Biochemical Pharmacology, Vol. 39, No. 3, pp. 399–405, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

COMMENTARY

NEW PERSPECTIVES ON THE BIOCHEMISTRY OF SUPEROXIDE ANION AND THE EFFICIENCY OF SUPEROXIDE DISMUTASES

CAROL DEBY* and ROLAND GOUTIER
Laboratoire de Biochimie et Radiobiologie, Institut de Chimie, Sart Tilman, 4000 Liège, Belgium

History of a controversy

The earliest hypothesis concerning the existence of superoxide anion (O_2^-) was put forth in 1931 by Haber and Willstätter [1] during water radiolysis. McCord and Fridovich [2] later postulated that it was generated during the activity of milk xanthine-oxidase, an enzyme capable of oxidizing various organic structures efficiently. The formation of O_2^- by an endothermic reaction appeared to be an intermediate step in the reduction of O_2 to hydrogen peroxide:

$$O_2 + e^- \rightarrow O_2^- . \quad (1)$$

In 1969, superoxide anion was unmistakably identified by electron spin resonance [3] during xanthine-oxidase activity. That same year, McCord and Fridovich [4] proved that erythrocuprein, a blue cuprozinc protein present in erythrocytes [named superoxide dismutase (SOD) by these authors], speeded up the spontaneous dismutation of O_2^- which occurs in protonated media:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2. \quad (2)$$

At first, it appeared that superoxide dismutase provided effective protection against chemical oxidations and cytotoxic phenomena mediated by xanthine-oxidase. For this reason Fridovich and coworkers postulated that O_2^- was the main oxidant produced by xanthine-oxidase. A manganese SOD, in addition to the cytosolic zinc–copper variety, was isolated in mitochondria. Other metalloproteins, functioning in the same way as SOD, were identified in the cells of all aerobic organisms. Subsequently, numerous investigators used SOD to show that O_2^- does indeed play a role in several oxidation processes. To cite only one example, Pederson and Aust [5] claimed that lipoperoxidations mediated by xanthine-oxidase were slowed by SOD. Babior *et al.* [6] demonstrated that bacterial SOD played a protective role during phagocytosis as well. These early observations were later corroborated by many investigators. Furthermore, it was clearly established that O_2^- can be generated by enzymes other than xanthine-oxidase. It was then that members of the staff of B. Chance [7] made an important breakthrough: disturbances in the mitochondrial respiratory chain resulting from an anoxia of varying duration in turn led to the univalent reduction of O_2^- (cf. Eqn 1) [8].

At the same time, though, O_2^- seemed to be a good reducing agent [9] of substances such as ferricytochrome *c*, nitroblue tetrazolium, and tetranitromethane. Elsewhere, these same experiments were conducted to substantiate and measure the generation of O_2^- under biological conditions. The apparent ambivalence of O_2^- under identical conditions puzzled investigators. In an attempt to ascertain the exact physical and chemical properties of O_2^-, many studies were carried out in the 1970s, employing the most rigorous of methods: pulsed radiolysis in combination with fast spectrometry, thermodynamic calculations, etc.

At the end of the decade, Sawyer *et al.* [10] summarized the main findings of these investigations: "O_2^- is a pitifully weak oxidant." One study found, for example, that lipoperoxidations mediated by O_2^- were impossible [11]. As a counterbalance, the nucleophilicity of O_2^-, of apparently limited importance in biological mediums rich in H^+, was found to be of greater significance in aprotic conditions [12]. The role of O_2^- in oxidizing phenomena was thus believed to be of lesser importance, with H_2O_2, produced by its dismutation, now being considered the true agent involved in oxidase activities [13].

As the current decade got under way, then, it was not uncommon to come across articles with sarcastic titles such as "How super is superoxide?" [14]. In 1980, Nanni *et al.* [15] wrote that ". . . the studies to date indicate that O_2^- is fairly innocuous." At the same time, it was widely believed that superoxide dismutases, speeding up as they do the formation of H_2O_2 formation, did not offer protection against oxygen toxicity.

Thus, two opposing opinions concerning superoxide reactivity met head on: the first held that O_2^- was completely inactive and therefore merely a chemical curiosity [16], the second that it was highly reactive, extremely oxidant, and involved in various biological processes [4].

This difference of opinion aside, it was impossible to refute the effectiveness of SOD in substantially eliminating many of the oxidizing effects of oxidases (including lipoperoxidations) and many toxic phenomena linked to oxygen activities. In an attempt to reconcile these apparently opposing schools of thought regarding the efficiency of SOD in combatting oxygen activation toxicity, it was proposed

* Corresponding author.

