The Biological Radicals

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Past and present knowledge on the most important biological radicals, the superoxide radical anion and the nitrogen monoxide radical, are briefly compiled. The contribution covers the history of their detection, their enzymology, their physiological role and their detrimental effects, if they are produced in an unbalanced way. An in-depth understanding of their formation and metabolic fate is considered to improve our understanding of important biomedical problems such as host defense, blood circulation, inflammation and oxidative tissue damage.

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1. Introduction

In the 18th and 19th century, the term radical indicated any group or substituent such as ethyl or carboxyl that was attached to a larger molecule[1]. The use of this term changed gradually, after Moses Gomberg synthetized a free and persistent radical for the first time, the triphenylmethyl[2]. Now, the term radical is restricted to compounds harboring one or more unpaired electrons and, in consequence, are paramagnetic. Compounds meeting these criteria are by no means uncommon in nature. In particular, enzymes or other proteins containing transition metals are often paramagnetic but are usually not named radicals. However, also low molecular weight radicals, e. g. thiyl radicals, have been reported to be formed in nature under extreme conditions such as x-ray irradiation.

Sometimes an unpaired electron resides in amino acid residues of the protein and is involved in the catalytic mechanism. The prototypes of the latter enzymes are the ribonucleotide reductases, which had been discovered in 1960 and the following years by Peter Reichard (1925–2018) and colleagues[3][4]. In 1972, Ehrenberg and Reichard provided the first evidence that the enzyme of *Eschericha coli* contained a free radical[5]. In 1978, finally the radical was identified as tyrosyl radical by electron spin resonance technology[6]. Depending on species and/or culture condition, the types of ribonucleotide reductases differ, but all make use of radical chemistry to eliminate the 2´-OH group of ribose in the ribonucleotide. In class Ia and Ib, an Fe-O-Fe bridge-stabilized tyrosyl radical attacks the ribose via a cysteyl radical, in class II the cysteyl radical is formed with the aid of adenosylcobalamine and class III works with a glycyl radical. The typical reductant of the ribonucleotide reductases is thioredoxin[7][8], glutaredoxin[9], other redoxins such as tryparedoxin[10] or formate (reviewed in[9][11][12]). Radical chemistry is, however, also observed in other enzymatic processes. Well known examples are the univalent reduction of the ferryl iron in heme peroxidases[13] and the enzymatic formation of lipid hydroperoxides (LOOH)[14], which is presumed to be preceded by hydrogen abstraction to first yield a carbon–centered radical and then, by addition of oxygen, to yield a superoxy radical (LOO•).

A fairly stable free radical, ubisemiquinone, was detected in 1931 by Leonor Michaelis (1875–1949)[15]. In mitochondria, its oxidized and reduced forms are associated with complex I (NADH: ubiquinone oxidoreductase; EC 1.6.5.3) and complex II (succinate: coenzyme Q oxidoreductase; EC 1.3.5.1). They are, therefore, also called coenzyme Q, yet despite defined binding sites in the proteins of mitochondrial complexes, ubiquinone and ubiquinol can almost freely move within the mitochondrial membrane. The reduction of ubiquinone in complex I and II starts with a two-electron transition. In contrast, the cytochromes of complex III (coenzyme QH_2: cytochrome c oxidoreductase; EC 1.10.3.2) and IV (cytochrome c oxidase; EC 1.9.3.1) transfer single electrons, which implies that somewhere in complex III or earlier a separation of electrons must take place, and ubisemiquinone would be a reasonable candidate to fulfill this job (but see below).

However, the focus here is on the really free radicals, i.e., those built by the organism on purpose, released from their site of generation and free to cause harm or benefit, wherever their life time allows them to diffuse. These are the superoxide radical anion ($•O_2^-$), its conjugate acid, the superoxide radical ($•O_2H$), and the nitrogen monoxide radical (•NO; also called nitric oxide). The discovery of each of them came as an unanticipated surprise.

