase activity in the newborn, *Aust. Paediatr. J.*, 13, 25, 1977.

542. **Rotilio, G., Rigo, A., Bracci, R., Bagnoli, F., Sargentini, I., and Brunori, M.,** Determination of red blood cell superoxide dismutase and glutathione in newborns in relation to neonatal hemolysis, *Clin. Chim. Acta*, 81, 131, 1977.
543. **Bracci, R., Ciccoli, L., Falciani, G., De Donno, M., and Dettori, M.,** Vitamin E glutathione peroxidase and superoxide dismutase as possible factors inhibiting oxidative hemolysis in the neonate, *Boll. Soc. Ital. Biol. Sper.*, 54, 879, 1978.
544. **Saik, L. A., Hsieh, H.-L., Baricos, W. H., and Shapira, E.,** Enzymatic and immunologic quantitation of erythrocyte superoxide dismutase in adults and in neonates of different gestational ages, *Paediatr. Res.*, 16, 933, 1982.
545. **Russanov, E. M., Kirkova, M. D., Setchenska, M. S., and Arnstein, H. R. V.,** Enzymes of oxygen metabolism during erythrocyte differentiation, *Biosci. Rep.*, 1, 927, 1981.
546. **Haga, P.,** Ceruloplasmin levels and erythrocyte superoxide dismutase activity in small preterm infants during the early anemia of prematurity, *Acta Paediatr. Scand.*, 70, 861, 1981.
547. **Shields, G. S., Markowitz, H., Klassen, W. H., Cartwright, G. E., and Wintrobe, M. M.,** Studies on copper metabolism. XXXI. Erythrocyte copper, *J. Clin. Invest.*, 40, 2007, 1961.
548. **Gartner, A. and Weser, U.,** Erythrocuprein (Cu_2Zn_2 superoxide dismutase) is the major copper protein of the red blood cell, *FEBS Lett.*, 155, 15, 1983.
549. **Williams, D. M., Lynch, R. E., Lee, G. R., and Cartwright, G. E.,** Superoxide dismutase activity in copper-deficient swine, *Proc. Soc. Exp. Biol. Med.*, 149, 534, 1975.
550. **Bettger, W. J., Fish, T. J., and O'Dell, B. L.,** Effects of copper and zinc status of rats on erythrocyte stability and superoxide dismutase activity, *Proc. Soc. Exp. Biol. Med.*, 158, 279, 1978.
551. **Okahata, S., Nishi, Y., Hatano, S., Kobayashi, Y., and Usui, T.,** Changes in erythrocyte superoxide dismutase in a patient with copper dieficiency, *Eur. J. Paediatr.*, 134, 121, 1980.
552. **Scudder, P., Stocks, J., and Dormandy, T. L.,** The relation between erythrocyte superoxide dismutase activity and erythrocyte copper levels in normal subjects and in patients with rheumatoid arthritis, *Clin. Chim. Acta*, 69, 397, 1976.
553. **Banford, J. C., Brown, D. H., Hazelton, R. A., McNeil, C. J., Sturrock, R. D., and Smith, W. E.,** Serum copper and erythrocyte superoxide dismutase in rheumatoid arthritis, *Ann. Rheum. Dis.*, 41, 458, 1982.
554. **Crouch, R. K. and Chambers, J. K.,** Absence of abnormal erythrocyte superoxide dismutase, copper, or zinc levels in patients with retinitis pigmentosa, *Br. J. Opthalmol.*, 66, 417, 1982.
555. **Polidoro, G., Di Ilio, C., Ardini, A., La Rovere, G., and Federici, G.,** Superoxide dismutase, reduced glutathione and TBA-reactive products in erythrocytes of patients with multiple sclerosis, *Int. J. Biochem.*, 16, 505, 1984.
556. **Marceau, N. and Aspin, N.,** The association of the copper derived from ceruloplasmin with cytocuprein, *Biochim. Biophys. Acta*, 328, 351, 1973.