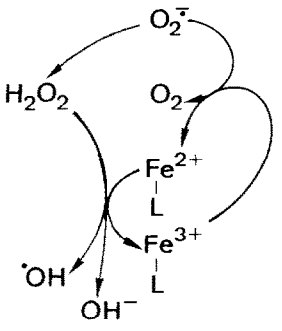


Fig. 1. The Haber–Weiss cycle. Superoxide anion ($O_2^{\cdot-}$) furnishes H_2O_2 and regenerates Fe^{2+}, the two partners of the Fenton reaction which produces hydroxyl radical $^{\cdot}OH$.

that superoxide anion and H_2O_2 interacted to generate hydroxyl radical $^{\cdot}OH$:

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^- + {}^{\cdot}OH. \quad (3)$$

This radical is a very strong oxidizer [17], especially familiar to radiochemists, and one capable of attacking various kinds of organic structures, including very stable molecules (e.g. alkanes and benzenes). Equation (3) became known as the "Haber–Weiss reaction" and offered conclusive proof as to the efficiency of SOD, which was shown to scavenge one of the reagents which form $^{\cdot}OH$. However, it was demonstrated that the detoxicating effect of SOD was enhanced by the addition of catalase, which destroys H_2O_2 in oxidase systems.

This seemed to demonstrate the validity of Eqn (3); however, there were still doubters, and the validity of Eqn (3) was soon questioned on theoretical grounds. Indeed, its velocity coefficient was found to be much smaller than that of the inverse reaction [18].

A new mechanism (actually the old Fenton reaction "revisited") [19] was then proposed, one which appeared to resolve the above-mentioned contradictions:

$$Me^{n+} + H_2O_2 \rightarrow Me^{(n+1)+} + {}^{\cdot}OH + OH^-. \quad (4)$$

Here, Me represents a transition metal such as iron, manganese, cobalt and copper. Iron was retained as being of particular interest because it is present in all biological media [20]. The role of $O_2^{\cdot-}$ then appeared in a clearer light: (a) it generates H_2O_2 by dismutation (Eqn (2)), and (b) it regenerates Me^{n+} (cf. Eqn (4)) by reduction. If iron is used, for instance:

$$Fe^{3+} + O_2^{\cdot-} \rightarrow O_2 + Fe^{2+}. \quad (5)$$

This allows the Haber–Weiss cycle to be set in motion (Fig. 1). $O_2^{\cdot-}$ thus appears to be a very effective reducing agent in various mechanisms of oxygen activation, especially in the case of Fe^{3+}. The disagreement concerning the role of $O_2^{\cdot-}$ in the peroxidation of unsaturated lipids is thereby resolved: $O_2^{\cdot-}$ regenerates the Fe^{2+} involved in lipoperoxidation, especially during the process of reactivation. If ROOH is a lipoperoxide of an unsaturated lipid (RH), Fe^{2+} catalyzes the formation of alkoxyl radical ($RO^{\cdot}$) and maintains auto-oxidation cycles [21] according to the following sequence:

$$Fe^{2+} + ROOH \rightarrow RO^{\cdot} + OH^- + Fe^{3+}$$

$$RO^{\cdot} + RH \rightarrow ROH + R^{\cdot}$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$$

$$ROO^{\cdot} + RH \rightarrow R^{\cdot} + ROOH.$$

As $O_2^{\cdot-}$ is also capable of reducing Fe^{3+} (cf. Eqn 5), $O_2^{\cdot-}$ and Fe^{3+} thereby combine to catalyze lipid peroxidation. It follows that SOD slows down the rate of peroxidation. Other reducing agents (e.g. ascorbate) [22] may be substituted for $O_2^{\cdot-}$ in peroxidations, but it is not known for certain whether such substitutions are possible under biological conditions.

Update on unresolved problems

The capacity of $O_2^{\cdot-}$ to regenerate Fe^{2+} by reducing Fe^{3+} (and thus the efficiency of SOD itself) is no longer disputed, but the other properties of $O_2^{\cdot-}$ and the problems related to it have received far less attention. However, much work has been done recently in these areas, a detailed account of which follows.

Does $O_2^{\cdot-}$ generate singlet oxygen? Unlike fundamental oxygen ($^3O_2$), which does not react directly with organic molecules because of its biradicalar structure [23], singlet oxygen ($^1O_2$), in which all the electrons are paired, is not subject to quantic interdiction and thus reacts strongly with organic matter [24] without the intervention of catalysts. However, the conversion of $^3O_2$ into $^1O_2$, implying a spin-inversion, requires a large energy supply (25 kCal/mol) uncommon in biochemical reactions. As early as 1970, Khan [25] had postulated that the spontaneous dismutation of $O_2^{\cdot-}$ would directly produce the potent oxidizer $^1O_2$ and that $O_2^{\cdot-}$ thus played a more direct role in oxygen toxicity than had been thought previously. If proved, this would be another way of corroborating the protective role of SOD, as catalytically accelerated dismutation produces only $^3O_2$ [26].

The theory maintaining that singlet oxygen is generated by $O_2^{\cdot-}$ dismutation met with strong criticism and had been virtually abandoned until very recently. In 1987, Corey (a Nobel Prize winner) and Khan (a pioneer in the study of singlet oxygen) published the results of a spectrophotometrical study which showed that $^1O_2$ was formed during the spontaneous dismutation of $O_2^{\cdot-}$ [27]. These authors studied the peak of 1260 nm highly characteristic of $^1O_2$ by means of a cooled germanium photodiode sensitive to infrared and a monochromator which selected the emission of 1260 nm. This potentially extremely important discovery must first be confirmed under biological conditions where the rates at which $^1O_2$ and $^3O_2$ are formed may be first accurately measured and then compared. It bears mentioning that a few years earlier, Arudi *et al.* [28], using thermodynamic calculations, maintained that it was not possible for $^1O_2$ to be generated during $O_2^{\cdot-}$ dismutation. At present, the matter is still under study.

