Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Invited Review Article

Reflections of an aging free radical

Barry Halliwell

Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Centre for Life Sciences, #05-01A, 28 Medical Drive, 117456, Singapore

ARTICLE INFO

Keywords:
Antioxidant
Free radical
Catalytic iron
Ferroptosis
Plants
Ascorbate-glutathione cycle
Hydrogen peroxide
Ergothioneine

ABSTRACT

In this mini-reflection, I explain how during my doctoral work in a Botany Department I first became interested in H$_2$O$_2$ and later in my career in other reactive oxygen species, especially the role of “catalytic” iron and haem compounds (including leghaemoglobin) in promoting oxidative damage. The important roles that H$_2$O$_2$, other ROS and dietary plants play in respect to humans are discussed.

I also review the roles of diet-derived antioxidants in relation to human disease, presenting reasons why clinical trials using high doses of natural antioxidants have generally given disappointing results. Iron chelators and ergothioneine are reviewed as potential cytoprotective agents with antioxidant properties that may be useful therapeutically. The discovery of ferroptosis may also lead to novel agents that can be used to treat certain diseases.

1. Introduction

I was honoured and delighted to be asked to give a special lecture at the meeting of the Society for Free Radical Research International (SFRRI) to be held in Taiwan March 17–20, 2020, and settled on a lecture title of “Reflections of an Ageing Free Radical”. I was even more honoured when asked to write an article based around that talk for Free Radic. Biol. Med., a leading journal in which I have already published 35 papers in my career, cited a grand total of 6259 times (based on Google Scopus, Sep 2020). I was equally honoured when in 2008 I received the Lifetime Achievement Award from the Society for Redox Biology and Medicine and the paper I wrote subsequently for Free Radic. Biol. Med. “The Wanderings of a Free Radical” [1] has also been highly cited (511 times as of Google Scopus on Sep 14, 2020). Unfortunately, COVID-19 led to the cancellation of the Taiwan meeting, like many others, and has caused consternation worldwide. There is hope that cytoprotective agents with antioxidant properties may have some value in treating the many side effects of infection by this horrible virus [2–4]. The editors of Free Radic. Biol. Med. asked me to still go ahead with the manuscript, so here it is.

2. My early days

As “The Wanderings of a Free Radical” explains [1], my D. Phil. (the Oxford term for PhD) from the Botany School of Oxford University was on plant metabolism (Fig. 1). During it, I showed that H$_2$O$_2$ plays a key role in plant metabolism (Fig. 1); thus I became deeply interested in the field of H$_2$O$_2$ and oxygen radicals at a time when few people cared about it [1,5,6]. Of course, interest in the field has exploded since. Cross contamination of organelles with catalase from different subcellular compartments was a key part of my work (Fig. 1) and contamination of reagents (including commercial catalase with SOD [7]) is still a problem that plagues us today [1,7,8]. We also discovered during my thesis work that there is a chemical reaction between Good’s buffers and flavin mononucleotide (FMN) that can generate H$_2$O$_2$ [9]; the buffer you use can often affect your results.

3. Hydrogen peroxide is ubiquitous

I always find it amusing when I read papers where a few μM H$_2$O$_2$ is allegedly causing severe cytotoxicity. H$_2$O$_2$ is a very important signaling molecule in vivo: the late Professor Roy Burdon in Scotland was an early pioneer in this work [10] and many others have made substantial contributions [11–14]. This employment of H$_2$O$_2$ for useful biochemical roles is in part because of its limited reactivity (at least in the absence of transition metal ions, as discussed below). Like superoxide [8,15,16], H$_2$O$_2$ is very selective in the molecules that it can react with directly. Few people realize that H$_2$O$_2$ is present at high levels (sometimes >100 μM) in such beverages as tea and coffee, although adding milk decreases the levels (Fig. 2) [17,18]. H$_2$O$_2$ is readily generated in the laboratory in cell culture media (the amounts depending on the media used) [19–21] and by photochemical reactions involving flavins [9,22].
The front page of my D. Phil. thesis and a photograph of the graduating class.

Can you spot the younger me in the photo? (Note the thesis had to be hand-typed with carbon paper; the original went to the examiners and mine is a carbon copy).