557. **Bohnenkamp, W. and Weser, U.,** Copper deficiency and erythrocuprein (2Cu, 2Zn superoxide dismutase), *Biochim. Biophys. Acta*, 444, 396, 1976.
558. **Alexander, N. M. and Benson, C. D.,** Superoxide dismutase activity (erythrocuprein) in Wilson's disease, *Life Sci.*, 16, 1025, 1975.
559. **Panchenko, L. F., Lamchingiin, T., Gerasimov, A. M., Sukhanov, Yu. S., and Konoplina, L. A.,** Activity of superoxide dismutase in the blood of children with iron deficiency anemia, *Vopr. Med. Khim.*, 25, 181, 1979.
560. **Okahata, S., Kobayashi, Y., and Usui, T.,** Erythrocyte superoxide dismutase activity in Fanconi's anaemia, *Clin. Sci.*, 58, 173, 1980.
561. **Kobayshi, Y., Yoshimitsu, Y., Okahata, S., and Usui, T.,** Superoxide dismutase activity in rabbit reticulocytes, *Experientia*, 39, 69, 1983.
562. **Auclair, C., Dhermy, D., and Boivin, P.,** Intravascular hemolysis in patients with erythrocyte superoxide dismutase deficiency: further evidence for the protective role of superoxide dismutase in aerobic cells, *Clin. Resp. Physiol.*, 17(Suppl.), 207, 1981.
563. **Agostoni, A., Gerli, G. C., Beretta, L., Bianchi, M., Vignoli, M., and Bombelli, F.,** Superoxide dismutase, catalase and glutathione peroxidase activities in maternal and cord blood erythrocytes, *J. Clin. Chem. Clin. Biochem.*, 18, 771, 1980.
564. **Kobayashi, Y., Okahata, S., Tanabe, K., Ueda, K., and Usui, T.,** Erythrocyte superoxide dismutase activity in hemolytic uremic syndrome, *Hiroshima J. Med. Sci.*, 27, 181, 1978.
565. **Concetti, A., Massei, P., Rotilio, G., Brunori, M., and Rachmilewitz, E. A.,** Superoxide dismutase in red blood cells: method of assay and enzyme content in normal subjects and in patients with β-thalassemia (major and intermedia), *J. Lab. Clin. Med.*, 87, 1057, 1976.
566. **Gerli, G. C., Beretta, L., Bianchi, M., Pellegatta, A., and Agostoni, A.,** Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in β-thalassaemia (major and minor), *Scand. J. Haematol.*, 25, 87, 1980.

567. **Gerli, G. C., Beretta, L., Bianchi, M., and Agostoni, A.,** Erythrocyte superoxide dismutase, catalase and glutathione peroxidase in conditions of augmented oxidant stress, *Clin. Resp. Physiol.*, 17(Suppl.), 201, 1981.
568. **Vanella, A., Rizza, V., Li Volti, S., Pinturo, R., Musumeci, S., and Mollica, F.,** Effect of polyamines on autohemolysis: studies on normal and thalassaemic children, *Acta Haematol.*, 63, 226, 1980.
569. **Yenchitsomanus, P. and Wasi, P.,** Increased erythrocyte superoxide dismutase activities in β^0-thalassaemia/haemoglobin E and in haemoglobin H diseases, *J. Clin. Pathol.*, 36, 329, 1983.
570. **Das, S. K. and Nair, R. C.,** Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes, *Br. J. Haematol.*, 44, 87, 1980.
571. **Sree Kumar, K., Rowse, C., and Hochstein, P.,** Copper-induced generation of superoxide in human red cell membranes, *Biochem. Biophys. Res. Commun.*, 83, 587, 1978.
572. **Chan, P. C., Peller, O. G., and Kesner, L.,** Copper(II)-catalyzed lipid peroxidation in liposomes and erythrocyte membranes, *Lipids*, 17, 331, 1982.
