Methemoglobin—It's Not Just Blue: A Concise Review

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Hemoglobin has functions besides carrying oxygen to the tissues, and regulates vascular tone and inflammation via a redox couple with methemoglobin. Hemoglobin has iron in the reduced valance Fe(II) and methemoglobin has iron in the oxidized valance Fe (III), with a free energy capable of producing water from oxygen. In generating methemoglobin the couple functions as a nitrite reductase. The degree of oxidation of hemoglobin senses the oxygen level in the blood and uses its ability to produce nitric oxide from nitrite to control vascular tone, increasing blood flood when the proportion of oxygenated hemoglobin falls. Additional cardiovascular damage is produced by methemoglobin mediated oxidation of light density lipoproteins, accelerating arteriosclerosis. In addition, the release of heme from methemoglobin is an important factor in inflammation. These physiologic functions are paralleled by the well-described role in the oxidation of various drugs resulting in methemoglobinemia. Am. J. Hematol. 82:134–144, 2007. © 2006 Wiley-Liss, Inc.

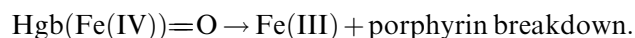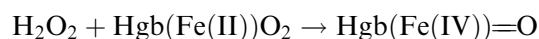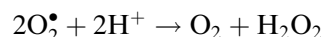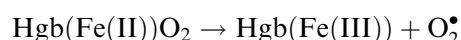Key words: inflammation; atherosclerosis; methylene blue

INTRODUCTION

Hemoglobin's function in oxygen carriage is so overwhelmingly important that it has obscured some of the other functions hemoglobin plays in physiology. The heme iron is carried in an (approximately) ferrous state (Fe(II)), the reduced form that can be oxidized to the ferric Fe(III) form (methemoglobin), analogous to the cytochrome system. It is coupled to redox cycles in the cell, and is recycled itself. This allows for the generation of two types of cyclic pathways. In the first, driven by the NAD-cytochrome b5 reductase, hemoglobin and methemoglobin are cycled (Fig. 1). In the second, a cell redox cycle system is driven by the oxidation of hemoglobin, with methamoglobin as the product (Fig. 2). These complicated systems have important roles in inflammation and vascular regulation.

REACTION OF HEMOGLOBIN WITH OXYGEN

The transport of oxygen requires oxygen reversibly bound to ferrous hemoglobin, HgbFe(II). The oxygenated hemoglobin $Hgb(Fe(II))O_2$ is a very stable molecule but does slowly auto-oxidize at a rate of about 3%/day. This rate is accelerated at lower oxygen tensions if the hemoglobin is partially oxygenated. The chemistry is actually quite complex.

The auto-oxygenation generates Hgb(Fe(III)), called methemoglobin, and superoxide. At least in vitro the superoxide undergoes dismutation to hydrogen peroxide and oxygen. The hydrogen peroxide is rapidly decomposed by catalase. The 6th coordinate of the methemoglobin is occupied with water. If not immediately destroyed the hydrogen peroxide would react with $Hgb(Fe(II))O_2$ to produce ferrylhemoglobin, $Hgb(Fe(IV))=O$ with a rhombic heme that reacts with further hydrogen peroxide to produce free Fe(III) and porphyrin degradation products [1].