Photograph courtesy of Professor Chris Leaver.

Abstract

Leaves of spinach (Spinacia oleracea L.) and other plants readily catalyse the oxidation of formate to CO$_2$. The enzymic mechanisms by which leaves convert formate to CO$_2$ have not been fully established.

In the first part of this thesis, a detailed study of the properties and mechanism of formate oxidation in leaves of spinach and spinach-beet is described. Studies of the effects of catalase, catalase inhibitors and H$_2$O$_2$ led to the conclusion that the peroxidatic action of catalase was responsible for formate oxidation at pH 5 in all fractions. In vivo, this activity takes place in peroxisomes. Its apparent location in chloroplasts and mitochondria is shown to be an artefact due to the generation of H$_2$O$_2$ by these organelles and the presence of contaminating catalase.

The pathways by which formate is produced in plant tissues have been the subject of much previous investigation. Glyoxylate is a good precursor of formate when supplied to plant tissues (Cossins & Sinha, 1965), and so formate could be produced from this compound during photorespiration. It was found that peroxisomes could catalyse the decarboxylation of glycollate and glyoxylate. Detailed studies of these reactions, led to the conclusion that these activities were due to the generation of H$_2$O$_2$ in peroxisomes by the action of glycollate oxidase, followed by non-enzymic reaction of H$_2$O$_2$ with glyoxylate, producing CO$_2$ (from the carboxyl group) and formate. This occurs because the peroxisomal catalase is unable to completely destroy H$_2$O$_2$, consistent with the observations of other workers on different systems.
Adding 10% (v/v) milk decreases H$_2$O$_2$ levels. Data reproduced from Ref. [18] with publisher’s permission.

Having read an earlier paper by Varma et al. [23], we decided to use an oxygen electrode and other methods to investigate levels of H$_2$O$_2$ in human urine (Fig. 3). Levels were very variable between subjects (e.g. Table 1) and increased on standing, to an extent decreased by adding SOD, suggesting that some of the H$_2$O$_2$ generation was by O$_2^-$-dependent autoxidation reactions within the urine [24]. The levels we observed were substantial: Varma et al. [23] found higher levels, probably because their assay involved 30 min incubation, whereas our experiments used a shorter time (Fig. 3). Many other groups have since confirmed the presence of H$_2$O$_2$ in human urine (e.g. Ref. [25–27]). The actual levels of H$_2$O$_2$ in urine in the bladder in vivo are uncertain: much of the H$_2$O$_2$ may be generated when the urine is exposed to atmospheric O$_2$ levels when voided and any H$_2$O$_2$ generated in the bladder in vivo may diffuse into the epithelial cells for catabolism. Interestingly, levels of urinary H$_2$O$_2$ increase after drinking instant coffee [28–30], probably because autoxidisable compounds from the coffee are excreted in the urine [30]. By contrast, green tea contains lower (but still substantial [Fig. 2]) levels of H$_2$O$_2$ than coffee but drinking it does not increase urinary H$_2$O$_2$ levels [30].

Indeed, H$_2$O$_2$ and other reactive oxygen species (ROS) played key roles in human evolution (reviewed in Ref. [8,31–33]). They continue to do so throughout human development, starting from a gleam in your father’s eye, leading to fertilization and all that follows [8,34–39]; I have attempted to summarize this in Fig. 4. Reactive oxygen species are also essential in protection against infection and in the regulation of inflammation, sometimes pro-inflammatory and sometimes the opposite [40,41].

4. In praise of plants, and a failed experiment with bananas

I am never sorry that I completed a D. Phil. in a Botany School, because plants are of the greatest importance in the ROS/antioxidant field (Table 2). Working with Christine Foyer as my first PhD student, we postulated the ascorbate-glutathione cycle as the major mechanism by which chloroplasts dispose of H$_2$O$_2$ [1,42,43], see Fig. 5. Before working on this cycle we were attempting to purify an oxalate oxidase enzyme from banana. Extracts of this fruit had high oxalate oxidase activity, but we could never get it purified, so made the lucky decision (in retrospect) to change topic to the ascorbate-glutathione cycle. During this work, we found that oxalate can easily be decarboxylated non-enzymically in the presence of flavins under normal laboratory lighting conditions, and H$_2$O$_2$ is involved yet again [22]. My laboratory later proposed that H$_2$O$_2$ serves to regulate CO$_2$ fixation in the chloroplast by inactivation of the Calvin cycle enzyme fructose bisphosphatase, whose activity can then be restored by the thioredoxin system [46]. Christine (Fig. 5) has gone on to have a very distinguished career in the plant field, with numerous awards and impactful publications [47].