Release of Fe^{2+} from ferritin by $O_2^{\cdot-}$. During the last 10 years, the prominent role played by iron

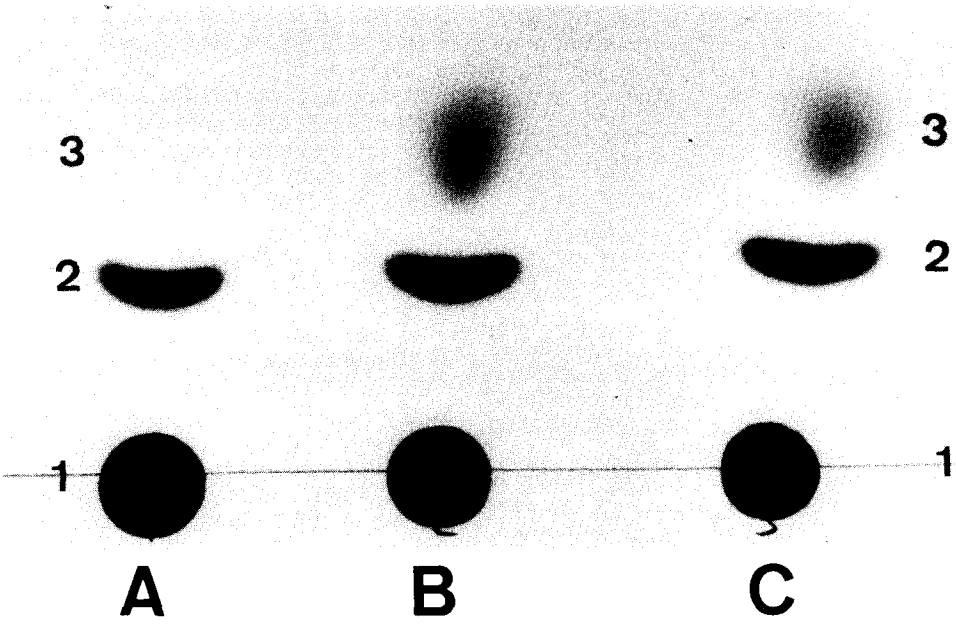


Fig. 2. Separation by thin-layer chromatography of the lipid constituents of erythrocyte ghosts. Key: (A) after incubation with dimethylformamide (DMF); (B) after incubation with O_2^- (4×10^{-4} M in DMF); (C) after incubation with O_2^- (10^{-4} M in DMF); (1) phospholipids; (2) cholesterol; and (3) non-esterified fatty acid.

in *in vitro* and *in vivo* radicalar phenomena and lipoperoxidations has provoked much interest [29, 30]; particular attention has been devoted to the mechanisms by which it could be made available *in vivo* to participate in the Haber–Weiss or lipoperoxidation cycles. It is now generally accepted that the main source of iron in cells is ferritin [30], a multi-subunit protein shell surrounding a mineral core. Channels lead to the central core, where iron is stored at oxidation degree 3. At least at this point in time, it seems that iron linked to ferritin is unable to participate in oxidation processes until it is mobilized from the storage protein [20].

The displacement of iron linked to ferritin must be preceded by reduction into Fe^{2+} [20]. Reductants are selected according to their shapes, as they must enter the ferritin central cavity [31]. Superoxide anion was first considered an efficient mobilizing agent of the iron stored in ferritin [32, 33]. More recent studies have demonstrated that the ascorbate-mediated release of Fe^{2+} from ferritin is inhibited by SOD. It has been proposed here as well that it is O_2^-, generated by the Fe^{3+} induced oxidation of ascorbate, which is the real reductant of ferritin iron [34].

Ferritin promotes, while SOD inhibits, the peroxidation of phospholipids in liposomes since the Fe^{2+} necessary for lipoperoxidation to take place is released by O_2^- [35].

Deesterification of membrane phospholipids by O_2^-. We owe our extensive knowledge of the nucleophilic properties of O_2^- to the numerous studies carried out during the 1970s [12]. Nucleophilic reactions involving O_2^- may not occur in an aqueous medium because it dismutates rapidly in protonated media [14].

The general schema of a nucleophilic reaction is

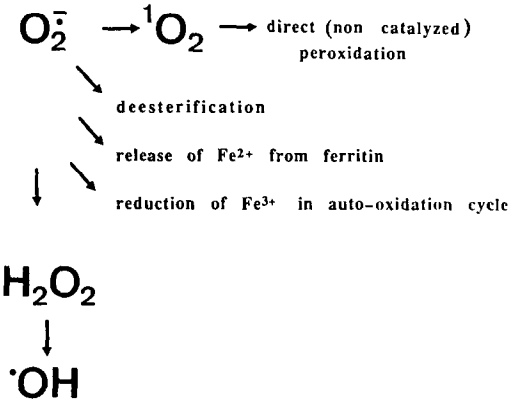


Fig. 3. Facts evidencing the importance of O_2^- and, consequently of SOD, in biology.