573. **Beretta, L., Gerli, G. C., Ferrarese, R., Agostoni, A., Gualandri, V., and Orsini, G. B.,** Antioxidant system in sickle cells, *Acta Haematol.*, 70, 194, 1983.
574. **Schacter, L. P., Del Villano, B. C., Gordon, E. M., and Klein, B. L.,** Red cell superoxide dismutase and sickle cell anemia sympton severity, *Am. J. Hematol.*, 19, 137, 1985.
575. **Schacter, L. P.,** Enhanced free radical oxygen generation by red cells from individuals with sickle cell trait, *Prog. Clin. Biol. Res.*, 165, 547, 1984.
576. **Allison, A. C. and Eugui, E. M.,** The role of cell-mediated immune responses in resistance to malaria, with special reference to oxidant stress, *Annu. Rev. Immunol.*, 1, 361, 1983.
577. **Clark, I. A. and Hunt, N. H.,** Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria, *Infect. Immun.*, 39, 1, 1983.
578. **Suthipark, U., Krungkrai, J., Jearnpipatkul, A., Yuthavong, Y., and Panijpan, B.,** Superoxide dismutase (SOD) in mouse red blood cells infected with *Plasmodium berghei*, *J. Parasitol.*, 68, 337, 1982.
579. **Kulkarni, A. B., Renapurkar, D. M., and Sharma, K. D.,** Hepatic superoxide dismutase in the mouse infected with *Plasmodium berghei*, *Folia Parasitol. Prague*, 31, 89, 1984.
580. **Joenje, H., Eriksson, A. W., Frants, R. R., Arwert, F., and Houwen, B.,** Erythrocyte superoxide dismutase deficiency in Fanconi's anaemia, *Lancet*, 1, 204, 1978.
581. **Nordenson, I.,** Effect of superoxide dismutase and catalase on spontaneously occurring chromosome breaks in patients with Fanconi's anaemia, *Hereditas*, 86, 147, 1977.
582. **Raj, A. S. and Heddle, J. A.,** The effect of superoxide dismutase, catalase and L-cysteine on spontaneous and on mitomycin C induced chromosomal breakage in Fanconi's anaemia and normal fibroblasts as measured by the micronucleus method, *Mutat. Res.*, 78, 59, 1980.
583. **Nagasawa, H. and Little, J. B.,** Suppression of cytotoxic effect of mitomycin-C by superoxide dismutase in Fanconi's anemia and dyskeratosis congenita fibroblasts, *Carcinogenesis*, 4, 795, 1983.
584. **Emerit, I.,** Chromosome breakage factors: origin and possible significance, *Prog. Mutat. Res.*, 4, 61, 1982.
585. **Joenje, H., Arwert, F., Eriksson, A. W., De Koning, H., and Oostra, A. B.,** Oxygen-dependence of chromosomal aberrations in Fanconi's anaemia, *Nature (London)*, 290, 142, 1981.
586. **Joenje, H., Frants, R. R., Arwert, F., De Bruin, G. J. M., Kostense, P. J., Van de Kamp, J. J. P., de Koning, J., and Eriksson, A. W.,** Erythrocyte superoxide dismutase deficiency in Fanconi's anaemia established by two independent methods of assay, *Scand. J. Clin. Lab. Invest.*, 39, 759, 1979.
587. **Mavelli, I., Ciriolo, M. R., Rotilio, G., De Sole, P., Castorino, M., and Stabile, A.,** Superoxide dismutase, glutathione peroxidase and catalase in oxidative hemolysis. A study of Fanconi's anaemia erythrocytes, *Biochem. Biophys. Res. Commun.*, 106, 286, 1982.
588. **Lammi-Keefe, C. J., Hegarty, P. V. J., and Swan, P. B.,** Effect of starvation and refeeding on catalase and superoxide dismutase activities in skeletal and cardiac muscles from 12-month-old rats, *Experientia*, 37, 25, 1981.