One of the few things (and there aren’t many!) that nutritionists are fairly sure of is that plant-rich diets seem to promote health, lessening the risk of developing such age-related diseases as dementia, type 2 diabetes, and certain cancers. No one knows why, although the antioxidants present are often implicated as an explanation (Table 2). It could
even be the opposite: plants can contain high levels of phytoprostanes and aldehydes [48–50], perhaps upregulating endogenous defence systems in a hormetic response. Certain amounts of ascorbate and vitamin E, which both originate from plants, are essential in the diet but the optimal amounts in the human diet are uncertain. The RDA set for ascorbate in different countries is variable, indicative of the modest scientific database on which it is calculated [51–53], whereas the RDA set for vitamin E (~15 mg/day) is still uncertain but has some scientific support [54,55]. However, administering higher doses of ascorbate or vitamin E to humans has not shown convincing evidence of general benefit in the prevention or treatment of human disease, although there may be some benefit of vitamin E in cardiovascular disease for small sub-groups of patients [1,8,56–59]. Whereas, in my view, the only clearly-established in vivo role of vitamin E is as an antioxidant [8,55], ascorbate may well be more important in vivo as an enzyme cofactor: the most recent emphasis on this has been on the Tet dioxygenases, which start the process of DNA demethylation and hence regulate the metagenome [60,61]. The lack of ascorbate in many cell culture media (rarely added because it is unstable) is yet another example of how the cell culture process can lead to misleading results [19–21,62], in this case artefactually altering the metagenome. Perhaps the most important antioxidant role of ascorbate in vivo is its ability to scavenge oxidizing air pollutants in the respiratory tract [63,64].

5. A failure of antioxidants does not negate the importance of oxidative damage

Why have epidemiological studies administering high doses of vitamin E, C or β-carotene for the prevention or amelioration of human disease been so disappointing? Is it because oxidative damage is unimportant in the origin and progression of human disease? Data indicate that this is not so, especially for certain cancers, such as those related to chronic inflammation [8,65–67] and for neurodegenerative diseases such as Alzheimer [8,68–71] and Parkinson disease [8,72–74]. So why then have high-dose antioxidants failed to deter the development or progression of these conditions? Table 3 lists some possible explanations. To me, one of the most plausible explanations, as I have argued several times earlier [8,76–78] is that these high doses simply fail to further decrease oxidative damage levels in vivo (see Refs. [53,79–81] for other examples). Hence they couldn’t be expected to be effective against diseases where oxidative damage is a key contributor, simply because they don’t decrease it. Limited amounts of vitamins E and C are of course essential in the diet, but it may in fact be fortunate that mega-doses of them do not seem to exert greater antioxidant capacity in vivo, as otherwise the widespread use of supplements of them might cause side effects by interfering with essential cell signaling processes, human development (Fig. 4), and/or with the key role of ROS in our defence against pathogens by phagocytes and in the regulation of inflammation [8,40,41,82,83]. It is also true that, if ROS are important in human diseases, agents or approaches that do decrease oxidative damage should be beneficial, although they might create side-effects. Indeed, several factors that are known to affect the risk of human disease also modulate oxidative damage (Table 4). It may be that some (perhaps even all?) of the beneficial effects of controlling blood glucose, cholesterol, body weight etc are indeed due to decreased oxidative damage [77]. Thus if you are an obese diabetic, losing weight, controlling blood glucose and lipids and exercising more may be the best
antioxidant strategies rather than consuming high doses of antioxidants (Table 4).

