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Review Article

Reactive oxygen species and the free radical theory of aging

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ABSTRACT

The traditional view in the field of free radical biology is that free radicals and reactive oxygen species (ROS) are toxic, mostly owing to direct damage of sensitive and biologically significant targets, and are thus a major cause of oxidative stress; that complex enzymatic and nonenzymatic systems act in concert to counteract this toxicity; and that a major protective role is played by the phenomenon of adaptation. Another part of the traditional view is that the process of aging is at least partly due to accumulated damage done by these harmful species. However, recent workers in this and in related fields are exploring the view that superoxide radical and reactive oxygen species exert beneficial effects. Thus, such ROS are viewed as involved in cellular regulation by acting as (redox) signals, and their harmful effects are seen mostly as a result of compromised signaling, rather than due to direct damage to sensitive targets. According to some followers of this view, ROS such as hydrogen peroxide and superoxide are not just causative agents of aging but may also be agents that increase the life span by acting, for example, as prosurvival signals. The goal of this review is to recall that many of the effects of ROS that are interpreted as beneficial may actually represent adaptations to toxicity and that some of the most extravagant recent claims may be due to misinterpretation, oversimplification, and ignoring the wealth of knowledge supporting the traditional view. Whether it is time to abandon the free radical (oxidative stress) theory of aging is considered.

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There are enzymes and other agents that defend against the damaging effects of reactive oxygen species (ROS): by minimizing their formation, scavenging those that are formed, and repairing the damage that they cause. Many of these protective factors are upregulated under oxidative stress, thus providing adaptation [1–11]. This is by itself an indication that superoxide, hydrogen peroxide, and other oxidants are more often damaging than beneficial.

H_2O_2 and $O_2^{\bullet-}$: friends or foes?

In a recent review [8], why organisms have not only catalases but numerous peroxidases to deal with H_2O_2 is considered. The idea that peroxidases are the first line of defense against H_2O_2 because they have lower K_m than catalases and that the latter become important only when peroxidases are overwhelmed is well supported in that review. More strictly the statement that catalases have high K_m means just that it is essentially impossible to saturate them with their substrate. Nevertheless, the relevant point is that at low levels of hydrogen peroxide peroxidases and peroxireductins outcompete catalases [2,8]. In one special case the authors [8] wonder if cytochrome *c* peroxidase, in addition to having a role

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only in the removal of H_2O_2, has another role, namely to use H_2O_2 as a final electron acceptor, when other electron sinks are scarce.

The view that H_2O_2 is a normal metabolite, although a potentially toxic one, allows these problems to be addressed.

Thus why should the peroxidases consume cellular reductants, when the elimination of H_2O_2 can be achieved by catalase-catalyzed disproportionation? Well, if H_2O_2 elimination were the only goal, evolution might have favored catalases having a low K_m for H_2O_2. Perhaps all peroxidases have biological significance beyond just the removal of H_2O_2. Indeed, in some cases, such as myeloperoxidase, iodoperoxidase, and the production of benzoquinone by the bombardier beetle, this is self-evident. But then what is the additional significance of the NADH peroxidase of *Escherichia coli?* Suppose that the transport of electrons in the respiratory chain has been inhibited by such natural substances as rotenone, thus limiting the oxidation of NADH. In this as well as in other situations the oxidation of NADH by the peroxidase will generate NAD, which will allow pathways such as glycolysis and the tricarboxylic acid cycle to run.

Hence, peroxidases can eliminate the potentially toxic H_2O_2 with concomitant benefits. This does not negate the extremely important role peroxidases and peroxiredoxins play in keeping H_2O_2 concentrations sufficiently low [2,8].

That H_2O_2 has a good side as a normal and useful metabolite, which is tolerable when its concentration is kept low, is also demonstrated by the existence of numerous oxidases having very important functions and that reduce O_2 by two electrons. Even xanthine oxidase (dehydrogenase) produces directly more H_2O_2 than $O_2^{\bullet-}$ under aerobic assay conditions.

Maybe the best illustration of the view that hydrogen peroxide is a useful and normal metabolite is the role it plays in protein disulfide formation. In this process, important roles are played by hydrogen peroxide-producing oxidases such as oxireductin 1 and hydrogen peroxide-consuming peroxidases such as peroxiredoxin 4 [12].

Yet, the damaging side of hydrogen peroxide should always be kept in mind. In the presence of loosely complexed iron, the failure to keep H_2O_2 as low as possible would result in considerable toxicity due to the production of highly reactive species, such as the hydroxyl radical [6,7,11].