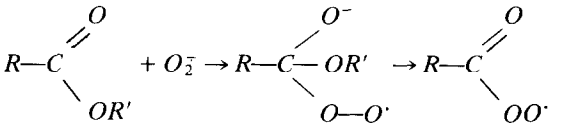
the following, where $[Nu:]^-$ is a nucleophilic agent provided with an electron doublet:

$$[Nu:]^- + b\text{—}\overset{\overset{a}{|}}{\underset{\underset{c}{|}}{C}}\text{—}A \Rightarrow b\text{—}\overset{\overset{a}{|}}{\underset{\underset{c}{|}}{C}}\text{—}Nu + [A:]^- . \quad (6)$$

This equation is transformed as follows when O_2^- is substituted for $[Nu:]^-$.

$$[^{\cdot}O{:}O:]^- + b\text{—}\overset{\overset{a}{|}}{\underset{\underset{c}{|}}{C}}\text{—}A \Rightarrow b\text{—}\overset{\overset{a}{|}}{\underset{\underset{c}{|}}{C}}\text{—}O_2^- + [A:]^- . \quad (7)$$

A mechanism accounting for the deesterification of carboxylic acid esters where the nucleophilic property of O_2^- is involved was postulated by San Filippo *et al.* [36] in 1976. A peroxyl radical was formed during the first step:



$$+ R'O^- \quad (8)$$

The formation of new O_2^- molecules in subsequent reaction steps led to net ester hydrolysis, yielding carboxylic acid anions and alcohol [37].

This schema was confirmed by other authors as well [38, 39]. Niehaus [40] hypothesized that O_2^--mediated deesterification could occur in phospholipids in aprotic media. He subjected a synthetic phosphatide, dilauroylphosphatidylcholine, to an attack of potassium superoxide (KO_2) in a dimethyl sulfoxide solution, in the presence of crown ether. Niehaus [40], observing that free fatty acids were released, proposed that a similar mechanism could occur in the aprotic region of cell membranes, delimited by the two phospholipid layers: O_2^- could thus accumulate and survive in that hydrophobic medium. Earlier, it had been demonstrated that in the absence of protons, the half-life of O_2^- was several hours [41, 42]. Before this hypothesis could be accepted, it had to be shown that electrically-charged superoxide anions could cross through cell membranes. Some of the many experiments conducted in this area did indeed show the capacity of O_2^- to do so. Experiments performed with "resealed" erythrocyte ghosts containing xanthine-oxidase and subjected to external xanthine-oxidase substrates demonstrated that the O_2^- formed inside these vesicles moved across the ghost membrane, since the reduction of ferricytochrome *c* outside the vesicles would not have occurred had SOD and xanthine-oxidase been sealed inside beforehand [43]. Lynch and Fridovich, the authors in question, also showed that O_2^- crossed the erythrocyte membrane through the anion channels [44] (transmembrane polypeptides), carrying out the rapid transport of anions as it did so [45]. Anion channels are covalently bound by 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene (DIDS), a specific blocker [46]. Resealed erythrocyte ghosts filled with ferricytochrome *c* were permeable to the O_2^- generated in the surrounding medium, as demonstrated by the reduction of ferricytochrome inside the vesicles. Adding DIDS to the medium inhibited this reduction. It was concluded from these experiments that O_2^- was released from erythrocytes and that this process was blocked by DIDS, offering conclusive proof that O_2^- crosses through the anion channels [44, 46].

Babior *et al.* [47] observed that the O_2^- generated close to neutrophil polymorphonuclear leucocyte membranes was partially released into their phospholipid bilayer. Petrone *et al.* [48], also working with neutrophil polymorphonuclears, demonstrated that superoxide was secreted out of these leucocytes, thereby forming leucotaxic agents by reaction with plasma peptides which were inhibited by SOD.

The speed at which H_2O_2 and O_2^- were released into the surrounding medium by endothelial cells exposed to menadione or nitrazepam demonstrated the permeability of cell membranes to these two substances. In fact, superoxide is released from the cells approximately 2 min after the drugs are added to the culture medium [49].

To summarize the findings of these observations, Niehaus' hypothesis that O_2^- can diffuse across membranes and accumulate in their apolar regions has not been disproven experimentally [40].

In our laboratory, we have recently begun conducting deesterification experiments using a somewhat less artificial membrane model than KO_2 acting on dilauroylphosphatidylcholine. We prepared human erythrocyte membranes ("ghosts"), devoid of hemoglobin and carefully dessicated, and allowed them to react with an O_2^- solution electrochemically generated in dimethylformamide, an aprotic solvent. An aprotic medium was chosen so as to prolong the half-life of O_2^- considerably [41]. O_2^- can also survive for several minutes in solutions containing as much as 10% water. It was observed that erythrocyte phospholipids are cleaved by O_2^- under apolar conditions [50] by the release of unesterified fatty acid (Fig. 2). It must be stressed that no lipoperoxides were detectable during these experiments, proving that peroxyl radicals (cf. Eqn 8) are extremely transient intermediates which ultimately produce carboxylic anions, exactly as was the case in short chains as seen above [36, 37].