$$Hgb(Fe(II))O_2 \rightarrow Hgb(Fe(III)) + O_2^{\bullet}$$

$$2O_2^{\bullet} + 2H^+ \rightarrow O_2 + H_2O_2$$

$$H_2O_2 + Hgb(Fe(II))O_2 \rightarrow Hgb(Fe(IV))=O$$

$$Hgb(Fe(IV))=O \rightarrow Fe(III) + porphyrin\ breakdown.$$

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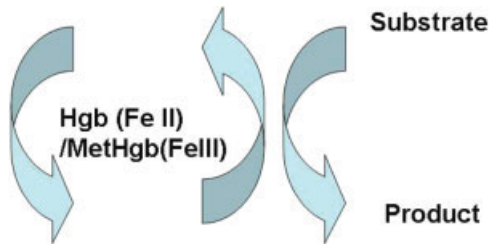
Fig. 1.   The hemoglobin/methemoglobin reaction results in the production of defined products. The methemoglobin is recycled by a reductase. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
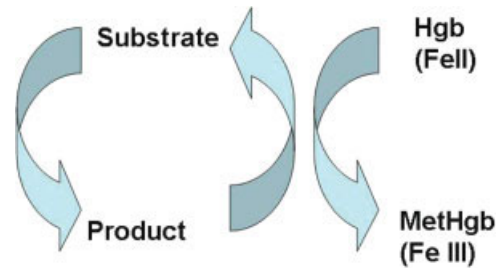

Fig. 2.   A redox couple in the cell can result in the production of methemoglobin. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

It has been difficult to detect ferrylhemoglobin, Hgb (Fe(IV)) in vivo, likely because of a "comproportionation" between ferrylhemoglobin and oxyhemoglobin, Hgb(Fe(II))O$_2$, to produce Hgb(Fe(III))O$_2$ [2].

$$Hgb(Fe(IV)){=}O + Hgb(Fe(II))O_2 \rightarrow$$
$$Hgb(Fe(III))O_2$$
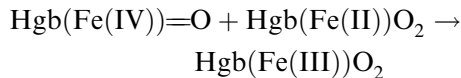
The ferrylhemoglobin is very oxidative, and the hydroxgen peroxide breakdown products accumulate in vivo and may initiate further oxidative damage [3]. These are not thought to be the major pathway for hemoglobin or heme degradation under most physiologic conditions.

When the hemoglobin, Hgb(Fe(II))O$_2$, is auto-oxidized to methemoglobin, Hgb(Fe(III)), the methemoglobin, is recycled back to hemoglobin Hgb(Fe(II)) so that in the steady state the amount of intracellular methemoglobin is <1%. The methemoglobin is reduced by the NADH-cytochrome b5-metHgb reductase. In addition, reduction can be done by several alternative pathways such as NADPH-dependent MetHgb reductase and direct reduction by intracellular ascorbate and glutathione [4].

NADH-CYTOCHROME b5 REDUCTASE

There are two forms of the NADH-cytochrome b5 reductase (diaphorase 1, DIA1) in humans. A soluble, erythrocyte restricted form, which is active in methemoglobin reduction, and a ubiquitous membrane associated form involved in lipid metabolism [5]. In the rat the two forms are generated from alternative transcripts differing in the first exon. In the rat exon I has an in-frame initiation codon, which is used inefficiently and allows the use of a downstream AUG in the second exon, generating the soluble form. Likewise, in humans there are two transcripts, M and S. The S transcript is not detectable, except in erthrocytes. The position of the first AUG is shifted in man, but analogous to the rat there seems to be an internal initiation

codon. The membrane form of the reductase also comes in two forms, localized in the ER or the mitochondrion inner membrane. In the ER the enzyme participates in lipid metabolism (including biosynthesis of cholesterol and in P450 mediated drug metabolism), but in the mitochondrion membrane it mediates the regeneration of ascorbate from the ascorbate radical. In the absence of myristolation the N-terminus acts as a signal recognition peptide and targets the protein to be inserted into the ER. Myristolation lowers the ability to interact with the signal recognition particle and makes the protein available for post-translational targeting to the mitochondrial membrane.

Those cases of congenital methemoglobinemia due to an enzyme defect in the reductase have been classified into 4 types [6]. Type 1 is a deficiency in cytochrome b5 reductase limited to erythrocytes [7,8]. Type 1 has few symptoms other than visible cyanosis, such as occasional complaints of headache, fatigue, and exertional dyspnea. The methemoglobin levels exceed 25% of the total hemoglobin. Type 2 is more pervasive and is associated with a generalized systemic deficiency due to the alterations in lipid metabolism affecting a multitude of tissues, particularly the central nervous system [9,10]. There is an unremitting, progressive neurological deterioration with mental retardation, microcephaly, opisthotonus, athetoid movements, and generalized hypertonia. Type 3 is no longer recognized as a separate entity since it was shown [11] to be identical to type 1. Type 4 was described in a single case, and is manifested by an attenuated concentration of cytochrome b5 [12].