2. The Superoxide Radical

The superoxide radical was known to researchers interested in atmospheric chemistry or physico-chemists working with simplified clean systems[16]. As in the case of H_2O_2, the superoxide radical found its role in biology after its metabolism appeared at the horizon with the discovery of superoxide dismutase (SOD). The history of this discovery has been masterly reviewed by Irwin Fridovich (1929–2019). In the introductory chapter of the proceedings of the famous Banyuls symposium on “Superoxide and Superoxide Dismutases” (Banyuls, France; 1976), he amusingly describes the frustrated search for the explanation of a mysterious ferricytochrome c reduction that, strangely enough, depended on the presence of oxygen. The phenomenon had been observed in various biochemical reactions, the search for its chemical basis took decades, but no hypothesis could be experimentally verified. Finally, a youngster, Joe McCord, entered Fridovich’s laboratory, postulated that the reductant of cytochrome c could be superoxide, and identified SOD, which abolished the strange phenomenon[17]. McCord’s hypothesis[18] indeed marks the beginning of superoxide research in biochemistry.

In 1969, superoxide dismutase was isolated from bovine erythrocytes[19]. It was the copper/zinc type that was known for years under different names for green proteins of unknown function such as hemocuprein, hepatocuprein[20], erythrocuprein[21] or cerebrocuprein[22]. The bimolecular rate constant for SOD-catalyzed dismutation of $\bullet O_2^-$ is about 2×10^9 M^{-1} s^{-1}[23] and, thus, is seven orders of magnitude faster than the non-catalyzed reaction (<100 M^{-1} s^{-1} [23]). The spontaneous dismutation at physiological pH is faster (~2×10^5 M^{-1} s^{-1} [24]), since $\bullet O_2^-$ is partially associated ($pK_a = 4.8$) and the dismutation of the protonated superoxide is faster (k for $\bullet O_2H + \bullet O_2H = 7.6 \times 10^5$ M^{-1} s^{-1} and for $\bullet O_2H + \bullet O_2^-$ k = 8.5×10^7 [24]). However, still SOD accelerates the dismutation by four orders of magnitude[24]. The rate constant of Cu/Zn-SOD is indeed the fastest ever reported for a bimolecular enzymatic reaction. The entire surface charge of the enzyme[25], and in particular an electrostatic gradient directed towards the reaction center guides the negatively charged superoxide radical anion towards the positive histidine-complexed copper ion[26][27], which explains the incredible efficiency of these enzymes.

In the following years, different types of superoxide dismutases were discovered: manganese- containing SODs in bacteria[28]and mitochondria of higher organisms[29], iron-containing SODs in bacteria[30] and protozoa[31] and extracellular forms of the Cu/Zn-SOD in mammals[32]. Cu/Zn-SODs were also sporadically found in bacteria. The first one was the enzyme of *Photobacterium leiognathi*, which lives as symbiont in the teleost pony fish. The unusual occurrence of a Cu/Zn-SOD in a symbiotic bacterium was suspected to be the result of a natural gene transfer[33]. However, sequencing of the Cu/Zn-SOD of *P. leiognathi* and comparison with known sequences falsified this assumption[34], and Cu/ Zn-SODs were soon discovered also in non-symbiotic bacteria[35].