A second important factor could be the timing of antioxidant administration. For example, antioxidants have been shown to protect against stroke-induced brain damage in a multiplicity of animal models (reviewed in Ref. [8,88,89]). However, they haven’t proven effective in the treatment of stroke in humans [8,89–91]. In an animal model, the antioxidants are usually given prior to vessel occlusion, sometimes at carefully chosen time points afterwards [8,88,89]. However, when my research group worked with neurologists to study levels of oxidative damage in stroke patients, using a range of plasma biomarkers, levels were already very high when the patients were first examined (Fig. 6). Stroke onset (Fig. 6) is defined as the time when the patient is hospitalized and consent has been obtained to take samples. Usually it is difficult to pinpoint the exact onset of the stroke, and much oxidative damage may already have been done by the time the patient enters the hospital, rendering antioxidant administration ineffective [92]. On a brighter note, we also observed that levels of oxidative damage remained high for a considerable time, which suggests that longer-term therapy with appropriate antioxidants (i.e. these that do decrease oxidative damage) might be beneficial (Fig. 6).

6. Revisiting irony

A second reason for the use of H₂O₂ to regulate cellular processes is its ability to cross membranes, in part by diffusion through the lipid bilayer and in addition direct passage through some of the aquaporin proteins [8,96]. Iron and other transition metal ions play a key role in promoting oxidative damage, by converting fairly-benign species such as H₂O₂ into more reactive and damaging ones (Table 5) and by promoting “autoxidation” reactions, speeding up the oxidation of thiols, NAD(P)H, ascorbate, dopamine and a multiplicity of other biomolecules [8,98,99]. Thus if H₂O₂ passing through a lipid bilayer meets a “stray” Fe²⁺ ion, OH⁻ can be generated and lipid peroxidation can be initiated [8]. If the same thing happened during passage of H₂O₂ through an aquaporin, the OH⁻ would immediately attack the protein, damaging it and affecting water transport. The body attempts to prevent such reactions (Table 5) by ensuring the safe sequestration of transition metal ions, especially iron, in non-redox-active forms. This is a very important antioxidant defence [8,98–100]. Indeed, the release of “catalytic iron” during tissue injury is a key promoter of oxidative damage [9,98–100]. This was a major early focus of my research, much of it with John Gutteridge, who is retired now but still thinks critically about the field [32,101].
Table 4

If antioxidants such as vitamins E, C or β-carotene rarely change oxidative damage levels in humans, what does?

| Scenario                              | Suggested Strategy to minimize |
|---------------------------------------|-------------------------------|
| Obesity (Humans)                      | Lose weight, exercise more*   |
| Hyperglycaemia (Humans)               | Control blood glucose         |
| High plasma LDL Cholesterol (Humans)  | Control LDL cholesterol       |
| High Cholesterol Diet (Rabbits and rats, humans possibly) | Eat diet rich in fruits and vegetables |
| Zinc Intake (Rabbits, some other animals, human data inconclusive) | Maintain adequate zinc intake |
| Body Iron Levels (Rabbits, rats, mice, maybe humans) | Avoid iron supplements unless deficient |
| Certain foods* (Humans, e.g. dark soy sauce, tomato) | Eat diet rich in fruits and vegetables |
| Diabetes (in some studies, not others)* |                                |
| Intake of polyunsaturated fatty acids (PUFAs)* | Eat fish regularly |
| (docosahexaenoic acid, possibly eicosapentaenoic acid) |                             |
| Cigarette smoking                     | Avoid or minimize             |
| Sunlight (eye, skin)                  | Avoid too much exposure       |
| Other environmental toxins? (such as O₃, NO₂, arsenic in water) | Avoid if possible |

* Increases oxidative damage, decreases oxidative damage.

Adapted from Ref. [77].

Footnotes.

* Exercise has benefits other than weight loss, some of them may be “antioxidant” by upregulating endogenous antioxidant defences [82,84,85].
* May depend on how well glucose and lipids have been normalized in the diabetic cohorts studied.

° It is essential to do appropriate controls in testing effects of foods, because the consumption of any food (antioxidant or not) can sometimes alter levels of certain biomarkers [86,87].

Despite the propensity of PUFAs to oxidize in vitro, growing evidence suggests that they can minimize oxidative damage in vivo.

