in Marburg, to Karl Berggren in Sweden, to Hiroshi Kamimura in Japan, to Mike Pollak, Hellmut Fritzsche, and many others in the United States, and of course to Phil Anderson.

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The Biology of Oxygen Radicals

The superoxide radical is an agent of oxygen toxicity; superoxide dismutases provide an important defense.

Irwin Fridovich

The aerobic life-style offers great advantages, but is fraught with danger. Complete reduction of a molecule of oxygen to water requires four electrons, and in a sequential univalent process several intermediates will be encountered. These are the superoxide anion radical, hydrogen peroxide, and the hydroxyl radical, and they are too reactive (*1*) to be well tolerated within living systems. Nevertheless, the univalent pathway of oxygen reduction does occur and these dangerously reactive intermediates must somehow be accommodated. The primary defense is provided by enzymes that catalytically scavenge the intermediates of oxygen reduction. The superoxide radical is eliminated by superoxide dismutases, which catalyze its conversion to hydrogen peroxide plus oxygen (*2*), and hydrogen peroxide is removed by catalases (*3*), which convert it to water plus oxygen, and by peroxidases (*4*), which reduce it to water, using a variety of reductants available to the cell. Figure 1 illustrates the univalent pathway of oxygen reduction and the catalytic scavenging of intermediates. It is clear that efficient removal of the first two intermediates of oxygen reduction, O_2^- and H_2O_2, will prevent formation of the third, OH·. This is fortunate, since the hydroxyl radical reacts avidly with many substances (*5*) and its specific enzymatic scavenging would be impossible.

Multiple Defenses

Molecular oxygen, now so abundant in our atmosphere, is the product of photosynthesis. Blue-green algae are the most primitive organisms capable of true photosynthesis, in which light energy is used to derive reducing power from water, with the evolution of molecular oxygen (*6, 7*). Blue-green algae are not the simplest of organisms and they must have been preceded by many other life-forms. The appearance of the first blue-green algae, approximately 2×10^9 years ago (*8*), and the subsequent oxygenation of the biosphere imposed a stringent evolutionary pressure on the many organisms that, up to then, had lived and evolved in an anaerobic world. While evolving mechanisms for the utilization of oxygen, they had to develop defenses against its toxicity. Considering the situation of a common evolutionary pressure applied to a varied biota, it is not surprising that multiple defenses arose and have persisted.

At present there are superoxide dismutases with either iron or manganese at the active site, and still others with both copper and zinc (*2*). There are catalases that are hemoproteins (*3*), and others, found in organisms incapable of heme synthesis, that may be flavoproteins (*9*). There are heme-containing peroxidases (*4*) that can utilize a wide variety of electron donors for the reduction of H_2O_2, and others that contain selenium and specifically utilize reduced glutathione as the reducing substrate (*10*). The biological production of hydrogen peroxide and the existence of catalases and peroxidases have been known for more than a century (*3*). In contrast, the corresponding production of superoxide radical and the existence of superoxide dismutases have been appreciated for approximately one decade. I will devote the remainder of this article to recent findings in this newer field of investigation.

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Summary. The reactive superoxide radical, O_2^-, formerly of concern only to radiation chemists and radiobiologists, is now understood to be a normal product of the biological reduction of molecular oxygen. An unusual family of enzymes, the superoxide dismutases, protect against the deleterious actions of this radical by catalyzing its dismutation to hydrogen peroxide plus oxygen.

Sources of Superoxide

The superoxide radical is a minor, but not a trivial, product of biological oxygen reduction. The dictates of quantum mechanics lead to a spin restriction that hinders the divalent reduction of O_2 and favors the univalent pathway (*11*). Oxidative enzymes have been evolved that circumvent the spin restriction and accomplish the divalent and even the tetravalent reduction of O_2, without the release of intermediates. Thus, most of the oxygen consumed by respiring cells is utilized by cytochrome oxidase, which reduces oxygen to water without releasing either O_2^- or H_2O_2 (*12*). The strategy of minimizing the problem of oxygen toxicity by avoiding the production of O_2^- and H_2O_2 has clearly been employed. Nevertheless, O_2^- is made in respiring cells. We cannot easily learn the extent of its production in vivo because of the ubiquity of superoxide dismutases. However, in extracts of *Streptococcus faecalis* whose superoxide dismutase activity was suppressed by the addition of a specific inhibiting antibody, 17 percent of the oxygen consumption resulted in O_2^- production (*13*). In whole cells the proportion of univalent oxygen reduction is probably smaller.