As mentioned, the superoxide radical was discovered as a reductant, but it made its way in biology as an oxidant, since it can initiate and sustain free radical chains. With the availability of SODs, it became quite easy to prove the participation of superoxide in biological systems. The first pathogenic effect of superoxide formation was lipid peroxidation in biomembranes. As early as 1972, Fee and Teitelbaum described that oxidative hemolysis, as induced by dialuric acid, could be inhibited by SOD[36]. The basis of related experiments by Zimmermann and colleagues [37][38]were the rediscovery of catalase and glutathione peroxidase as contraction factor I and II by Albert Lehninger (1917–1986) and colleagues[39] and studies on high amplitude swelling of mitochondria induced by GSH [40][41]. These phenomena were shown to be associated with, and possibly caused by, lipid peroxidation in mitochondrial membranes. SOD indeed inhibited GSH-induced oxidative destruction of isolated mitochondrial membranes[42]. How the superoxide radical contributes to lipid peroxidation in this and similar artificial experimental settings, remains unclear. Certainly, GSH here does not act as an antioxidant; deprived of its enzymatic environment, it rather autoxidizes in the presence of traces of transition metals with formation of superoxide. Already in 1974, Misra had observed that autoxidizing thiols produce superoxide[43]. The superoxide radical (more likely than the superoxide radical anion) might abstract a hydrogen atom from a methylene group between two double bonds of a polyunsaturated fatty acid, which is the usual start of a free radical chain in membrane lipids. Accordingly, catalase and GPx1 inhibited loss of volume control and contractibility and lipid peroxidation[37][38][41][42].

These observations pointed to an essential contribution of H_2O_2 or any other hydroperoxide, respectively. A superoxide-driven formation of the hydroxyl radical ($\bullet OH$) from H_2O_2 in the presence of traces of iron, according to Haber and Weiss[16], might cause lipid peroxidation in simplified models such as washed mitochondria and isolated membranes. $\bullet OH$ is indeed a very aggressive oxidant. It reacts with a realm of naturally occurring compounds with rate constants higher than 10^9 M^{-1} s^{-1}, i.e., at rates near or at control by diffusion[44]. Strong oxidative power of H_2O_2 in the presence of Fe^{2+} had already been observed in the 19th century by the British chemist Henry J. Horstman Fenton (1854–1929)[45], but Fenton never mentioned the involvement of a radical, and the precise mechanism of the “Fenton chemistry” is still being

debated. Most recently even a participation of singlet oxygen ($^1O_2$; the least excited species, $^1\Delta_gO_2$, also occurs in biological systems) in such redox processes has been postulated[46]. This way, another oxidant would be added to the scenario of $\bullet O_2^-$ products.

In short, even in simplified model systems of biomembrane destruction, we have to consider various initiators, propagators and amplifiers of free radical chains. Homolysis of H_2O_2 will yield two molecules of the hydroxyl radical, the most likely initiator of lipid peroxidation By analogy, homolysis of a fatty acid hydroperoxide would yield one hydroxyl radical and an alkoxyl radical (LO•), which implies that the radical chain would be accelerated due to branching. More Likely, however, •OH is generated from H_2O_2 or LOOH and Fe^{++} according to Haber and Weiss or a Haber/Weiss-like reaction, respectively. In the latter case also an alkoxyl radical (LO•) may be formed, which is almost as aggressive as •OH[47]. After hydrogen abstraction (initiation), the polyunsaturated fatty acids usually add molecular dioxygen, which yields the lipid superoxyl radical (LOO•). The latter can in turn abstract a hydrogen atom from another unsaturated fatty acid residue (propagation) or react with a chain-breaking scavenger such as vitamin E (termination). Singlet oxygen, as discussed in[38], is not involved, because spontaneous dismutation of $\bullet O_2^-$ yields ground state oxygen[24]. In principle, however, also $^1\Delta_gO_2$ may contribute to lipid peroxidation, if $^1\Delta_gO_2$ is formed by myeloperoxidase products[48][50]. Apart from the canonical way of initiating lipid peroxidation, $^1O_2$ tends to produce cyclic peroxides[51].