A novel and exciting aspect of the interplay of iron and ROS is the recent discovery of ferroptosis [123], which is turning out to play a key role in the pathology of many diseases, such that inhibitors of this process may have therapeutic potential [123–125]. Studies of ferroptosis have unearthed a novel mechanism by which the sequestration of iron can be achieved to decrease oxidative damage. Some breast cancer cell lines resistant to ferroptosis have increased the expression of prominin2, which promotes the formation of ferritin-containing multivesicular bodies and exosomes that transport iron out of the cell, decreasing the intracellular level of catalytic iron and thus inhibiting ferroptosis [126]. Studies in Drosophila and mice have revealed yet another mechanism; oligodendrocytes provide antioxidant defence to neurons by supplying them with ferritin heavy chain that can sequester catalytic iron [127].

7. Ironic burgers

A question asked in the early days of studying iron-ROS interactions was whether hemoglobin and other haem proteins are biological Fenton reagents, e.g. can they react with H₂O₂ to produce OH*/[128]. If they did, the OH* would immediately attack the haem ring or the adjacent amino acid residues and would be unlikely to be released into free solution [8]. Nevertheless, it is indeed the case that haem and, in the presence of H₂O₂, haem proteins such as myoglobin, haemoglobin and cytochrome c can be powerful pro-oxidants, a process that is important in vivo, the kidney and brain being especially sensitive targets of oxidative damage by haem proteins [129,130]. There are two aspects to these pro-oxidant effects (Fig. 7). Haem proteins react with H₂O₂ to generate Fe(IV) (ferryl) species that can damage biomolecules, but an excess of peroxide can degrade the haem and release catalytic iron capable of promoting OH* formation [131–133]. Interestingly, ascorbate can reduce ferryl species and prevent the former, but it can promote oxidative damage by released catalytic metal ions (Fig. 7).

In the 1980s, a distinguished plant biochemist, Alan Pupp, visited my laboratory (then at King’s College London), for a short period. He was very interested in the biochemistry of the legume root nodule, which contains the protein leghaemoglobin and suffers oxidative stress during senescence [134] (this paper was co-authored with Christine Foyer, a small world!). Alain and I were very interested in the biochemistry of the legume root nodule, which contains the protein leghaemoglobin and suffers oxidative stress during senescence [134] (this paper was co-authored with Christine Foyer, a small world!). Alain and I were very interested in the biochemistry of the legume root nodule, which contains the protein leghaemoglobin and suffers oxidative stress during senescence [134] (this paper was co-authored with Christine Foyer, a small world!). Alain and I were very interested in the biochemistry of the legume root nodule, which contains the protein leghaemoglobin and suffers oxidative stress during senescence [134] (this paper was co-authored with Christine Foyer, a small world!). Alain and I were very interested in the biochemistry of the legume root nodule, which contains the protein leghaemoglobin and suffers oxidative stress during senescence [134] (this paper was co-authored with Christine Foyer, a small world!).
Fig. 6. Oxidative damage in human ischemic stroke: a biomarker study.
Data taken from Ref. [92].
Note that levels of oxidative damage (as measured by plasma biomarkers) were already high when the first samples were taken and remained high even after clinical improvement. Control refers to age-matched healthy subjects. F2- and F4-isoprostanes are well established biomarkers of oxidative lipid damage and allantoin is a biomarker of oxidative degradation of urate [8,92–95].
Most studies with desferrioxamine are carried out with the commercially available desferal (desferrioxamine B methanesulphonate).

Adapted from Ref. [8].

Table 5
Iron promotes oxidative damage Adapted from Ref. [97] by courtesy of Oxford University Press.

| Starting agent | More reactive species produced on addition of iron ions |
|----------------|------------------------------------------------------|
| H₂O₂ | Hydroxyl radical (FENTON REACTION) (and possibly reactive oxo-metal ion species) |
| HCl | OH* |
| Lipid peroxides | Peroxy radicals, alkoyl radicals, cytotoxic aldehydes |
| Thiols (R-SH) | O₂•-, H₂O₂, R•, OH*, oxy sulphur radicals |
| NAD(P)H | NAD(P)•+, O₂•, OH* |
| Ascorbic acid | OH*, possibly O₂•, semidehydroascorbate radical |
| Dopamine, serotonin, DOPA | OH•, O₂•, carbon-centred or other radicals derived from the compound |
| Peroxynitrite (ONOO⁻) | NO₂⁻ |

Table 6
Properties of desferrioxamine.