589. **Kar, N. C. and Pearson, C. M.,** Catalase, superoxide dismutase, glutathione peroxidase and thiobarbituric acid-reactive products in normal and dystrophic human muscle, *Clin. Chim. Acta*, 94, 277, 1979.
590. **Mizuno, Y.,** Superoxide dismutase activity in early stages of development in normal and dystrophic chickens, *Life Sci.*, 34, 909, 1984.
591. **Hunter, M. I. S., Brzeski, M. S., and De Vane, P. J.,** Superoxide dismutase, glutathione peroxidase and thiobarbituric acid-reactive compounds in erythrocytes in Duchenne muscular dystrophy, *Clin. Chim. Acta*, 115, 93, 1981.
592. **Burri, B. J., Chan, S. G., Berry, A. J., and Yarnell, S. K.,** Blood levels of superoxide dismutase and glutathione peroxidase in Duchenne muscular dystrophy, *Clin. Chim. Acta*, 105, 249, 1980.
593. **Matkovics, B., Laszlo, A., and Szabo, L.,** A comparative study of superoxide dismutase, catalase and lipid peroxidation in red blood cells from muscular dystrophy patients and normal controls, *Clin. Chim. Acta*, 118, 289, 1982.

594. **Matkovics, B., Gyurkovits, K., Laszlo, A., and Szabo, L.,** Altered peroxide metabolism in erythrocytes from children with cystic fibrosis, *Clin. Chim. Acta*, 125, 59, 1982.
595. **Sinet, P. M., Debray, Q., Carmagnol, F., Pelicier, Y., Nicole, A., and Jerome, H.,** Normal erythrocyte SOD values in two human diseases: schizophrenia and cystic fibrosis, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevir, New York, 1983, 302.
596. **Chauhan, D. P., Gupta, P. H., Nampoothiri, M. R. N., Singhal, P. C., Chugh, K. S., and Nair, C. R.,** Determination of erythrocyte superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, reduced glutathione and malonyldialdehyde in uremia, *Clin. Chim. Acta*, 123, 153, 1982.
597. **Del Villano, B. C., Miller, S. I., Schacter, L. P., and Tischfield, J. A.,** Elevated superoxide dismutase in black alcoholics, *Science*, 207, 991, 1980.
598. **Storozhok, S. A.,** Lipid hydroperoxide content, activity of superoxide dismutase, and glucose-6-phosphate dehydrogenase in erythrocytes in alcohol intoxication, *Vopr. Med. Khim.*, 29, 31, 1983.
599. **Boveris, A., Fraga, C. C., Varsavsky, A. I., and Koch, O. R.,** Increased chemiluminescence and superoxide production in the liver of chronically ethanol-treated rats, *Arch. Biochem. Biophys.*, 227, 534, 1983.
600. **Dreosti, I. E., Manuel, S. J., and Buckley, R. A.,** Superoxide dismutase (EC 1.15.1.1), manganese, and the effect of ethanol in adult and foetal rats, *Br. J. Nutr.*, 48, 205, 1982.
601. **Takahara, S. and Ogata, M.,** Metabolism in Japanese acatalasemia with special reference to superoxide dismutase and glutathione peroxidase, in *Biochemical and Medical Aspects of Active Oxygen*, Hayaishi, O. and Asada, K., Eds., University of Tokyo Press, Tokyo, 1977, 275.
602. **Medeiros, M. H. G., Marchiori, P. E., and Bechara, E. J. H.,** Superoxide dismutase, glutathione peroxidase, and catalase in the erythrocytes of patients with intermittent acute porphyria, *Clin. Chem.*, 28, 242, 1982.
603. **Hagglof, B., Marklund, S. L., and Holmgren, G.,** CuZn superoxide dismutase, Mn superoxide dismutase, catalase and glutathione peroxidase in lymphocytes and erythrocytes in insulin-dependent diabetic children, *Acta Endocrinol.*, 102, 235, 1983.