$O_2^{\bullet-}$ may be considered the ultimate danger for the aerobic life, because it is unavoidably produced and is potentially toxic [1–9,11,13–16]. Thus, almost all organisms use enzymes that dismute the bulk of the superoxide made in the cells. Some anaerobes that only occasionally encounter oxygen may have superoxide reductases (SORs), which reduce $O_2^{\bullet-}$ to H_2O_2 while consuming cellular reductants. SORs of some organisms have some superoxide dismutase (SOD) activity, whereas other species have separate SOR and SOD, and it has been suggested that in such cases, perhaps, SODs play a role when O_2 and $O_2^{\bullet-}$ concentrations are elevated [16].

Superoxide and hydrogen peroxide can act as villains not only in partnership with each other, but individually as well. $O_2^{\bullet-}$ and NO react to form peroxynitrite. This depletes NO while generating a powerful oxidant. In the presence of CO_2, H_2O_2 forms peroxymonocarbonate, which, at least in some cases, behaves as an even stronger oxidant [17]. In the presence of both metal complexes and Cu,ZnSOD, and of carbon dioxide, increased concentration of H_2O_2 can lead to formation of the carbonate radical, yet another strong oxidant [18,19]. Evidence that at least part of the toxicity of hydrogen peroxide in vivo may be carbon dioxide dependent has been reported [20].

The free radical theory of aging: dead or alive?

An illustration of the complexity of ROS-related processes is the ongoing puzzle concerning the role of ROS in aging [21–24]. Recently, it has been reported, for example, that the life span of a mutant of *Caenorhabditis elegans* lacking all of its SODs and especially mitochondrial SODs does not seem to be seriously affected [23]. Furthermore the (mitochondrial) SOD2 single mutant of this organism had longer life span than the wild type (without paraquat). Yet additional oxidative stress (imposed by paraquat), as well as osmotic, cold, and heat stresses, decreased the life span of (killed) the SOD mutants, much faster than it killed the wild type [23]. Moreover, the metabolism, growth, and reproduction of the SOD-lacking mutants were clearly impaired even in the absence of paraquat. More interestingly, low levels of paraquat extended the life span of the wild type, but shortened that of a mutant lacking all five SODs of this species [23].

A sensible explanation for these intriguing observations is likely to be that moderately increased superoxide stress causes adequate adaptations that are sufficient to protect these mutants from permanent damage caused by endogenously generated ROS, in the absence of additional imposed oxidative stress. The authors explain this with a phenomenon termed "prosurvival signaling," which is descriptively correct, yet it does not become clear whether the authors [23] consider it to be an adaptation. Indeed, adaptations such as significant upregulation of the peroxide-removing systems in the mutants were observed [23]. In *E. coli* such adaptations include induction of MnSOD, of iron–sulfur cluster repairing enzymes, of stable isoenzymes of the oxidatively sensitive aconitases and fumarases, and of catalase. Moreover these adaptations are only part of the strategic responses, not just toward superoxide and hydrogen peroxide, but toward oxidative stress and perturbations in the cellular redox state that are governed by regulons such as SoxRS and OxyR [4,6,10]. That overproduction of SODs suppresses the adaptations provided by SoxRS and has other detrimental effects for *lag*-phase but not for *log*-phase *E. coli* is known [5,13]. That paraquat is more toxic for SOD mutants of *E. coli* than for the wild type has been known for the past 30 years.

Hence, this author was temporarily misled by the conclusions and by the title of the paper [23], "Superoxide dismutase is dispensable for normal animal lifespan," because this contradicts the several decades long persuasion that the opposite is true!

It should be said from the outset that this bold title is misleading. The legitimate question is whether ROS and specifically superoxide contribute significantly to permanent damage that causes, rather than is just associated with, aging. Surely, any claim that endogenous production of free radicals and ROS is the sole cause of aging cannot be taken seriously.

Thus, it is reasonable to suppose that the extension of the life span of wild-type *C. elegans* by low to moderate paraquat concentrations is the result of adaptations triggered by the imposed oxidative stress. In contrast in the situation of partial or full SOD deficiency, paraquat not only did not extend the life span but rapidly killed the *C. elegans* mutants. In the mutants with partial SOD deficiency, such as the SOD2 mutant, the adaptations, such as the upregulation of catalase and other peroxide-consuming enzymes, was probably sufficient in the absence of paraquat to counterbalance the killing efficiency of the moderately increased $O_2^{\bullet-}$ concentration. The induced peroxide-removing system in the mutant lacking all SODs probably explains the equal resistance of the mutant and the wild type toward the toxicity of peroxide. In addition, redox-cycling agents such as paraquat not only deplete NAD(P)H but also generate additional hydrogen peroxide from the dismutation of the generated superoxide. The generation of hydrogen peroxide by the redox cycling of intracellular paraquat is different from adding hydrogen peroxide to the medium. In the former case the peroxide is produced at a constant rate, which results in an increased steady-state level of hydrogen peroxide, whereas in the

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