The deesterification process induced by O_2^- provides explanations for various phenomena. Superoxide anion can be considered as a mediator of drugs (e.g. naphthoquinone) which induce SOD-inhibited erythrocyte hemolysis by a pathway other than lipoperoxidation [51, 52]. O_2^- increases the fluidity of erythrocyte stroma, an effect [53] also inhibited by SOD, but decreased by $^{\cdot}OH$ [54]. Deesterification and fluidity thus seem to be related, whereas peroxidation appears to increase rigidity by the formation of bridges and stroma alteration.

The release of a large number of non-esterified fatty acids (NEFA) is observed in tissues first exposed to a relatively prolonged anoxia and later reoxygenated (ischemia-reperfusion) [55, 56]. It has been shown elsewhere that lysophosphatides are also released [57]. O_2^- is produced during reoxygenation along at least two pathways: (a) disorders of electron-transport in anoxied-reoxygenated mitochondria [7, 8], and (b) activation of xanthine-dehydrogenase into xanthine-oxidase [58].

The release of NEFA could at least partially result from reactions to O_2^- inside the membranes, where it acts as a phospholipase. This same phenomenon could also be caused by phospholipase activation induced by intracellular hypercalcemia resulting from anoxia. We strongly suggest that the realease of NEFA during ischemia reperfusion be reexamined, this time using phospholipase inhibitors and O_2^- scavengers.

Finally, the deesterification of phospholipids by O_2^- could also account for the increase in permeability observed in ischemia-reperfusion.

Biological importance of SOD

Whatever importance SOD may have is directly related to that of O_2^-. Referring to the Haber–Weiss cycle (Fig. 1), proponents of the chemical inertia of O_2^- have also claimed that SODs serve no biological function: they maintain that O_2^- dismutation accelerates the generation of H_2O_2 and that ascorbate or another reductant could play the role of reducer ascribed to O_2^- in the cycle.

However, Fridovich has postulated recently [53] that O_2^- appears to act directly and could possess a toxicity of its own, thereby justifying the importance of SOD; however, he did not give precise chemical details concerning these direct effects, except for the release of Fe^{2+} from ferritin by O_2^- (inhibited by SOD). To support his position, we have attempted above to show that O_2^- can indeed exert a direct nucleophilic action. The question of singlet oxygen generation is still awaiting a definitive answser, but the ability of SOD to inhibit singlet oxygen production would serve as conclusive proof.

Superoxide dismutases have been found in virtually all oxygen-tolerant organisms [59]. Adaptation to hyperoxia occurs at the same time as an increase in SOD, both in procaryotes and eucaryotes [60, 61]. However, there are some exceptions: for example, Carlioz and Touati [62], by isolating an *Escherichia coli* mutant completely devoid of SOD, demonstrated that the latter was not strictly necessary for the aerobic survival of this particular bacteria. The slowdown in growth observed, however, suggests that serious cell damage had taken place. Even if the mutant survived aerobically, it would be rapidly eliminated under conditions of oxidative stress.

The importance of SOD in protecting yeast against O_2^- toxicity was the focus of a recent paper [63]. In the experiments, oxidant stress was produced by paraquat, used to generate O_2^-. The authors considered O_2^- directly responsible for the cytotic effects of oxygen.

In addition to these theoretical and *in vitro* approaches, there exists a great deal of published material attesting to the protective role played by SOD under experimental conditions: the correlation between the amount of SOD present in cells and the degree of resistance to oxygen [53]; an increase in the sensitivity to O_2^- toxicity by diethyldithiocarbamate, which decreases the amount of SODs present in the cells [51]; and the beneficial role played by SOD when added to SOD-less mutants during exposure to O_2 or O_2^- [64].

Pharmacological importance of SOD

SOD is able to weaken or eliminate altogether a wide variety of toxic effects produced by exposure to O_2^--generating systems, as illustrated by Fridovich [53, 59]. Clinical uses of SOD were the object of a paper by Michelson [65] and its role in human pathology was described clearly by Marklund [66].

It is not possible here to provide a detailed description of all the therapeutic uses suggested for SOD since its discovery. For a detailed treatment of this subject, see the Marklund article cited above [66].

The SOD locked in liposomes, when administered intraveneously along with catalase, was found to offer protection against oxygen toxicity [67]. It has also been reported that SOD reduced inflammation, which is a secondary effect of irradiation, and helps to counter the side-effects of chemotherapy. Intra-articular injections of SOD aid in the treatment of joint diseases. Michelson [15] reported that its main application lays in its anti-inflammatory activity, especially on polymorphonuclear leucocytes.

Over the past few years, the most significant progress in SOD therapy has probably been made in two areas: the treatment of ischemia-reperfusion, and the practice of grafts and transplantations. It is generally accepted that free radical generation plays a key role in these pathological conditions. SOD, together with catalase, is currently administered to eliminate the O_2^- partially responsible for the destructive phenomena of ischemia-reperfusion observed in transplantations, with improved results [68].

The therapeutic use of SOD also improves changes in the small intestine occurring during ischemia and after reperfusion [69]; protects against ischemia-induced hepatocellular injury (in combination with catalase); reduces the effects of renal dysfunction following ischemia [69]; reduces the size of infarcts induced by coronary arterial occlusion [70]; when administered along with catalase, plays a beneficial role during the reperfusion of an excised heart [71]; and improves the renal function of excised and reperfused kidneys [72].