In vivo, lipid peroxidation is even more complicated. In mammals, up to eight lipoxygenases (COX and LOX) differing in reaction and substrate specificity contribute to lipid peroxidation (reviewed in[52][53][54]). They contain a non-heme iron and are usually dormant enzymes. Activation is achieved by oxidation of the catalytic iron, as has first been demonstrated for cyclooxygenase (COX1) in 1971 by William Lands and colleagues[55], and later extended to 5-LOX[56], 12-LOX[57] and 15-LOX[58]. Therefore, enzymatic lipid peroxidation is under the control of all enzyme families involved in hydroperoxide metabolism (reviewed in[54]), and some of the glutathione peroxidases (GPx) and peroxiredoxins also reduce the products of LOXs, the hydroperoxides, and, thus may act as terminators by preventing •OH formation from LOOH in a Haber/Weiss-like reaction. Most of the thiol peroxidases require the support of a phospholipase, since, with the notable exception of GPx4, they can only reduce free fatty acid hydroperoxides efficiently, and the specificity for free fatty acids also holds true for most of the LOXs. Thus, biosynthesis and metabolism of lipid peroxides is under the control of lipases, in particular of phospholipase A_2 and its regulator Ca^{++}. The couple 15-LOX and GPx4 is an important exception, since 15-LOX appears unique in acting on complex phospholipids in membranes, thus producing the products that are specifically handled by GPx4[59].

In 1971 Gerriet Loschen had discovered that pigeon heart mitochondria produce H_2O_2[60], in particular when the respiratory chain was poisoned with antimycin A, and in 1974 Christoph Richter demonstrated that the superoxide anion was the precursor of the mitochondrial H_2O_2[61]. The possible source of this H_2O_2 was heavily discussed. Gerriet Loschen and Angelo Azzi argued that the most likely source of the mitochondrial H_2O_2 was an autoxidizing cytochrome b[62], which, because of a maximum in the spectrum upon reduction at $\lambda = 566$, was called cytochrome b_{566}. In contrast, Britton Chance (1913-2010), Alberto Boveris (1940–2020) and Enrique Cadenas insisted on autoxidation of the ubiquinols[63]. It was quite clear that $\bullet O_2^-$ formation in mitochondria happened somewhere at the substrate site of the antimycin A block. Antimycin A blocks the respiratory chain at the oxygen site of cytochrome b_{566}, which implies that all components at the substrate site of this block become reduced and can theoretically produce $\bullet O_2^-$ by autoxidation. The problem is that there are so many components: the flavine of succinate dehydrogenase, non-heme iron proteins, ubiquinols and cytochrome b_{566}. In 1986, finally, Hans Nohl (1940–2010) and Werner Jordan reinvestigated the antimycin-induced superoxide formation. They first showed that ubiquinol does not readily autoxidize and does not produce $\bullet O2^-$ in aprotic media such as mitochondrial membranes. Then, they made use of a novel inhibitor, myxothiazol[64], which had been isolated by Reichenbach and colleagues from *Myxococcus fulvus*. In contrast to antimycin A, myxothiazol blocks the respiratory chain at the substrate site of cytochrome b_{566}[65]. By means of this inhibitor, Nohl and Jordan could create a functional state of the respiratory chain with completely reduced ubiquinol and completely oxidized cytochrome b_{566}. In contrast to antimycin A, myxothiazol did not induce any $\bullet O_2^-$ production and antimycin A was no longer active in the presence of myxothiazol[66]. In particular the last quoted experiment unambiguously demonstrates that antimycin-induced $\bullet O_2^-$ / H_2O_2 production, as detected by Loschen et al.[60][61], occurs in complex III, more precisely by autoxidation of cytochrome b_{566}. Yet by now, almost a dozen different sites of mitochondrial superoxide production are being discussed, and the mechanisms differ[67][68][69]. An involvement of ubiquinols or flavin radicals must therefore still be considered.

An important beneficial role of $\bullet O_2^-$ was reported in 1973. Bernhard Babior (1935–2004) et al.[70] demonstrated that granulocytes produced $\bullet O_2^-$, and they already reasoned that this phenomenon was an essential part of the body's defense system against pathogenic bacteria. The discovery was soon confirmed and extended to other phagocytes [71][72][73][74]. It complemented three fields of already advanced research: the respiratory burst known since 1933[75], inflammation and phagocytosis known for more than a century by Elie Metchnikoff's (1845–1916) milestone paper [76].