604. **Babior, B. M.,** Oxygen-dependent microbial killing by phagocytes, *N. Engl. J. Med.*, 298, 659 and 721, 1978.
605. **Badwey, J. A. and Karnovsky, M. L.,** Active oxygen species and the function of phagocytic leukocytes, *Annu. Rev. Biochem.*, 49, 695, 1980.
606. **Fantone, J. C. and Ward, P. A.,** Role of oxygen-derived free radicals and metabolites in leucocyte-dependent inflammatory reactions, *Am. J. Pathol.*, 107, 397, 1982.
607. **Halliwell, B.,** Production of superoxide, hydrogen peroxide and hydroxyl radicals by phagocytic cells: a cause of chronic inflammatory disease?, *Cell Biol. Int. Rep.*, 6, 529, 1982.
608. **Weiss, S. J. and Lo Buglio, A. F.,** Phagocyte generated oxygen metabolites and cellular injury, *Lab. Invest.*, 47, 5, 1982.
609. **Lynch, R. E.,** The metabolism of superoxide anion and its progeny in blood cells, *Top. Curr. Chem.*, 108, 35, 1983.
610. **Huber, W. and Saifer, M. G. P.,** Orgotein, the drug version of bovine Cu-Zn superoxide dismutase. I. A summary account of safety and pharmacology in laboratory animals, in *Superoxide and Superoxide Dismutases*, Michelson, A. M., McCord, J. M., and Fridovich, I., Eds., Academic Press, London, 1977, 517.
611. **Oyanagui, Y.,** Participation of superoxide anions at the prostaglandin phase of carrageenan foot-oedema, *Biochem. Pharmacol.*, 25, 1465, 1976.
612. **McCord, J. M., Stokes, S. H., and Wong, K.,** Superoxide radical as a phagocyte-produced chemical mediator of inflammation, *Adv. Inflammation Res.*, 1, 273, 1979.
613. **Huber, W., Saifer, M. G. P., and Williams, L. D.,** Superoxide dismutase pharmacology and orgotein efficacy: new perspectives, *Dev. Biochem.*, 11B, 395, 1980.
614. **Hirschelmann, R. and Bekemeier, H.,** Effects of catalase, superoxide dismutase and 10 scavengers of oxygen radicals in carrageenan aedema and in adjuvant arthritis of rats, *Experientia*, 37, 1313, 1981.
615. **Bragt, P. C., Bansberg, J. I., and Bonta, I. L.,** Antiinflammatory effects of free radical scavengers and antioxidants. Further support for proinflammatory roles of endogenous hydrogen peroxide and lipid peroxides, *Inflammation*, 4, 289, 1980.
616. **Petrone, W. F., English, D. K., Wong, K., and McCord, J. M.,** Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 1159, 1980.
617. **Pyatak, P. S., Abuchowski, A., and Davis, F. F.,** Preparation of a polyethylene glycol:superoxide dismutase adduct, and an examination of its blood circulation life and anti-inflammatory activity, *Res. Commun. Chem. Pathol. Pharmacol.*, 29, 113, 1980.
618. **Boccu, E., Velo, G. P., and Veronese, F. M.,** Pharmacokinetic properties of polyethylene glycol derivatized superoxide dismutase, *Pharmacol. Res. Commun.*, 14, 113, 1982.

619. **Beauchamp, C. O., Gonias, S. L., Menapace, D. P., and Pizzo, S. V.,** A new procedure for the synthesis of polyethylene glycol protein adducts: effects on function, receptor recognition, and clearance of superoxide dismutase, lactoferrin and α_2-macroglobulin, *Anal. Biochem.*, 131, 25, 1983.