In a recently published paper, Hernandez and Granger [73] stated that SOD was most beneficial when used as an anti-oxygen agent in organ preservation and transplanatation. In another recent article, Bolli accentuated the favourable effect of the SOD–catalase combination on post-ischemic myocardial dysfunction in dogs [74].

Outlook and conclusions

The lively controversy surrounding the direct toxicity of O_2^- is based on its so-called poor reactivity and, consequently, on the role of superoxide dismutases themselves [14]. New approaches have been made to this problem since Sawyer and Valentine [14], not that long ago, wrote: "Extensive studies of the chemistry of O_2^- have not as yet revealed how superoxide may affect biological systems."

There has been a renewed interest in SOD research in recent years. Investigations demonstrating the importance of SOD in protecting microorganisms and grafts and transplantations provide a strong argument in support of the discovery by McCord and Fridovich of O_2^- toxicity. Currently, several papers have appeared attesting to the direct effects of O_2^-, ones that are either reduced in number of eliminated entirely by O_2^- scavengers or superoxide dismutases. As summarized in Fig. 3, these effects are:

(a) Membrane deesterification occurring in the apolar environment constituted by the hydrophobic region of cell membranes;
(b) the release of Fe^{2+} directly involved in lipoperoxidations; this mechanism explains how the administration of SOD inhibits lipoperoxidations under specific conditions; and
(c) the production of singlet oxygen during spon-

taneous dismutation, a phenomenon which could not occur during the catalytic dismutation of O_2^-.

New vistas on oxygen toxicity therapeutics have thus been opened: the scavenging of O_2^-; the promise of recombinant SOD; and the synthesis of small molecules which create an SOD-like effect. Electrophilic agents, acting in apolar mediums, could also offer efficient protection for membranes by capturing an electron and restoring an O_2 molecule from superoxide anion. The struggle against superoxide anion appears more and more as a pharmacological necessity.