It may be helpful to mention reactions known to produce substantial amounts of O_2^-. The autoxidations of hydroquinones (*14*), leukoflavins (*14, 15*), catecholamines (*16, 17*), thiols (*18*), and tetrahydropterins (*19*) have all been shown to generate O_2^-. Reduced ferredoxins are also subject to spontaneous oxidation, which produces O_2^- (*20*). Hemoglobin and myoglobin, in their oxygenated forms, have classically been considered to be ferro-oxy compounds, but there are good reasons for thinking of them as ferri-superoxy compounds, and they do slowly liberate O_2^- as they are converted to methemoglobin and metmyoglobin (*21, 22*). The production of methemoglobin is sufficiently substantial that erythrocytes contain a methemoglobin reductase to carry out a net reversal of the process. A number of enzymes, including xanthine oxidase, aldehyde oxidase, and dihydro-orotic dehydrogenase, produce O_2^-, as do several flavin dehydrogenases (*2*). The superoxide radical has also been demonstrated as an intermediate in the mechanisms of action of galactose oxidase (*23*), indoleamine dioxygenase (*24*), and 2-nitropropane dioxygenase (*25*), and other enzymes will undoubtedly be found to produce it. Fragments of subcellular organelles, such as mitochondria (*26, 27*) and chloroplasts (*28–30*), produce O_2^-. Polymorphonuclear leukocytes or granulocytes have also been shown to liberate large amounts of it during the respiratory burst that accompanies active phagocytosis (*31*). Thus, we can confidently conclude that O_2^- is made during biological oxygen reduction, although we can neither specify the predominant responsible reaction in any particular cell nor precisely quantitate its extent. Superoxide radical must now concern biochemists, as it has long concerned radiation chemists (*1*).

Dangers of Superoxide

Fluxes of O_2^-, generated enzymatically or photochemically, have been shown to inactivate virus (*32*), induce lipid peroxidation (*33*), damage membranes (*34, 35*), and kill cells (*36*). There are indications that O_2^- was not itself the species that caused these effects, but was the precursor of a more potent oxidant, whose generation depended on the simultaneous presence of H_2O_2. For example, methional [CH_3-S-CH_2-CH_2-CHO], when exposed to an enzymatic source of both O_2^- and H_2O_2, was oxidatively attacked, resulting in the production of ethylene. Superoxide dismutase inhibited ethylene production, indicating the importance of O_2^-, and catalase did likewise, indicating the importance of H_2O_2. Since superoxide dismutase does not scavenge H_2O_2 and catalase does not scavenge O_2^-, we concluded that both O_2^- and H_2O_2 were needed (*37*). Haber and Weiss (*38*), in earlier studies of the catalytic decomposition of H_2O_2 by iron salts, had deduced a free radical mechanism, one of whose component reactions was $O_2^- + H_2O_2 \rightarrow OH^- + OH\cdot + O_2$. This suggested that O_2^- and H_2O_2 had cooperated in the production of OH·, which then attacked methional and produced ethylene. In accord with this supposition was the observation that compounds, such as ethanol and benzoate, that were known to scavenge OH· and to be unreactive toward O_2^- or H_2O_2 were able to inhibit ethylene production (*37*).

Similar observations have since been made by many workers. Yet studies performed under carefully controlled conditions have demonstrated that the direct reaction of O_2^- with H_2O_2 is a slow process (*39*). One explanation for this apparent impasse invokes catalysis by iron compounds. Thus, O_2^- could reduce a ferric compound [$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$] and the resulting ferrous compound could then reduce H_2O_2, as it does in the well-known Fenton's reaction [$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot$]. In accord with this proposal, an iron-EDTA complex was shown to catalyze the hydroxylation of tryptophan in the presence of O_2^- plus H_2O_2 (*40*). Whatever the actual mechanism, it is clear that O_2^- and H_2O_2 do conspire in the production of an oxidant more potent than themselves. In this light, it seems possible that the greatest danger of O_2^- is that posed by its interaction with hydrogen peroxide or with organic peroxides (*41*), with the generation of highly reactive entities that can then attack DNA, membrane lipids, and other essential cell components.