To date, with new mutations being described often, there are 33 different mutations known in unrelated patients of different ethnicity with recessive congenital methemoglobinemia [13]. For the 28 exon mutations, 17 have been associated with Type 1, 15 with Type 2, and one mutation was common to both 1 and 2. The Type 1 typically consists of missense mutations related to enzyme stability. Type 2 are typically deletions or premature stop codons resulting in truncated
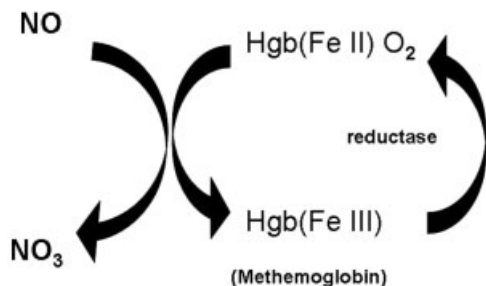
Fig. 3. Oxygenated hemoglobin uses the oxygen to produce nitrate, and in the process methemoglobin. The methemoglobin is recycled by the reductase.

or unstable proteins, and those associated with FAD binding residues [14,15].

Recently another factor controlling methemoglobin was reported, an antioxidant protein AOP2 that protects against methemoglobin formation. It is associated with the hemoglobin complex and may be involved in the normal protection of the heme pocket [16].

REACTION WITH NITRIC OXIDE, NITRITE, AND NITRATE

Nitric oxide is a gas that is continuously produced by nitric oxide synthetase in the endothelial cells using L-arginine as the substrate. The constitutive enzyme requires calcium and calmodulin. NO is responsible for control of the systemic [17] and coronary artery vascular [18,19] tone, acting as an endothelial relaxing factor [20,21]. The effects of NO are however limited to the local area surrounding the cells producing the gas.

NO rapidly diffuses from the endothelial cell into the vessel lumen and enters the red blood cell. It reacts differently with oxyhemoglobin and deoxyhemoglobin. With oxy-hemoglobin it produces nitrate (NO_3^-) and methemoglobin (probably via an initial chemical oxidation to nitrite) (Fig. 3).

The nitrate represents a metabolic end point, and is excreted as an inert salt. The reaction with $HgbO_2$ limits the half-life of the NO. The reaction with free hemoglobin is so fast that any NO produced would be consumed immediately. Under most circumstances the hemoglobin is separated from the plasma by being confined to the interior of the red cell. This functions as a barrier due to (1) the red cell membrane itself [22,23], (2) the unstirred layer around the erythrocyte [24], and the (3) erythrocyte-free zone along the surface of the vascular endothelium [25–27], conditioned by the rheological flow patterns of blood. In the absence of flow, the rate of NO capture would be increased, and dilation of the vessels decreased. These barriers to the uptake and destruction of NO decrease the rate of hemoglobin reaction with NO by about 600-fold, allowing local concen-
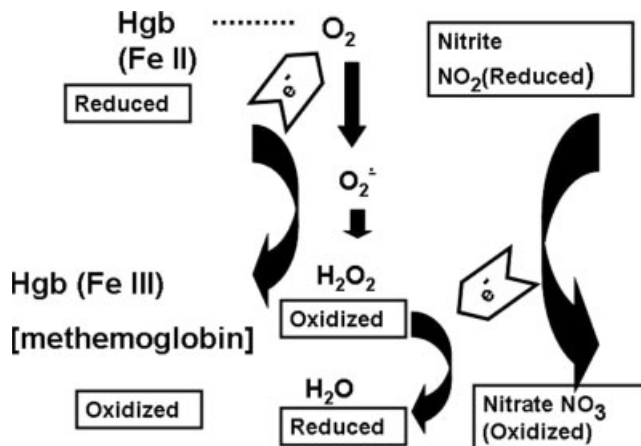
Fig. 4. Mechanism for oxidation of nitrite. While the mechanism is still unclear, one electron is taken from Fe(II) to form Fe(III) and one from the substrate and the two electrons added to oxygen to eventually reduce oxygen to water.