REFERENCES

1. Haber F and Willstätter R, Unpaarigkeit und radikalketten in reaktions mechanismus organischer und enzymatischer Vorgänges. *Ber Dt Chem Ges* **64**: 2844–2856, 1931.
2. McCord JM and Fridovich I, The reduction of cytochrome *c* by milk xanthine oxidase. *J Biol Chem* **243**: 5753–5760, 1958.
3. Knowles PF, Gibson JF, Pick FM and Bray RC, Electron-spin-resonance evidence for enzymic reduction of oxygen to a free radical, the superoxide ion. *Biochem J* **111**: 53–58, 1969.
4. McCord JM and Fridovich I, Superoxide dismutase. An enzyme function for erythrocuprein (hemocuprein). *J Biol Chem* **244**: 6049–6055, 1969.
5. Pederson TC and Aust SD, The role of superoxide and singlet oxygen in lipid peroxidation promoted by xanthine-oxidase. *Biochem Biophys Res Commun* **52**: 1071–1078, 1973.
6. Babior BM, Kipnes RS and Curnutte JT, Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* **52**: 741–744, 1973.
7. Boveris A and Turrens JF, Production of superoxide anion by the NADH-dehydrogenase of mammalian mitochondria. In: *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase* (Eds. Bannister JV and Hill HAO), pp. 84–92. Elsevier, North Holland, 1981.
8. Naqui A, Chance B and Cadenas E, Reactive oxygen intermediates in biochemistry. *Annu Rev Biochem* **55**: 137–166, 1986.
9. Green MJ and Hill HAO, Chemistry of dioxygen. In: *Methods in Enzymology* (Ed. Parker L), Vol. 105, pp. 3–22. Academic Press, Orlando, FL, 1984.
10. Sawyer DT, Gibian MJ, Morrison MM and Seo ET, On the chemical reactivity of superoxide ion. *J Am Chem Soc* **100**: 627–628, 1978.
11. Gebicki JM and Bielski BHJ, Comparison of the capacities of the perhydroxyl and the superoxide radicals to initiate chain oxidation of linoleic acid. *J Am Chem Soc* **103**: 7020–7022, 1981.
12. Lee-Ruff E, The organic chemistry of superoxide. *Chem Soc Rev* **16**: 195–214, 1977.
13. Sawyer DT and Nanni EJ, Redox chemistry of O_2 and peroxides. In: *Oxygen and Oxy-radicals in Chemistry and Biology* (Eds. Rodgers MAJ and Powers EL), pp. 15–44. Academic Press, New York, 1981.
14. Sawuer DT and Valentine JS, How super is superoxide? *Acc Chem Res* **14**: 393–400, 1981.
15. Nanni EJ, Stallings MD and Sawyer JT, Does superoxide anion oxidize catechol-α-tocopherol and ascorbic acid by direct electron transfer? *J Am Chem Soc* **102**: 4481–4485, 1980.
16. Czapski G, Radiation chemistry of oxygenate aqueous solutions. *Annu Rev Phys Chem* **22**: 171–208, 1971.
17. Cohen G, In defense of Haber–Weiss. In: *Superoxide and Superoxide Dismutases* (Eds. Michaelson MA, McCord JM and Fridovich I), pp. 317–321. Academic Press, New York, 1977.
18. Koppenol WH, Butler J and Van Leeuwen JW, The Haber–Weiss cycle. *Photochem Photobiol* **28**: 655–660, 1978.
19. Walling C, Fenton's reagent revisited. *Acc Chem Res* **8**: 125–131, 1975.
20. Crichton RR and Charlotteaux-Wauters M, Iron transport and storage. *Eur J Biochem* **168**: 485–506, 1987.
21. Frankel EM, Lipid oxidation. *Prog Lipid Res* **19**: 1–22, 1980.
22. Hochstein P and Ernster L, ADP-activated lipid peroxidation coupled to the TNPH oxidase system of microsomes. *Biochem Biophys Res Commun* **12**: 388–394, 1963.
23. Malmström BG, Enzymology of oxygen. *Annu Rev Biochem* **51**: 21–59, 1982.
24. Rawls HR and Van Santen PJ, Singlet oxygen: a possible source of the original hydroperoxides in fatty acids. *J Am Oil Chem Soc* **171**: 135–138, 1970.
25. Khan AV, Singlet molecular oxygen from superoxide anion and sensitized fluorescence of organic molecules. *Science* **168**: 476–477, 1970.
26. Koppenol WH, Reactions involving singlet oxygen and the superoxide anion. *Nature* **262**: 420–421, 1976.
27. Corey EJ, Mehrotra MM and Khan AU, Water-induced dismutation of superoxide anion generates singlet molecular oxygen. *Biochem Biophys Res Commun* **145**: 842–846, 1987.
28. Arudi RL, Bielski BHJ and Alen AO, Search for singlet oxygen luminescence in the disproportionation of HO_2/O_2^-. *Photochem Photobiol* **39**: 703–706, 1984.
29. Aust SD and White BC, Iron chelation prevents tissue injury following ischema. *Adv Free Radical Biol Med* **1**: 1–17, 1985.
30. Braughler JM, Duncan LA and Chase RL, The involvement of iron in lipid peroxidation. *J Biol Chem* **261**: 10282–10289, 1986.
31. Theil EC, Ferritin: structure, function and regulation. In: *Iron-Binding Proteins without Cofactors or Sulfur Clusters* (Eds. Theil EC, Eichorn GL and Marzili LG), pp. 1–38. Elsevier, New York, 1983.
32. Thomas CE and Aust SD, Release of iron from ferritin by cardiotoxic anthracycline antibiotics. *Arch Biochem Biophys* **248**: 684–699, 1986.
33. Babbs CF, Role of iron ions in the genesis of reperfusion injury following successful cardiopulmonary resuscitation. *Ann Emerg Med* **14**: 777–783, 1985.
34. Boyer RF and McCleary CJ, Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. *Free Radic Biol Med* **3**: 389–395, 1987.
35. Thomas CE, Morehouse LA and Aust SD, Ferritin and superoxide dependent lipid peroxidation. *J Biol Chem* **260**: 3275–3280, 1985.
36. San Filippo J, Romano LJ, Chern CI and Valentine JS, Cleavage of esters by superoxide. *J Org Chem* **41**: 586–587, 1976.
37. San Filippo J Jr, Chern C-I and Valentine JS, Oxidative cleavage of α-keto, α-hydroxy, and α-haloketones, esters, and carboxylic acids by superoxide. *J Org Chem* **41**: 1077–1078, 1976.
38. Magno F and Bontempelli G, On the reaction kinetic of electro generated superoxide ion with aryl benzoate. *J Electroanal Chem* **68**: 337–344, 1976.
39. Gibian MJ, Sawyer DT, Ungermann T, Tangpoonpholvivat R and Morrison MM, Reactivity of superoxide ion with carbonyl compounds in aprotic solvents. *J Am Chem Soc* **101**: 640–644, 1979.
40. Niehaus WG Jr, A proposed role of superoxide anion as a biological nucleophilic in the deesterification of phospholipids. *Bioorg Chem* **7**: 77–84, 1978.
41. Valentine JS and Curtis AB, A convenient preparation