Superoxide Dismutases: Comparative Aspects

Three distinct types of superoxide dismutases have been described. They all catalyze the same reaction and do so with comparable efficiency. The iron-containing (FeSOD) and manganese-containing (MnSOD) enzymes are characteristic of prokaryotes and are closely related, as shown by homologies in their amino acid sequences. The enzymes that contain both copper and zinc (CuZn-SOD) are characteristic of eukaryotes and appear to have been independently evolved, since they have no sequences homologous to those of FeSOD and MnSOD (*42*). The distribution of these enzymes must tell a fascinating story of evolutionary events, but it is a tangled skein and difficult to unravel. Thus FeSOD and MnSOD are found in bacteria, and recent surveys (*13*) showed that Gram-positive bacteria most frequently contain only MnSOD, whereas Gram-negative bacteria generally contain both FeSOD and MnSOD. Yet some Gram-positive bacteria, such as *Staphylococcus aureus*, contain FeSOD plus MnSOD, and some, such as *Bacillus*

cereus, contain only FeSOD. Furthermore, there are Gram-negative bacteria, such as *Alcaligenes faecalis*, that contain only FeSOD. The CuZnSOD is characteristic of the cytosol of eukaryotes, yet the symbiotic bacterium *Photobacterium leiognathi* has been shown to contain CuZnSOD in addition to FeSOD (*43*). Since this organism is one partner in a long-standing symbiosis (*44*) and is the only bacterium thus far found to contain CuZnSOD, it is tempting to speculate that it obtained this enzyme through a gene transfer from the host fish.

Eukaryotes generally contain both CuZnSOD and MnSOD. These are readily distinguished, even in crude extracts, since the former is inhibited by CN^- and is stable to treatment by a mixture of chloroform and ethanol, whereas the latter is resistant to CN^- but is denatured by chloroform and ethanol. Yeast (*45*), plants (*46, 47*), chicken liver (*48*), rat liver (*49*), and pig heart (*48*) have been shown to contain MnSOD in the mitochondrial matrix and CuZnSOD in the cytosol. Human and baboon liver also contain both MnSOD and CuZnSOD. Only MnSOD is found in the mitochondrial matrix, but the cytosol contains both enzymes (*50*). The mitochondrial MnSOD and the bacterial MnSOD have homologous amino acid sequences (*42*), in accord with the proposal that mitochondria evolved from an endocellular symbiosis between a prokaryote and a protoeukaryote.

Superoxide Dismutases: Mechanisms

The superoxide radical is unstable with respect to O_2 and H_2O_2 and it spontaneously goes over to these products by dismutation. Since O_2^- is the conjugate base of a weak acid, $HO_2\cdot$, whose pK_a is 4.8, we must actually consider three dismutation reactions. These reactions and their rate constants (k_2) are (*51, 52*)

$$HO_2\cdot + HO_2\cdot \rightarrow H_2O_2 + O_2 \quad k_2 \simeq 8 \times 10^5 M^{-1}\ \text{sec}^{-1} \quad (1)$$

$$HO_2\cdot + O_2^- + H^+ \rightarrow H_2O_2 + O_2 \quad k_2 \simeq 8 \times 10^7 M^{-1}\ \text{sec}^{-1} \quad (2)$$

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad k_2 < 0.2 M^{-1}\ \text{sec}^{-1} \quad (3)$$

The spontaneous dismutation is thus most rapid at pH 4.8, and the rate decreases by a factor of 10 for each unit increase in pH above 4.8. The dismutation between O_2^- and O_2^- (reaction 3) is probably so slow because electrostatic repulsion prevents the close approach that would allow electron transfer. Therefore the simplest mechanism for catalysis of

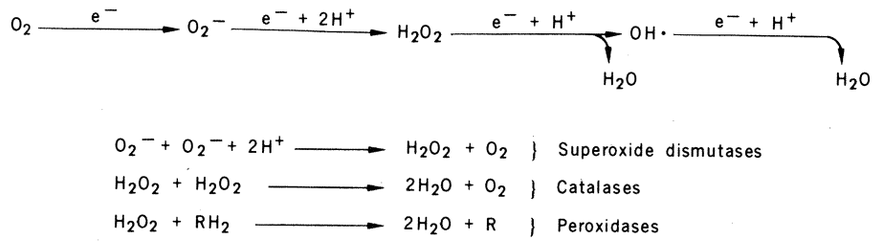

Fig. 1. The univalent pathway of oxygen reduction and the enzymatic defenses against the intermediates encountered.