trations of NO to form to permit some vaso-dilation [28,29]. This allows a portion of the NO to escape both the diffusion into the vascular smooth muscle and the reaction with hemoglobin in the RBC. This NO is able to react with oxygen to form nitrite, or to nitrosylate free thiols, amines, and other unidentified components [30].

$$2NO + O_2 \rightarrow 2NO_2$$

$$NO_2^- + Hgb(Fe(II)) - O_2 + 2H^+ \rightarrow Hgb(Fe(III)) + NO_3^- + H_2O$$
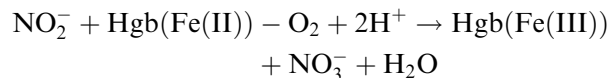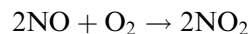
While the mechanism for this is complex and controversial, overall the reaction takes one electron from the iron (Fe(II)→Fe(III)) and one electron from the NO_2 and both are transferred to oxygen, reducing it eventually to water.

This is a counterintuitive reaction that is a common reaction for hemoglobin, since the nitrite is oxidized (loss of electron) and the iron is oxidized (loss of electron), which does not balance, unless the oxygen is accounted for, which becomes reduced (gains electrons) (Fig. 4). One model of the reaction involves the production of superoxide and hydrogen peroxide, which may not be correct, but illustrates the principle [31].

Obviously, reactions with deoxygenated hemoglobin must use another mechanism since there is no oxygen, and the reactions with the deoxygenated hemoglobin are reductive not oxidative. These also result in the production of methemoglobin. There are two major reactions of the nitrogenous compounds with Hgb(Fe(II)), one binding NO to the heme and the other reducing nitrite to NO.
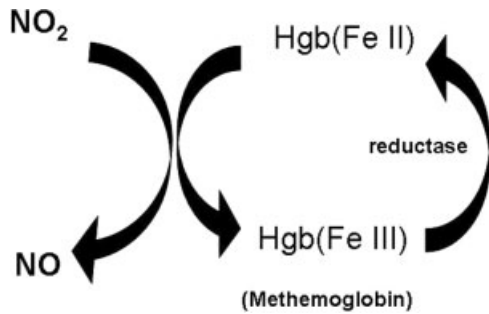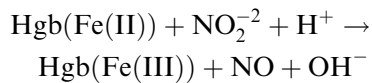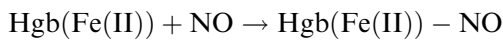
Fig. 5. Hemoglobin acts as a nitrite reductase, but only in the deoxygenated from. This is a reduction in contrast to the reaction of NO$_2$ with oxygenated hemoglobin (HgbFe(II)(O$_2$)), which is an oxidation.

$$Hgb(Fe(II)) + NO \rightarrow Hgb(Fe(II)) - NO$$

$$Hgb(Fe(II)) + NO_2^{-2} + H^+ \rightarrow$$
$$Hgb(Fe(III)) + NO + OH^-$$

Nitrite could potentially be produced from dietary nitrates, and a salivary gland nitrate reductase (probably due to bacterial) rapidly converts nitrates to nitrites. Nitrite in the stomach is acidified and nitrosylates thiols, forming S-nitrosothiols, and react with amines to form N-nitrosoamines. With electron donors such as ascorbate or excess nitrite the acidified nitrite generates NO gas, which may help in mucosal blood flow regulation and protection. In the plasma the majority of nitrite (NO$_2$) is derived from the auto-oxidation of NO.