620. **Veronese, F. M., Boccu, E., Shiavon, O., Velo, G. P., Conforti, A., Franco, L., and Milanino, R.,** Anti-inflammatory and pharmacokinetic properties of superoxide dismutase derivatized with polyethylene glycol via active esters, *J. Pharm. Pharmacol.*, 35, 757, 1983.
621. **Wong, K., Cleland, L. G., and Poznansky, M. J.,** Enhanced anti-inflammatory effect and reduced immunogenicity of bovine liver superoxide dismutase by conjugation with homologous albumin, *Agents Actions*, 10, 231, 1980.
622. **Kelly, K., Boux, H., Petkau, A., and Sehon, A.,** On the safety of treatment with bovine superoxide dismutase: production of a humoral antibody response in rabbits with repeated treatment, *Can. J. Physiol. Pharmacol.*, 60, 1374, 1982.
623. **Cleland, L. G., Bielicki, J., Vernon-Roberts, B., and Betts, H. W.,** Superoxide dismutase (SOD) and albumin conjugates with delayed clearance from plasma and body cavities. Is SOD anti-inflammatory?, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 268.
624. **Huber, W. and Menander-Huber, K. B.,** Orgotein, *Clin. Rheum. Dis.*, 6, 465, 1980.
625. **Baret, A., Jadot, G., Valli, M., Brugerolle, B., Puget, K., and Michelson, A. M.,** Pharmacokinetic and anti-inflammatory properties in the rat, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 274.
626. **Chiu, P., Davis, P., and Wong, K.,** Rheumatoid arthritis and superoxide generation in neutrophils, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 379.
627. **Strauss, R. G., Snyder, E. L., Wallace, P. D., and Rosenberger, T. G.,** Oxygen-detoxifying enzymes in neutrophils of infants and their mothers, *J. Lab. Clin. Med.*, 95, 897, 1980.
628. **Rister, M., Bauermeister, K., Gravert, U., and Gladtke, E.,** Superoxide dismutase and glutathione peroxidase in polymorphonuclear leukocytes, *Eur. J. Paediatr.*, 130, 127, 1979.
629. **Rister, M. and Bauermeister, K.,** Superoxide-dismutase and superoxide-radical release in juvenile rheumatoid arthritis, *Klin. Wochenschr.*, 60, 561, 1982.
630. **Salin, M. L. and McCord, J. M.,** Free radicals and inflammation. Protection of phagocytosing leukocytes by superoxide dismutase, *J. Clin. Invest.*, 56, 1319, 1975.
631. **Flohe, L., Martin, W., Loschen, G., and Gunzler, W. A.,** Is leukotriene B_4-induced chemotaxis mediated by superoxide?, *Life Chem. Rep.*, Suppl. 2, 318, 1984.
632. **Martin, W., Loschen, G., Gunzler, W. A., and Flohe, L.,** Superoxide dismutase inhibits LTB_4-induced leukotaxis, *Agents Actions*, 16, 48, 1985.
633. **Parente, L.,** Study on the effect of superoxide dismutase on arachidonic acid metabolism, *Prostaglandins*, 23, 725, 1982.
634. **Oyanagui, Y.,** Steroid-like anti-inflammatory effect of superoxide dismutase in serotonin-, histamine- and kinin-induced edemata of mice: existence of vascular permeability regulating protein(s), *Biochem. Pharmacol.*, 30, 1791, 1981.
635. **Oyanagui, Y.,** Physiological regulation of vascular permeability by endogenous glucocorticoids and active oxygen, *Inflammation*, 7, 81, 1983.
636. **Oyanagui, Y.,** Anti-inflammatory effects of polyamines in serotonin and carrageenan paw edemate — possible mechanism to increase vascular permeability inhibitory protein level which is regulated by glucocorticoids and superoxide radical, *Agents Actions*, 14, 228, 1984.
637. **Del Maestro, R. F., Bjork, J., and Arfors, K.-E.,** Increase in microvascular permeability induced by enzymatically generated free radicals. I. *In vivo* study, *Microvasc. Res.*, 22, 239, 1981.