- Powerful chelator of Fe(III) (stability constant −10^{11})
- Chelates several other metal ion with stability constants several orders of magnitude lower (e.g. Al(III): −10^{2}; Cu(II): −10^{4}; Zn(II): −10^{12}; has been used to remove aluminium from dialysis patients.
- Reacts slowly with O₂•+ or HO• (k₂ ≈ 10^{-9} M^{-1} s^{-1}) to form nitroxide radicals that can oxidize ascorbate, GSH and NAD(P)H
- Substrate for myoglobin/H₂O₂ systems, nitroxide radical forms as ferryl haem is reduced
- Moderately good scavenger of peroxyl (RO₂•) radicals in aqueous solution, again a nitroxide results.
- Reacts with tyrosyl radical (k₄ ≈ 6 × 10^{-9} M^{-1} s^{-1}), a nitroxide again results
- Reacts fast with OH• (k₃ ≈ 10^{8} M^{-1} s^{-1}); nitroxide radicals are again produced, so desferrioxamine is an excellent OH• scavenger; ferrioxamine scavenges OH• with the same rate constant
- Does not react directly with ONOO•/ONOOC but can protect against damage by them by reacting quickly with COO• (k₀ ≈ 1.7 × 10^{-9} M^{-1} s^{-1}) and NO₂• radicals (k₂ ≈ 7.6 × 10^{-9} M^{-1} s^{-1}). Nitroxide produced again.
- Inhibits iron-dependent lipid peroxidation and OH• generation from H₂O₂ in most systems; ferrioxamine does not inhibit, so a control with this substance can distinguish protection by iron binding from protection by direct scavenging of RS
- Accelerates the oxidation of Fe^{2+} solutions, by binding the resulting Fe(s) more tightly than does Fe^{3+}; this is the ferroxidase action of DFO, which produces O₂•⁻ (Fe^{2+} → DFO + O₂ → Fe(III) → DFO + O₂•⁻)
- Penetrates only slowly into most animal cells; may enter by pinocytosis into lysosomes; poorly absorbed from gut but oral administration could conceivably be used to interfere with iron absorption.
- Can raise levels of HIF-1α and promote a cellular ‘hypoxic response’

Most studies with desferrioxamine are carried out with the commercially available desferal (desferrioxamine B methanesulphonate).

Adapted from Ref. [8].

8. Hope for the future?

Ergothioneine, a thione-thiol with significant cytoprotective properties in vitro, some (but by no means all) of which may involve its antioxidative activity, was first discovered in the ergot fungus, hence the name (reviewed in Refs. [4,143-145]). It was extensively studied in the 1950s to early 1990s [142,146-148], including by us [149,150]. Interest in it waned until 2005, when a selective transporter (OCTN1) that brings ergothioneine into the body and distributes it to key tissues [151-153] was identified by Gründemann et al. [151,152]. Indeed, some evidence suggests that expression of OCTN1 may increase at sites of tissue injury to bring in more ergothioneine and raise its levels as a cytoprotective mechanism [154]. Levels of ergothioneine are decreased in several human diseases, and ergothioneine administered to animals seems to enter all body tissues and fluids, including the brain [146,147,155]. Ergothioneine appears safe for human consumption and accumulates in the animal and human body when administered [146-148,155,156]. Several questions remain, including whether it can really act as a significant antioxidant in vivo [156], perhaps only when it is accumulated to high levels [154]. Ergothioneine is, from our current knowledge, only made by fungi (especially several types of mushroom), certain bacteria and certain yeasts. Yet it is found in many foods; higher plants cannot make it but some of them may acquire it from fungi in the soil (reviewed in Ref. [144]). We are continuing to investigate its therapeutic potential [4,143,157]; it has also been suggested by others to protect against frailty [158,159], be elevated during human fasting as part of an antioxidant defence response [160] and be a “longevity vitamin” [161].