Already Metchnikoff had observed that phagocytosis was not only directed against bacteria, but the phagocytes attacked practically everything that is sick, dead or foreign, thus triggering an inflammatory response. Up to Babior's discovery, H_2O_2 formed by the oxidative burst and halogen atoms (or hypohalous acids) arising from the myeloperoxidase reaction were widely considered the only bactericidal agents of phagocytosing leukocytes[77][78]. Initially, Babior appeared to believe that $\bullet O_2^-$ itself was the predominant killing agent[70]. In the meantime we have learned that $\bullet O_2^-$ is definitely the indispensable precursor of the H_2O_2 that is associated with phagocytosis, but the white blood cell use it also to make a highly toxic cocktail to cope with a bacterial invasion. It comprises $\bullet O_2^-$, H_2O_2, •OH possibly derived from Haber/Weiss chemistry, •NO, peroxynitrite formed from •NO and $\bullet O_2^-$ (see below), hypohalous acids or halogen atoms from a myeloperoxidase reaction, $^1\Delta_gO_2$ and likely more, and the composition of the cocktail differs depending on the cell type. Moreover, oxidative burst and superoxide formation may occur independently from phagocytosis, if phagocytes are stimulated, e.g., by pro-inflammatory cytokines, immune complexes or the complement component C5a (compiled in[79]).

It appears needless to state that the bactericidal cocktail does not work without any collateral damage to the environment of a fighting leukocyte. It causes tissue damage and, in consequence, inflammation. Already before the superoxide dismutase became known, erythrocuprein was rediscovered as an anti-inflammatory protein under the name "orgotein", which is in line with the pro-inflammatory role of $\bullet O_2^-$ [80]. Orgotein was finally developed up to marketing approval in several countries for treatment of osteoarthritis, interstitial cystitis and induration penis plastic. Some years later, the drug had to be abandoned, because the promise of complete lack of antigenicity of the bovine protein turned out to be too optimistic. As a substitute, the recombinant human Cu/Zn-SOD was prepared in a hurry by Grünenthal GmbH (Aachen, Germany) and the Chiron corporation in Emeryville (CA; USA) [81][82]. The human SOD showed exciting promise in animal models of septicemia[83] or reperfusion injury[84], yet the general aversion against recombinant products in these years and the costs involved let the project die. In short, the hope for an improved clinical use of SOD remained a dream[85].

Babior's enzyme that produces superoxide radicals in phagocytes was first described by Sbarra and Karnowski in 1959, yet as an enzyme producing H_2O_2[86]. It is now known as NADPH oxidase type 2 (NOX2[87][88][89]. Its catalytic complex ($p91^{phox}$ and $p22^{phox}$) is a transmembrane protein. It contains an FAD and cytochrome b_{558} (discovered by Segal and Jones[90]). Its FAD moiety accepts the reduction equivalents of NADPH from the interior of the cell and releases $\bullet O_2^-$ preferentially into the phagocytic vacuole, but also into the extracellular space. Like the lipoxygenases, NOX2 is a dormant enzyme that needs to be activated by cytosolic factors: $p67^{phos}$, polyphosphorylated $p47^{phox}$, $p40^{phox}$, the GTPases Rac1 and Rac2, and Rap1. Any functional disturbance of this complex system leads to a severe clinical condition, chronic granulomatous disease, which is characterized by recurrent infections. The disease was first described in 1954[91] and underscores the importance of NOX2 in host defense[91][92][93]. Superoxide production by NOX-type enzymes was soon detected also in many non-phagocytic cells. The sources are other members of the NOX family. The common denominator of these enzymes is a homologue of the flavocytochrome $p91^{phox}$. However, their mode of activation and the pathologies in case of malfunction differ (compiled in[88]). In addition, not all NOX-type enzymes produce $\bullet O_2^-$. DUOX I and DUOX II can make H_2O_2 directly and NOX4 appears to obligatorily produce H_2O_2 without the help of any SOD[88].