reaction 3 would involve alternate reduction and reoxidation of the catalyst during successive encounters with O_2^-. The catalyst would thereby accomplish the transfer of an electron from one O_2^- to another without the necessity for close approach of the anions. This appears to be the actual mechanism of action of all of the superoxide dismutases that have been examined (*53-58*). This mechanism can be written

$$\text{E-Me}^n + O_2^- \rightarrow \text{E-Me}^{n-1} + O_2 \quad (4)$$

$$\text{E-Me}^{n-1} + O_2^- + 2H^+ \rightarrow \text{E-Me}^n + H_2O_2 \quad (5)$$

where E denotes enzyme and Me metal. In the case of CuZnSOD it is the copper that participates in the catalytic cycle and oscillates from the cupric to the cuprous state, while the zinc appears primarily to play a structural role. In MnSOD and FeSOD the trivalent and divalent states of the metals are involved in the catalytic cycle. The superoxide dismutases are extraordinarily efficient catalysts whose rate of interaction with O_2^- is approximately $2 \times 10^9 M^{-1}\ \text{sec}^{-1}$. This is close to the diffusion limit and, as expected for a diffusion-limited process, the rate shows a very small temperature effect (energy of activation) and is diminished by increasing the viscosity of the solvent.

Active Site of Copper-Zinc Superoxide Dismutase

X-ray crystallographic analysis has given us a reasonably detailed idea of the structure of the CuZnSOD from bovine erythrocytes (*59*). The major structural feature of each subunit is a cylinder composed of β structure, which may be called a β barrel. A pair of nonhelical loops, projecting from the top and bottom of one side of the β barrel, enclose and create the active site. The two subunits are joined by noncovalent, predominantly hydrophobic interactions between the β barrels. In the dimeric native enzyme the active sites are thus on opposite sides of the molecule, and they appear to operate independently (*60*).

The active site itself is composed of Cu(II) and Zn(II) in close proximity. Indeed, they appear to be bridged by a common ligand, the imidazole of histidine 61. The copper is liganded to the nitrogens of four imidazoles derived from histidines 44, 46, 61, and 118; while the zinc is liganded to three imidazoles and one carboxylate of histidines 61, 68, and 78 and aspartate 81. One face of the copper is exposed to the solvent, while the zinc is located between the copper and the interior of the molecule. It has been suggested that the bridging imidazolate functions in proton conduction. Thus reduction of Cu(II) to Cu(I), during one step of the catalytic cycle, might be accompanied by severance of the bond between the copper and the bridging imidazolate. This imidazolate could then take a proton from the solvent. During subsequent reoxidation of Cu(I) by O_2^-, the bridging interaction would be reestablished while the proton was donated to yield the product HO_2^-. This is an interesting mechanism, but at the moment it remains entirely speculative.

The enzymes MnSOD (*61*) and FeSOD (*62*) are also composed of subunits of identical size, but their chemical identity has yet to be established. Indeed there is an interesting question concerning the relationship between the number of subunits and the number of active sites. Most of the published data indicate that there is one Mn or one Fe per pair of subunits, although a few reports of one metal atom per subunit have appeared. If the former result is correct we must contemplate either dissimilar subunits, only one of which has a metal binding site, or identical subunits that cooperate in the creation of a single metal binding site. Studies of the amino acid sequences and structures of the enzymes may resolve this question.

Superoxide and Oxygen Toxicity

The conclusion that O_2^- is an important agent of oxygen toxicity and that superoxide dismutases provide an essential

defense is supported by several types of evidence. Purely circumstantial evidence was obtained by surveying a range of microorganisms (*63*). In general, aerobes contained superoxide dismutase and obligate anaerobes did not. One organism, *Lactobacillus plantarum*, was aerotolerant, yet did not contain this enzyme. However, it was unable to respire and thus in no need of a defense against O_2^-, since it could not reduce oxygen to O_2^- (*63, 64*).