Returning to the subject of plants, do their beneficial health effects involve decreasing oxidative damage? Sometimes they may do (e.g. tomatoes [162,163]), sometimes no. There is no great mass of data to support the view that “whole plants” act as antioxidants in vivo. Plants load themselves with antioxidants because they are subject to severe oxidative stress as they produce O₂ during photosynthesis; they don’t produce these antioxidants for the benefit of humans who eat plants [164].

9. Conclusion: an ageing free radical

I turn 71 years old in 2020 but my laboratory is still active, studying the biological role of ergothioneine [143,144] and the role of ROS in ageing, Parkinson disease, cardiotoxicity and dementia [157,165-167]. ROS do not cause ageing, but they regulate aspects of it carefully, as many other groups have shown. Nor will antioxidants prevent ageing. Our attempts to find antioxidants that slow ageing in C. elegans revealed that the ones that seemed to work were not acting by antioxidant mechanisms [165]. Can antioxidants that really work in vivo help human diseases? Maybe yes, maybe no, as Fig. 8 summarises. I have shown this figure before [1,8], but still think that it is worth repeating. Tissue
damage causes oxidative stress, which may worsen the damage (in which case agents that truly decrease oxidative damage in vivo will help) or trigger adaptations that minimize tissue injury (in which case such agents will not and may be deleterious). It all depends!

The field of free radicals/reactive species/antioxidants underpins all of human biology [8,11–14,31,164]. Just when I think the field is getting a bit old and boring, new things appear. One is ferroptosis, as mentioned earlier and more are likely in the works.

I have done all the h-index, papers in top journals, citations etc. They don’t go away. In my last few years of research, I can do what I want in the lab and publish where I like. My hope is that ergothioneine and derivatives of it will turn out to be important therapeutic agents for humans. Time will tell.

Acknowledgement

I thank the distinguished Tan Chin Tuan family for the funding support for my Centennial Professorship.

Fig. 8. What is the significance of oxidative stress in human disease? Adapted from Ref. [8] with permission of Oxford University Press.

References

[1] B. Halliwell, The wanderings of a free radical, Free Radic. Biol. Med. 46 (2009) 531–542.
[2] J.M. McCord, B.M. Hybertson, A. Cota-Gomez, K.P. Geraci, G. Gao, Nrf2 activator PB125® as a potential therapeutic agent against COVID-19, Antioxidants 9 (2020) 51.
[3] H. Sies, M. Parnham, Potential therapeutic use of ebselen for COVID-19 and other respiratory viral infections, Free Radic. Biol. Med. 156 (2020) 107–112.
[4] I.K. Cheah, B. Halliwell, Could ergothioneine aid in the treatment of coronavirus patients? Antioxidants 9 (2020) E595.
[5] B. Halliwell, V.S. Butt, Oxidative decarboxylation of glycollate and glyoxylate by leaf peroxisomes, Biochem. J. 138 (1974) 217–224.
[6] B. Halliwell, Oxidation of formate by peroxisomes and mitochondria from spinach leaves, Biochem. J. 138 (1974) 77–85.
[7] B. Halliwell, Superoxide dismutase: a contaminant of bovine catalase, Biochem. J. 135 (1973) 379–381.
[8] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, fifth ed., Clarendon Press, Oxford, 2015. UK.
[9] B. Halliwell, V.S. Butt, Flavin-monomonucleotide-sensitized photo-oxidation of glyoxylate in Good’s buffers, Biochem. J. 129 (1972) 1157–1158.
[10] R.H. Burdon, Control of cell proliferation by reactive oxygen species, Biochem. Soc. Trans. 24 (1996) 1028–1032.
[11] M.P. Murphy, A. Holmgren, N.G. Larsson, B. Halliwell, C.J. Chang, B. Kalyanaratna, S.G. Rhee, P.J. Thornalley, L. Partridge, D. Gems, T. Nystrom,
B. Halliwell

Free Radical Biology and Medicine 161 (2020) 234–245
antioxidant defense function for neurons by secreting ferritin heavy chain, Cell Metabol. 30 (2020) 259–272, e10.