$$2NO + O_2 \rightarrow 2NO_2$$
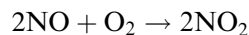
The nitrite is stable compared to NO, but can chemically react with NO to form NO$^+$, which can itself form S-nitrosothiols and N-nitrosamines. More importantly, it can be reduced by deoxy-hemoglobin to form methemoglobin and NO.

$$NO_2 + Hgb(Fe(II)) \rightarrow Hgb(Fe(III)) + NO$$
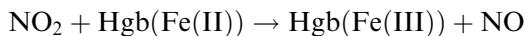
Since the methemoglobin (Hgb(Fe(III))) can then be recycled, it yields an enzymatic conversion of nitrite into vasoactive NO. Nitrite levels may be as high as 150 nM to 1 μM, but nitrite is oxidized by oxyhemoglobin to nitrate with a half life of about 11 min [32].

Hemoglobin therefore functions as a nitrite reductase (Fig. 5), resulting in the production of methhemoglobin.

The methemoglobin is regenerated to hemoglobin by the NADH-cytochrome b5 reductase system. But the deoxy-form of hemoglobin is not the nitrite reductase, because the maximal rate of NO production occurs at the oxy-to-deoxy transition [33]. The rate of NO synthesis is maximal at 40–60% hemoglobin saturation. This reductase couple might be the sensor for the oxygen tension to cause increased vasodilation and decrease in arteriole resistance. The hemoglobin directed production of NO would be effective at oxygen tension of 40–15 mmHg [34], and below about 10 mmHg direct nitrite reduction to NO by nonenzymatic disproportionation and enzymatic reduction by xanthine oxidoreductase might contribute to NO formation.

For completeness it can be noted that peroxynitrite will react with Hgb(Fe(II))O$_2$ to produce nitrate (NO$_3$) with a ferryl hemoglobin (Fe(IV)) as an intermediate and methemoglobin (Hgb(Fe(III))) as the final product, a reaction probably of significance in free radical scavenging [35].

So when fully oxygenated the HgbFe(II)O$_2$ can inactivate NO or nitrite, thereby allowing contraction of the vessels and decrease in blood flow. When partially deoxygenated electrons from NADPH are used to reduce NO$_2$ to produce NO, via the hemoglobin/methemoglobin couple, to decrease vascular tone and increase blood flow and therefore oxygen to the tissues. The hemoglobin/methemoglobin couple acts as the oxygen sensor and is therefore essential in maintenance of the vascular flow and the regulation of blood flow in response to oxygen tensions in the endothelial cells.

ROLE OF METHEMOGLOBIN IN INFLAMMATION

Beside the local control of blood flow, the hemoglobin-methemoglobin redox couple plays a role in inflammation and the development of atherosclerotic disease. Methemoglobin, or the heme-Fe(III) released from methemoglobin, promotes inflammation and oxidizes lipoproteins promoting atherosclerosis.

The production of methemoglobin is not always to the benefit of the organism. NO produced during inflammation and other oxygen reactive species results in conversion of hemoglobin to methemoglobin, and this consequently results in an increased rigidity of the RBC with increased RBC lysis [36–39]. This results in release of free methemoglobin (now without the reduction mechanism) [40]. Any free hemoglobin (HbgFe(II)) or heme (Fe(II)) released into the tissues or circulation is promptly converted by spontaneous oxidation to methemoglobin, Hgb(Fe(III)), or hemin, (heme Fe(III)) [41]. Methemoglobin is an activator of endothelial cells by stimulation of IL-6, IL-8, and E-selectin [42]. This is independent of the release of heme or iron and appears to be NF-κB mediated. The release of cytokines and the expression of adhesion molecules would intensify the inflammation response.