3. The Nitrogen Monoxide Radical

The discovery of the nitrogen monoxide (•NO; commonly called nitric oxide) did not only surprise, because it proved to be a radical — it also is a gas. The history has been reviewed by Salvador Moncada[94], Ferid Murad [95], Louis Ignarro[96], Robert Furchgott (1916–2009)[97][98] and Wilhelm Koppenol[99]. It started with the therapeutic use of nitro-vasodilators in the 19th century. A major push forward was the discovery of the endothelium-derived relaxing factor (EDRF) in the 1980s[100]. In many respects, EDRF mimicked the efficacy of compounds such as nitroglycerine or nitroprusside, but its chemical nature remained obscure. It was known that EDRF activated a guanylyl cyclase that had been extensively characterized by Murad[101], as did the nitro compounds, that it was inhibited by the superoxide anion radical, by hemoglobin and myoglobin and that it could be mimicked by •NO. In 1987, finally, two groups independently came to the very same conclusion: EDRF is •NO[102][103]. In 1998, Furchgott, Ignarro and Murad received the Nobel Prize "for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system"[104].

Many of the physiological functions of •NO were already known around the time of the mentioned Nobel Prize[105]. Its target is a guanylyl cyclase, where it binds to a heme moiety and produces cGMP as the second messenger that leads to smooth muscle relaxation in practically all animals. In mammals its biosynthesis is achieved by three distinct nitric oxide synthases (NOS; nNOS, eNOS and iNOS for neuronal, endothelial and inducible NOS, respectively). They use L-arginine, NADPH and O_2 as substrates and FAD, FMN, iron porphyrin IX, tetrahydrobiopterine and Zn^{2+} as cofactors. Their functions differ. The essential function of eNos is the regulation of blood flow via production of EDRF; it also contributes to inhibition of platelet aggregation. The neuronal isozyme is involved in neurotransmission and synaptic plasticity. The

inducible NOS is widely distributed, responds to exogenous stimuli such as bacterial lipopolysaccharides and phorbol esters and to endogenous pro-inflammatory cytokines. In macrophages, which typically lack myeloperoxidase, it complements the bactericidal cocktail with peroxynitrite, which is formed from •NO and $•O_2^-$ (see below). Apart from these canonical ways of •NO biosynthesis, the radical can also be produced by reduction of nitrite or nitrate[106].

In the meantime, •NO has reached the status of an approved drug to manage serious hypertension. A compound that inhibits the breakdown of its second messenger cGMP, sildenafil (Viagra®), has made its career as a lifestyle drug; it is used to improve penile erection. More recently, •NO was also discussed in plant and bacterial physiology. By mid July 2020, entering “nitric oxide” in EndNote yielded 88,863 hits, of which 10,853 were reviews. To compile the accumulated knowledge is simply impossible. We here can only highlight some aspects.

- •NO itself is a benign radical. Its biological effects are overwhelmingly beneficial. Its radical character, however, implies that it can react with a large variety of molecules and these down-stream processes may cause adverse effects. Fortunately, the history of nitrogen oxides can be traced back to Joseph Priestley (1733–1804), and a lot of the chemistry of •NO had been worked out before its presence in biological systems was detected[107]. The chemistry of the interaction of •NO with oxygen, thiols and other molecules is, however, very complex, and the relevance to biological systems still appears to be debated.

- Like $•O_2^-$, •NO can act as a reductant and as an oxidant.

- A prominent characteristic of •NO is its affinity to metal complexes. It is the basis of its physiological function as activator of guanylyl cyclase, but also of adverse effects resulting from binding to cytochrome P450 in the endoplasmic reticulum or to the cytochromes of the respiratory chain.

- The interaction of •NO with b-type cytochromes in complex III appeared to mimick antimycin A in triggering superoxide production (see above), which implies the formation of peroxynitrite ($ONOO^-$) due to the simultaneous presence of •NO and $•O_2^-$ and, in consequence, mitochondrial dysfunction[108].