[126] S.M. Sadrzadeh, E. Graf, S.S. Panter, P.E. Hallaway, J.W. Eaton, Hemoglobin. A biologic Fenton reagent, J. Biol. Chem. 259 (1984) 14354–14356.

[127] M. Hugelshofer, R.M. Buzzi, C.A. Schaer, H. Richter, K. Akeret, V. Anagnostakou, L. Mahmoudi, R. Vaccani, F. Vallerian, J.W. Deuel, F.W. Kronen, Z. Kulcsár, L. Regli, J.H. Baek, I.S. Pires, A.F. Palmer, M. Dentler, R. Humar, P.W. Buchler, P. R. Kircher, E. Schäfer, D.J. Schaer, Haptoglobin administration into the subarachnoid space prevents hemoglobin-induced cerebral vasospasm, J. Clin. Invest. 129 (2019) 5219–5235.

[128] M. Guerrero-Hue, C. García-Caballero, A. Palominos-Antolín, A. Rubio-Navarro, C. Vázquez-Carballo, C. Herencia, D. Martín-Sánchez, V. Farre-Alins, J. Egrea, P. Cannata, M. Praga, A. Ortíz, J. Egido, A.B. Sanz, J.A. Moreno, Currucmin reduces renal damage associated with rhabdomyolysis by decreasing ferroptosis-mediated cell death, Faseb. J. 33 (2019) 8961–8975.

[129] L. Eddy, A. Arduini, P. Hochstein, Reduction of ferythromoglobin in rat diaphragm, Am. J. Physiol. 259 (1990) C995–C997.

[130] J. Simoni, J. Villanueva-Meyer, G. Simoni, J.P. Moeller, D.E. Weissman. Control of oxidative reactions of hemoglobin in the design of blood substitutes: role of the ascorbate-glutathione antioxidant system, Artif. Organs 33 (2009) 115–126.

[131] J.M. Gutteridge, Iron promoters of the Fenton reaction and lipid peroxidation can be released from hemoglobin by peroxides, FEBS Lett. 201 (1986) 291–295.

[132] A. Pupo, K. Groten, F. Bastian, R. Carzaniga, M. Soussi, M.M. Lucas, M.R. de Sousa, M. Hugelshofer, R.M. Buzzi, C.A. Schaer, H. Richter, K. Akeret, V. Anagnostakou, S.M. Sadrzadeh, E. Graf, S.S. Panter, P.E. Hallaway, J.W. Eaton, Hemoglobin. A biologic Fenton reagent, J. Biol. Chem. 259 (1984) 14354–14356.

[133] F. Thies, L.M. Mills, S. Moir, L.F. Masson, Cardiovascular benefits of lycopene: role of pomegranate extract, J. Nutr. 141 (2011) 703–708.

[134] I.K. Cheah, L. Feng, R.M. Tang, K.H. Lim, B. Halliwell, Ergothioneine levels in an end-stage renal disease population, on the relationship to oxidative stress, Antioxidants 9 (2020) 638.

[135] I.K. Cheah, L. Feng, R.M. Tang, K.H. Lim, B. Halliwell, Ergothioneine levels in an end-stage renal disease population, on the relationship to oxidative stress, Antioxidants 9 (2020) 638.

[136] A. Macho-González, A. Garcimartín, M.E. López-Oliva, S. Bastida, J. Benedit, G. Ros, G. Nieto, F.J. Sánchez-Muniz, Can meat and meat-products induce oxidative stress? Antioxidants 9 (2020) 638.

[137] F. Qian, M.C. Riddle, J. Wylie-Rosett, F.B. Hu. Red and processed meats and health risk: how strong is the evidence? Diabetes Care 43 (2020) 265–271.

[138] N.K. Gopaul, B. Halliwell, E.E. Anggard, Measurement of plasma F2-isoprostanes as an index of lipid peroxidation does not appear to be confounded by diet, Free Radic. Res. 33 (2000) 115–127.

[139] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[140] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[141] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[142] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[143] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[144] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[145] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[146] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.

[147] I. Boshoven, B. Halliwell, Ergothioneine: antioxidant potential, physiological function and role in disease, Biochim. Biophys. Acta 1822 (2012) 784–793.