of solutions of superoxide anion and the reaction of superoxide anion with a copper (II) complex. *J Am Chem Soc* **97**: 224–226, 1975.
42. Frimer AA and Rosenthal I. Chemical reactions of superoxide anion radical in aprotic solvents. *Photochem Photobiol* **28**: 711–719, 1978.
43. Lynch RE and Fridovich I, Effects of superoxide on the erythrocyte membrane. *J Biol Chem* **253**: 1838–1845, 1978.
44. Lynch RE and Fridovich I, Presentation of the erythrocyte stroma by superoxide radical. *J Biol Chem* **253**: 4697–4700, 1978.
45. Bretscher MS, A major protein which spans the human erythrocyte membrane. *J Mol Biol* **59**: 351–357, 1971.
46. Roos D, Eckmann CM, Yazdandakhsh M, Hamers MN and de Boer M, Excretion of superoxide by phagocytes measured with cytochrome c entrapped in resealed erythrocyte ghosts. *J Biol Chem* **259**: 1770–1775, 1984.
47. Babior GL, Rosin RE, McMurrich BJ, Peters WA and Babior BM, Arrangement of the respiratory burst oxidase in the plasma membrane of the neutrophil. *J Clin Invest* **67**: 1724–1728, 1984.
48. Petrone WF, English DK, Wong K and McCord JM, Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. *Proc Natl Acad Sci USA* **77**: 1159–1163, 1980.
49. Rosen GM and Freeman BA, Detection of superoxide generated by endothelial cells. *Proc Natl Acad Sci USA* **81**: 7269–7273, 1984.
50. Boes M, Deby C, Pincemail J and Goutier R, Erythrocytes ghosts alteration induced by superoxide anion through a non radicalar mechanism. *Arch Int Physiol Biochim* **95**: S7–S8, 1987.
51. Goldberg B and Stern A, Superoxide anion as a mediator of drug-induced oxidative hemolysis. *J Biol Chem* **251**: 6468–6478, 1976.
52. Goldberg B and Stern A, The role of the superoxide anion as a toxic species in the erythrocyte. *Arch Biochem Biophys* **178**: 218–225, 1977.
53. Fridovich I, Biological effects of the superoxide radical. *Arch Biochem Biophys* **247**: 1–11, 1986.
54. Rosen GM, Barber MJ and Rauckman EJ, Disruption of erythrocyte membranal organization by superoxide. *J Biol Chem* **258**: 2225–2228, 1983.
55. Rehcrona S, Westerberg E, Akeson B and Siejö BK, Brain cortical fatty acids and phospholipids during and following complete and severe incomplete ischemia. *J Neurochem* **38**: 84–93, 1982.
56. Yoshida S, Abe K, Busto R, Watson BD, Kogure K and Grinberg MD, Influence of transient ischemia on lipid-soluble antioxidants, free fatty acids, and energy metabolites in rat brain. *Brain Res* **245**: 307–316, 1982.
57. Kinnaird AAA, Choy PC and Man RYK, Lysophosphatidylcholine accumulation in the ischemic canine heart. *Lipids* **23**: 32–35, 1988.
58. McCord JM, Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* **312**: 159–163, 1985.
59. Fridovich I, Superoxide radical: an endogenous toxicant. *Annu Rev Pharmacol Toxicol* **23**: 239–257, 1983.
60. Gregory EM and Fridovich I, Induction of superoxide dismutase by molecular oxygen. *J Bacteriol* **114**: 543–548, 1973.
61. Crapo JD and Tierny DF, Superoxide dismutase and pulmonary oxygen toxicity. *Am J Physiol* **226**: 1401–1402, 1974.
62. Carlioz A and Touati D, Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? *EMBO J* **5**: 623–630, 1986.
63. Bilinski T, Krawiec Z, Liczmanski A and Litwinska J, Is hydroxyl radical generated by the Fenton reaction *in vivo*? *Biochem Biophys Res Commun* **130**: 533–539, 1985.
64. Lynch RE and Cole BC, *Mycoplasma pneumonia*: a prokaryote which consumes oxygen and generates superoxide but which lacks superoxide dismutase. *Biochem Biophys Res Commun* **96**: 98–105, 1980.
65. Michelson AM, Free radicals and disease: treatment and clinical application with superoxide dismutase. In: *Free Radicals, Aging and Degenerative Diseases* (Eds. Johnson JE, Walford R, Harman D and Miquel J), pp. 263–291. Alan R. Liss, New York, 1986.
66. Marklund SL, Superoxide dismutase in human tissues, cells, and extracellular fluids: clinical applications. In: *Free Radicals, Aging and Degenerative Diseases* (Eds. Johnson JE, Walford R, Harman D and Miquel J), pp, 509–526. Alan R. Liss, New York, 1986.
67. Turrens JF, Crapo JD and Freeman BA, Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. *J Clin Invest* **73**: 87–95, 1984.
68. Atalla SL, Toledo-Pereyra LH, McKenzie GH and Cederna JP, Influence of oxygen-derived free radical scavengers on ischemic livers. *Transplantation* **40**: 584–590, 1985.
69. Korthuis RJ and Granger DV, Ischemia-reperfusion injury: role of oxygen-derived free radicals. In: *Physiology of Oxygen Radical* (Eds. Taylor A, Mahan S and Ward P), pp. 217–249. American Physiology Society, Bethesda, MD, 1986.
70. Jolly SR, Cane JW, Bailie MB, Abrams GD and Lucchesi BR, Canine myocardial reperfusion injury: its reduction by the combined administration of superoxide dismutase and catalase. *Circ Res* **54**: 277–286, 1984.
71. Bergsland J, Lo Balsamo L, Lajos P and Mookerjee B, Post-anoxic hemodynamic performance. The effect of allopurinol and superoxide dismutase/catalase. *Transplant Proc* **19**: 4165–4166, 1987.
72. Kojama I, Bulkley GB, William GM and Im MJ, The role of oxygen free radicals in mediating the reperfusion injury of cold-preserved ischemic kidneys. *Transplantation* **40**: 590–595, 1985.
73. Hernandez LA and Granger DN, Role of antioxidant in organ preservation and transplantation. *Crit Care Med* **16**: 543–549, 1988.
74. Bolli R, Oxygen-derived free radicals and post-ischemic myocardial dysfunction. *J Am Coll Cardiol* **12**: 239–249, 1988.