More direct evidence was provided by the observations that exposure to oxygen of facultative organisms such as *Streptococcus faecalis, Escherichia coli,* and *Saccharomyces cerevisiae* resulted in increased intracellular accumulation of the enzyme and that elevated levels of the enzyme were correlated with enhanced resistance to the lethal effect of hyperbaric oxygen. In the case of *E. coli*, which contains both FeSOD and MnSOD, the two superoxide dismutases responded very differently to oxygenation. The FeSOD was made whether or not oxygen was present, whereas the MnSOD was made only in the presence of oxygen (*65*). Transfer of cells from anaerobic to aerobic conditions resulted in prompt induction of the synthesis of MnSOD. Interference with the synthesis of MnSOD rendered the anaerobically grown cells susceptible to the toxicity of oxygen (*65*). Thus, we observed both induction of MnSOD and increase resistance to oxygen toxicity as a consequence of exposure to oxygen, and we concluded that the two are related. However, oxygenation of *E. coli* or of other cells certainly induces changes other than the synthesis of MnSOD, and one of these other changes might have been the cause of the enhanced resistance to oxygen toxicity.

Several lines of evidence support the correlation between MnSOD and resistance to oxygen toxicity. When *E. coli* were grown in a glucose-limited chemostat culture, with constant and abundant oxygenation, increasing the rate of inflow of fresh medium increased the rates of growth and respiration. The content of MnSOD increased in proportion to the rate of respiration and was correlated with resistance to the lethality of hyperbaric oxygen, even though oxygenation had remained constant during the growth of the cells (*66*). When the supply of nutrient was abruptly increased, the content of superoxide dismutase began to increase immediately, while the growth rate remained at the level characteristic of the previous limited supply of nutrient. When the cellular content of superoxide dismutase reached the level that is characteristic of the growth rate in the presence of an unlimited supply of nutrients, the growth rate abruptly increased. It thus appeared that under these conditions the growth rate was limited by the intracellular level of superoxide dismutase. It should be noted that the cellular content of catalase and of peroxidase did not increase under these conditions.

In another experiment, *E. coli* were grown in batch culture in an aerated rich medium containing glucose, amino acids, purines, pyrimidines, and vitamins (Trypticase Soy plus yeast extract). The energy needs of the cells were at first met by fermentation of the glucose. The rate of respiration was low and the medium was acidified by the accumulation of lactic and other organic acids. When the glucose was exhausted, the cells began to use the amino acids and the accumulated organic acids. The rate of respiration and the *p*H of the medium increased. If superoxide dismutase provides a defense against O_2^- produced during respiration, then the level of this activity should have been low during the fermentative phase of growth and elevated during the shift to more aerobic metabolism. This was observed (*67*) and the possible role of classical catabolite repression by glucose was eliminated.

Strong evidence in support of the superoxide theory of oxygen toxicity was obtained through the use of paraquat (methyl viologen) (*67, 68*). This compound, widely used as a herbicide, is easily reduced to a relatively stable radical, which then reacts with oxygen, generating O_2^-. Paraquat augments the production of O_2^- by chloroplasts and by lung microsomes, and this is probably one cause of its lethality to both plants and animals. When paraquat is administered to *E. coli* it subverts electron flow from the normal electron transport pathway. The result is an increase in cyanide-resistant respiration and in the rate of production of O_2^-. Under conditions of constant aeration, paraquat elicits a dramatic increase in the biosynthesis of MnSOD, whereas in the absence of oxygen it has no such effect. It is clear that O_2^-, directly or indirectly, increases the rate of synthesis of MnSOD in *E. coli*. When MnSOD was elevated as a consequence of aerobic exposure to paraquat, the cells were rendered resistant to the lethal effect of hyperbaric oxygen (*68*).

Mutants with modifications in superoxide dismutase activity have provided additional indications that O_2^- is an agent of oxygen toxicity and that superoxide dismutase is an essential defense. One mutant of *E. coli*, which had a temperature-sensitive defect in its ability to maintain normal intracellular levels of superoxide dismutase, showed a parallel temperature-sensitive defect in oxygen tolerance (*69*). Another mutant, selected on the basis of tolerance for hyperbaric oxygen, was found to contain an elevated level of FeSOD (*70*). Several mutants, selected on the basis of intolerance for oxygen, were found to lack MnSOD, catalase, and peroxidase, which suggests the possibility of a genetic linkage between these enzymes. Revertants to oxygen tolerance were selected and were found to fall into two classes. One group had regained the missing enzymatic activities, while the other group showed a diminished ability to respire (*71*). The latter type of reversion recalls the strain of *L. plantarum* that was tolerant of oxygen, although devoid of superoxide dismutase, because it did not respire (*63, 64*).