638. **Del Maestro, R. F., Bjork, J., and Arfors, K.-E.,** Increase in microvascular permeability induced by enzymatically generated free radicals. II. Role of superoxide anion radical, hydrogen peroxide and hydroxyl radical, *Microvasc. Res.*, 22, 255, 1981.
639. **Del Maestro, R. F.,** Role of superoxide anion radicals in microvascular permeability and leukocyte behaviour, *Can. J. Physiol. Pharmacol.*, 60, 1406, 1982.
640. **Del Maestro, R. F., Planker, M., and Arfors, K.-E.,** Evidence for the participation of superoxide anion radical in altering the adhesive interaction between granulocytes and endothelium, *in vivo*, *Int. J. Microcirc. Clin. Exp.*, 1, 105, 1982.
641. **Okabe, E., Todoki, K., Odajima, C., and Ito, H.,** Free radicals-induced changes in mesenteric microvascular dimensions in the anesthetized cat, *Jpn. J. Pharmacol.*, 33, 1233, 1983.
642. **Bjork, J. and Arturson, G.,** Effect of cimetidine, hydrocortisone, superoxide dismutase and catalase on the development of oedema after thermal injury, *Burns Incl. Therm. Inj.*, 9, 249, 1983.
643. **McCormick, J. R., Harkin, M. M., Johnson, K. J., and Ward, P. A.,** Suppression by superoxide dismutase of immune-complex-induced pulmonary alveolitis and dermal inflammation, *Am. J. Pathol.*, 102, 55, 1981.

644. **Sacks, T., Moldow, C. F., Craddock, P. R., Bowers, T. K., and Jacob, H. S.,** Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An *in vitro* model of immune vascular damage, *J. Clin. Invest.*, 61, 1161, 1978.
645. **McCord, J. M.,** Free radicals and inflammation. Protection of synovial fluid by superoxide dismutase, *Science*, 185, 529, 1974.
646. **Halliwell, B.,** Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. Its role in degradation of hyaluronic acid by a superoxide-generating system, *FEBS Lett.*, 96, 238, 1978.
647. **Puig-Parellada, P. and Planas, J. M.,** Synovial fluid degradation induced by free radicals. *In vitro* action of several free radical scavengers and antiinflammatory drugs, *Biochem. Pharmacol.*, 27, 535, 1978.
648. **Greenwald, R. A. and Moy, W. W.,** Effect of oxygen-derived free radicals on hyaluronic acid, *Arthritis Rheum.*, 23, 455, 1980.
649. **Greenwald, R. A.,** The role of oxygen-derived free radicals (ODFR) in connective tissue degredation. III. Studies on hyaluronic acid (HA) depolymerization in inflamed synovial fluids (SF), *Semin. Arthritis Rheum.*, 11 (Suppl. 1), 97, 1981.
650. **Greenwald, R. A. and Moy, W. W.,** Inhibition of collagen gelation by the action of superoxide radical, *Arthritis Rheum.*, 22, 251, 1979.
651. **Greenwald, R. A., Moy, W. W., and Lazarus, D.,** Degradation of cartilage proteoglycans and collagen by superoxide radical, *Arthritis Rheum.*, 19, 799, 1976.
652. **Carp, H. A. and Janoff, A.,** Potential mediator of inflammation. Phagocyte-derived oxidants suppress the elastase-inhibitory capacity of alpha$_1$-proteinase inhibitor *in vitro*, *J. Clin. Invest.*, 66, 987, 1980.
653. **Evans, C. H., Mears, D. C., and Cosgrove, J. L.,** Release of neutral proteinases from mononuclear phagocytes and synovial cells in response to cartilaginous wear particles *in vitro*, *Biochim. Biophys. Acta*, 677, 287, 1981.