Heme moieties that are oxidized (porphyrin-Fe(III)), such as methemoglobin, dissociate more readily from

the protein than the reduced heme (prophryin-Fe(II)) that is tightly bound to the proteins such as in hemoglobin. The heme in methemoglobin is more likely to dissociate from the pocket in the protein [43,44]. The free heme (as porphryin-Fe(III)) is extremely lipophilic and binds to lipids intercalating into the membranes of neighboring cells. Heme release or heme administration in vivo increases vasopermeability, adhesion molecule expression, and the infiltration of tissues by leukocytes [45]. Exposure to heme (Fe(III)) stimulates expression of adhesion molecules ICAM-1, VCAM-1, and E-selectin [46,47]. In addition to intercalating into membranes, the oxidized heme slips into the lipids of lipoproteins.

Methemoglobin becomes the mediator between hypoxia, red cell lysis, and increased inflammation.

Free hemoglobin released from cells rapidly dissociates into αβ dimmers. Hemoglobin released from cells, if not immediately oxidized to methemoglobin, is captured by the peptide complex haptoglobin (Hp). The clearance of the Hp-heme complex is actually a major macrophage function. The [Hp-Hgb(Fe(II))]$_n$ is cleared by special cells in the liver via the CD163 receptor. Endocytosis by macrophages results in lysosomal degradation of the protein and of the heme by heme oxygenase 1, releasing ferrous iron (Fe(II)), biliverdin, and CO [48]. Knock-out mice lacking Hp have no difficulty in clearing Hgb [49], rather the role of Hp would seem to be to protect against oxidative damage to lipids and LDL [50,51].

Hp also binds methemoglobin (Hgb(Fe(III))) [52], whose oxidative effect can be inhibited by the Hp [53], but there can still be an oxidation of LDL. Since in methemoglobin has a reduced capacity to bind hemin (Fe(III)), it can be transferred to ApoB in the LDL [54]. There is a linear oxidation of LDL by the methemoglobin and a burst due to hemin breakdown and oxidation by free iron. The Hp2-2 isoform of Hp fails to hold on to globin heme as well as Hp1-1. The Hp2-2 phenotype has more vascular disease than those with the Hp1-1 phenotype [55]. Since oxidized LDL is central to the progress of atherosclerosis, Hp can be considered to be antiatherosclerotic and methemoglobin proatherosclerotic.

Methemoglobin by itself, and by the facile release of hemin, (Fe(III))-prophryin, is a major cause of LDL oxidation and atherosclerotic damage (Fig. 6).

Hemin (Fe(III)) released from LDL or from circulating methemoglobin is bound to hemopexin, whose purpose should be to protect the vasculature form oxidation damage. Some is also bound to albumin and both proteins are transported to the endoreticular system in the liver and degraded [56]. Heme bound to hemopexin binds to a specific receptor, the low-density lipoprotein receptor-related protein (LRP)/
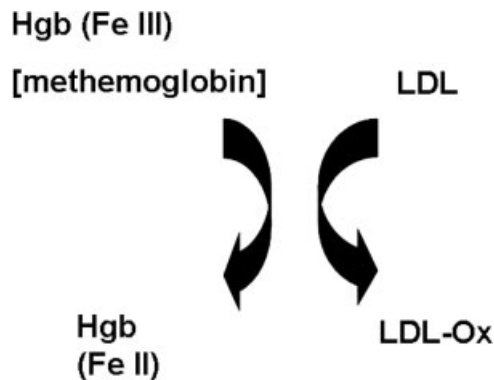
Fig. 6.   The hemoglobin/methemoglobin redox couple oxidizes LDL, leading to increase arteriosclerotic risk.

CD91, resulting in cell uptake and lysosomal degradation of the hemopexin and heme [57]. Like sCD163 this receptor appears to be recycled.