- •NO can interact with the biradical molecular dioxygen to form a realm of nitrogen oxidation products comprising radical and non-radical species such as, e.g., $•NO_2$, $•N_2O_2^-$, N_2O, N_2O_3, NO^-, NO_2^-, $ONOO^-$ and NO_3^-[25][107].

- In contrast to •NO, $•NO_2$ is a strong oxidant and is likely responsible for nitration of tyrosine in proteins[109]. The bimolecular rate constant for the reaction of $•NO_2$ with tyrosine at pH 7.5 is $3.2 \times 10^6\ M^{-1}\ s^{-1}$, $•NO_2$ will also nitrate unsaturated fatty acids[107][110].

- Nitrosothiol in proteins or low molecular compounds such as GSH is commonly considered as a hallmark of “nitrosative stress”. Of course, these derivatives could be formed by a reaction of •NO with thiyl radicals, yet the steady state concentration of thiyl radicals is too low to be of physiological relevance. Most likely, *S*-nitrosation is achieved by N_2O_3, the latter being built from •NO and $•NO_2$, with a rate constant of $1.1 \times 10^9\ M^{-1}\ s^{-1}$[107]. However, also other mechanisms are being discussed[25].

- In the context of lipid peroxidation, •NO can adopt controversial roles. Being a radical, it can terminate free radical chains, e.g., by interacting with an LOO• [111]. Its oxidation products, however, may also initiate a free radical chain by hydrogen abstraction from a poly-unsaturated fatty acid residue[110].

- The most important pathogenic reaction of •NO is probably its combination with $•O_2^-$ to form peroxynitrite. This reaction of two radicals proceeds with a rate constant of $1.9 \times 10^{10}\ M^{-1}\ s^{-1}$, which implies that it is limited by diffusion[25][112]. Peroxynitrite, although it is not a radical, is a highly aggressive oxidant, which prompted Beckmann and Koppenol to describe this reaction as one of the “good” (•NO) with the “bad” ($•O_2^-$) to make the “ugly” (peroxynitrite) [113].

- Peroxynitrite, apart from being detrimental by itself, had been proposed to decompose into NO^- and $^1\Delta_g O_2$, thus creating another oxidant[49]. This hypothesis was, however, falsified by two later publications [114][115].

- $•O_2^-$, by reacting with •NO to peroxynitrite, inhibits the beneficial effects of •NO, e.g., on the circulation [116][117], and simultaneously causes oxidative damage. In retrospect, therefore, the surprising results seen with SOD infusion in models of reperfusion injury and septicemia may be re-interpreted as resulting from •NO salvage and inhibition of the formation of peroxynitrite.

In short, •NO itself guarantees optimum blood flow and neuronal function, but when transformed to $•NO_2$ or peroxynitrite, it becomes Janus-faced: it creates an efficient bactericidal cocktail with the typical collateral oxidative tissue damage[109][118][119][120]. For recent developments and ramifications in the field see[106][121][122][123].

4. Conclusions

Free radicals are not per se detrimental in biological systems. Those here described in more detail, the superoxide radical anion and nitric oxide, are indispensable for normal life. The superoxide radical anion production by NADPH oxidase type 2 guarantees an efficient host defense, while the other NADPH oxidases plus superoxide dismutases provide H_2O_2, which is required for the regulation of signaling cascades. Nitric oxide, depending on the producing isoenzyme, regulates blood flow, optimizes brain function or supports host defense, particularly in macrophages. Usually, this radical formation is balanced. The defense is achieved by dismutases acting on $\bullet O_2^-$ directly, catalase dismutating H_2O_2, glutathione peroxidases and peroxiredoxins, which collectively reduce a large variety of hydroperoxides and peroxynitrite, and their auxiliary enzymes. Only if these enzymatic defense systems are overwhelmed, the biological radicals disclose their tendency to promiscuously react with each other and other cellular components and thereby may cause tissue damage, which usually is of oxidative nature.

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