Rationale

I have presented compelling evidence that superoxide dismutases are essential components of the biological defense against oxygen toxicity. It may nevertheless appear surprising that enzymes are needed to catalyze a reaction that is quite rapid, even in the absence of catalysis. This is easily explained. At *p*H 7.8, in an aqueous environment, O_2^- dismutes spontaneously at a rate of $8 \times 10^4 M^{-1}$ sec^{-1}. This is a large rate constant, but the reaction is second order in O_2^- and the first half-life is therefore a function of the steady-state level of O_2^-. Thus, at $1 \times 10^{-10} M$ O_2^- the reaction would be slow. In contrast, the reaction between O_2^- and superoxide dismutase is first order in O_2^- and first order in enzyme, and the enzyme is present in most tissues at approximately $1 \times 10^{-5} M$. At $1 \times 10^{-10} M$ O_2^- the enzyme-catalyzed dismutation would thus be 10^5-fold faster than the spontaneous reaction, even if the rate constant for the enzymatic reaction were the same as that for the spontaneous reaction. In fact, at physiological *p*H the rate constant for the enzymatic reaction is 10^4-fold greater than that for the spontaneous reaction. The net increase in the rate of dismutation of O_2^-, caused by intracellular levels of superoxide dismutase at a steady-state level of O_2^- of 0.1 n*M*, is thus 10^9-fold. The advantage provided by the enzyme would be even greater than this at the lower steady-state levels of O_2^- to be expected in a cell.

The dismutation of O_2^-, whether spon-

taneous or enzyme-catalyzed, produces H_2O_2, which is itself a dangerously reactive substance. However, as already described, there are catalases and peroxidases that scavenge it. The net effect of superoxide dismutase is to greatly lower the steady-state level of O_2^-, while the catalases and peroxidases do the same for H_2O_2. The likelihood that O_2^- or H_2O_2 will participate in deleterious reactions with other cell components is diminished in proportion to the decrease in their concentrations. The likelihood that O_2^- and H_2O_2 will collaborate in the production of even more reactive species, such as OH· or singlet oxygen, is diminished in proportion to the product of the decreases in their concentrations. These defensive enzymes are thus likely to exert a synergistic effect in protecting respiring cells against the consequences of the production of O_2^- and H_2O_2.

Oxygen Enhancements

Paraquat, which increases the rate of production of O_2^-, is much more toxic under aerobic than under anaerobic conditions. In effect, paraquat enhances the toxicity of oxygen and oxygen enhances the toxicity of paraquat. There are other oxygen enhancements that are probably also related to the production of O_2^-. Oxygen enhances the toxicity of several antibiotics including streptonigrin, mitomycin, daunomycin, adriamycin, and porfiromycin. In the case of streptonigrin this oxygen enhancement is clearly due to cyclic reduction and reoxidation, with the production of O_2^-. Increased levels of superoxide dismutase have been shown to protect against the oxygen enhancement of the lethality of streptonigrin (*65*). The structures of the other substances that exhibit oxygen enhancements also suggest that they divert normal electron flow and increase production of O_2^-.

Oxygen has long been known to enhance the lethality of ionizing radiation. The oxygen enhancement ratio quantitatively expresses this effect. Since ionizing radiation passing through water produces hydrogen atoms, hydrated electrons, and hydroxyl radicals which, in the presence of dissolved oxygen, secondarily yield O_2^- and H_2O_2, it appeared possible that O_2^- might be a factor in this oxygen enhancement. In one attempt to implicate O_2^-, *E. coli* containing a low level of superoxide dismutase, as a consequence of anaerobic growth, were compared with *E. coli* containing a high level of this enzyme, as a consequence of aerobic growth. No difference was noted (*72*). However, there have been several reports of superoxide dismutase protecting against radiation damage to DNA (*73*), viruses and mammalian cells in culture (*36*), suspensions of bacteria (*74*), and even whole mice (*75*). In these cases superoxide dismutase was effective when added to the suspending medium or when injected into the mice. This effect of extracellular enzyme recalled earlier studies of "medium effects" (*76*) and suggested that the question of the role of O_2^- in oxygen enhancement of radiation lethality needed to be reexamined. Dilute suspensions of *E. coli* in buffer exhibited an oxygen enhancement ratio of 2.4. The ratio was reduced to 1.5 when superoxide dismutase or catalase was added to the medium, and was reduced to 1.2 when these enzymes were present simultaneously (*77*). Controls with heat-inactivated enzymes or other proteins showed no effect. It thus appears that, in the case of dilute suspensions of *E. coli*, O_2^- and H_2O_2 are important agents of the oxygen enhancement.