654. **Blake, D. R., Hall, N. D., Treby, D. A., Halliwell, B., and Gutteridge, J. M. C.,** Protection against superoxide and hydrogen peroxide in synovial fluid from rheumatoid arthritis, *Clin. Sci.*, 61, 483, 1981.
655. **Igari, T., Kaneda, H., Horiuchi, S., and Ono, S.,** A remarkable increase of superoxide dismutase activity in synovial fluid of patients with rheumatoid arthritis, *Clin. Orthop. Relat. Res.*, 162, 282, 1982.
656. **Goebel, K.-M., Herbert, V., and Storck, U.,** Clinical and intrasynovial efficacy of orgotein treatment in rheumatoid arthritis of the knee joints, in *Oxy Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 255.
657. **Beckmann, R. and Flohe, L.,** The pathogenic role of superoxide radicals in inflammation: efficacy of exogenous superoxide dismutase, *Clin. Resp. Physiol.*, 17(Suppl.), 275, 1981.
658. **Granger, D. N., Rutili, G., and McCord, J. M.,** Superoxide radicals in feline intestinal ischemia, *Gastroenterology*, 81, 22, 1981.
659. **Parks, D. A., Bulkley, G. B., Granger, D. N., Hamilton, S. R., and McCord, J. M.,** Ischemic injury in the cat small intestine: role of superoxide raidcals, *Gastroenterology*, 82, 9, 1982.
660. **Roy, R. S. and McCord, J. M.,** Superoxide and ischemia: conversion of xanthine dehydrogenase to xanthine oxidase, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 145.
661. **Dalsing, M. C., Grosfeld, J. L., Shiffler, M. A., Vane, D. W., Hull, M., Baehner, R. L., and Weber, T. R.,** Superoxide dismutase: a cellular protective enzyme in bowel ischemia, *J. Surg. Res.*, 34, 589, 1983.
662. **Grogaard, B., Parks, D. A., Granger, D. N., McCord, J. M., and Forsberg, J. O.,** Effects of ischemia and oxygen radicals on mucosal albumin clearance in intestine, *Am. J. Physiol.*, 242, G448, 1982.
663. **Parks, D. A. and Granger, D. N.,** Oxygen-derived radicals and ischemia-induced tissue injury, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 135.
664. **Schoenberg, M. H., Younes, M., Muhl, E., Haglund, U., Sellin, D., and Schildberg, F. W.,** Free radical involvement in ischemic damage of small intestine, in *Oxy-Radicals and Their Scavenger Systems, Cellular and Medical Aspects*, Vol. II, Greenwald, R. A. and Cohen, G., Eds., Elsevier, New York, 1983, 154.
665. **Hammond, B., Kontos, H. A., and Hess, M. L.,** Oxygen radicals in the adult respiratory distress syndrome, in myocardial ischemia and reperfusion injury, and in cerebral vascular damage, *Can. J. Physiol. Pharmacol.*, 63, 173, 1985.
666. **McCord, J. M.,** Oxygen-derived free radicals in postischemic tissue injury, *N. Engl. J. Med.*, 312, 159, 1985.
667. **Guarnieri, C., Flamigni, F., and Caldarera, C. M.,** Role of oxygen in the cellular damage induced by reoxygenation of the hypoxic heart, *J. Mol. Cell. Cardiol.*, 12, 797, 1980.
668. **Shlafer, M., Kane, P. F., and Kirsh, M. M.,** Superoxide dismutase plus catalase enhances the efficacy of hypothermic cardioplegia to protect the globally ischemic, reperfused heart, *J. Thorac. Cardiovasc. Surg.*, 83, 830, 1982.

669. **Palma, J. M., Sandalio, L. M., and Del Rio, L. A.,** *J. Plant Physiol.*, 125, 427, 1986.
670. **Barra, D., Schinina, M. E., Bannister, W. H., Bannister, J. V., and Bossa, F.,** *J. Biol. Chem.*, 262, 1001, 1987.