Superoxide and Inflammation

The respiratory burst shown by activated granulocytes and the associated production of O_2^- have already been alluded to. Granulocytes are capable of chemotaxis, and they congregate at sites of injury or infection. Since a large fraction of the O_2^- produced during the respiratory burst escapes from the granulocytes, we might anticipate that a collection of activated phagocytes would damage each other as well as surrounding cells and connective tissue. Enzymatically generated O_2^- has been shown to depolymerize hyaluronate, an agent that lends viscosity and lubricating properties to synovial fluid (*78*). Moreover, the mortality of activated suspensions of granulocytes could be decreased by superoxide dismutase added to the suspending medium (*79*). The O_2^- produced by activated phagocytes, presumably to facilitate killing of engulfed bacteria, could thus exacerbate and prolong the inflammatory process. There is ordinarily very little superoxide dismutase in extracellular fluids, and the damaging effects of O_2^- released into such fluids would go largely unopposed. In that case injected superoxide dismutase should have an anti-inflammatory effect. Such effects have been reported (*80–82*).

Superoxide dismutase, injected as long as 1 hour after x-irradiation of mice, has been shown to diminish the lethality of such irradiation (*75*). Since the O_2^- generated during irradiation could not conceivably survive in the mouse for such a period, one must suppose that irradiation sets in motion some continuing physiological process, that is damaging to the animal and that involves the production of O_2^-. Granulocytes that are activated by the consequences of irradiation could provide the postulated continuing source of O_2^-, and the postirradiation protection of mice by injected superoxide dismutase could be an expression of the anti-inflammatory action of this enzyme.

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Mission-Oriented Research for Light Machinery

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Summary. The time of intelligent machines is upon us. But the United States is not actively pursuing this rich field of technological development. This is evidenced by the U.S. trade deficit of $9 billion in this market in 1977. The synergistic approach of Japan, Germany, Russia, and other countries to research, development, and demonstration among government, academic, and industrial groups is paying big dividends in vital U.S. markets. This article outlines a specific solution in terms of a U.S. national research policy for light machinery and robotics.

Recent reports on our weakening ability to compete in the market for consumer goods, many of which depend on new technology, indicate that the United States has rested on its industrial laurels for too long. In contrast to the $24 billion surplus of agricultural exports in 1977, manufactures produced a surplus of only $5 billion, dramatically down from $20 billion in 1975. Total research and development relative to the gross national product (GNP) is down 30 percent since 1963, while the percentage of industrial expenditure for basic research in the United States has dropped by a factor of 2.5. These are sobering statistics, since it has been documented that 45 percent of our economic growth from 1929 to 1969 has been due to technological innovation.

To be able to use energy resources to make products for world markets implies the use of machines. The purpose of this article is to show that high technology associated with production machinery is a means to the solution of our increasingly tenuous economic performance. Research for the development of a machine science has been ongoing for two centuries. It reached a functional level in its industrial application before World War II, but has become less effective relative to other technologies since that time. This article documents this lack of effectiveness of mechanical technology in terms of economic indicators and outlines the need to revitalize an important segment of our industrial manufacturing capacity (*1*, *2*).

A Technological Opportunity

It is well known that massive technological growth has occurred since World War II in electronics, particularly, microelectronics. During the same period, machine-based industries in the United States have made nominal progress, since the government has put insufficient emphasis on machine technology for the production of commercial goods. Other industrial nations have done the opposite (*3*). Computers or their smaller counterparts, microprocessors, can make it possible to create production machinery which "thinks," to make machines more versatile and reliable. It will be argued in this article that two major opportunities face the industrial community. These are (i) the development and use of modern research and design tools to establish a resurgence in the fundamental field of machine science, and (ii) the full use of distributed electronic sensors and computers combined with mechanical production devices to create more effective production systems.

Mechanical devices enter into the manufacture of a very large spectrum of commercial products. For example, the manufacture of our sophisticated microelectronic circuits is increasingly depen-

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