Hemin, released from methemoglobin, may be released in such quantities that it exceeds the binding ability of hemopexin and there the hemin (Fe(III)) is released extracellularly. Infused hemin is profoundly proinflammatory, and heme-oxygenase is antiinflammatory, but it is not clear if any free heme actually circulates, or if all the heme intercalated into membranes is derived directly from methemoglobin (Fig. 7). The hemopexin knockout mouse is viable, and it appears that the hemopexin is not required as protection against oxidative stress. The only apparent difference is a slowed recovery with more renal damage after hemolysis [58]. Heme-oxygenase is antiinflammatory due to the removal of heme, and the metabolism of heme results in the production of bilirubin and CO, both vasodilators. Hemeoxygenase knockouts have defects in iron metabolism [59], but exaggerated expression results in increased production of reactive iron species and cytotoxicity [60].

In a famous human case of HO-1, deficiency there was ongoing erythrocyte fragmentation and hemolysis with endothelial damage [61]. In the HO-1 and apoE double knockouts there is exacerbation of atherosclerotic lesion formation and defects in vascular remodeling [62] consistent with the critical role of hemin in oxidative damage and inflammation.

Methemoglobin is then a critical mediator of the inflammatory response, both as a product of NO and nitrate production, as a mediator of oxidative stress, and as the source of heme and iron with additional proinflammatory and oxidative proclivities.

ACQUIRED METHEMOGLOBINEMIA

The Hgb(Fe(II))O$_2$ methemoglobin Hgb(Fe(III)) couple can undertake redox transformations under a
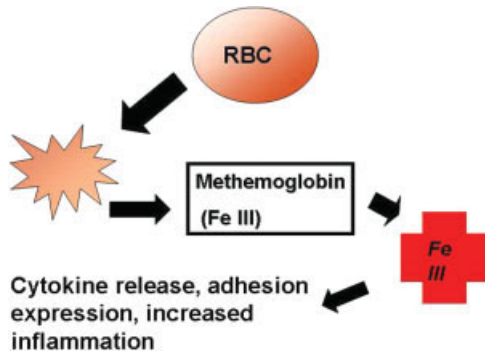
Fig. 7. Methemoglobin releases free heme and iron that stimulate the inflammatory process. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 8. Oxidation of the aniline derivative catalyzed by oxygenated hemoglobin.

number of important biological situations, and it is no surprise that xenobiotics are substrates, forming methemoglobin in the process. The acquired formation of methemoglobin can have consequences. While Hgb(Fe(II)) can be oxidized directly by some xenobiotics to form methemoglobin, resulting in the reduction of the xenobiotic, the usual reaction is the oxidation of the xenobiotic by Hgb(Fe(II))O$_2$, resulting in H$_2$O and methemoglobin.

The production of methemoglobin has clinical consequences. The presence of ferric iron in one or both of the pophyrins causes the oxygen-dissociate curve to shift to the left [63]. The combined effect of less oxygen carried and less released can result in respiratory failure [64]. Healthy individuals without anemia have few symptoms at 15% methemoglobin, but levels of 20–30% cause mental status changes, headache, fatigue, exercise intolerance, dizziness, and syncope, and levels of 50% result in dys-rhythmias, seizures, coma, and death [65]. Patients with anemia, lung disease, sepsis, abnormal hemoglobins, sickle cell, age less than 6 months, or the elderly are at greater risk.

There are two basic mechanisms for acquired methemoglobinemia: direct or indirect oxidation of the hemoglobin. Rarely the Fe(II) in hemoglobin, Hgb(Fe(II)), can be directly oxidized to Fe(III) to form methemoglobin. Direct oxidation can be caused by chlorates, hexavalent chromates, cobalt [66], and copper II salts. Ferricyanide has an adequate redox potential, but penetrates membranes poorly. But these high redox potential compounds are usually not implicated in methemoglobinemia, and exposure results in intravascular hemolysis rather than methemoglobinemia. A similar mechanism may be involved in the induction of methemoglobinemia by the ribonucleotide reductase inhibitor triapine (3-aminopyridine-2-carocaldehyde) [67]. This compound is an iron chelator, but unlike other iron chelators its inhibitory activity towards the non-heme iron protein ribonucleotide reductase is
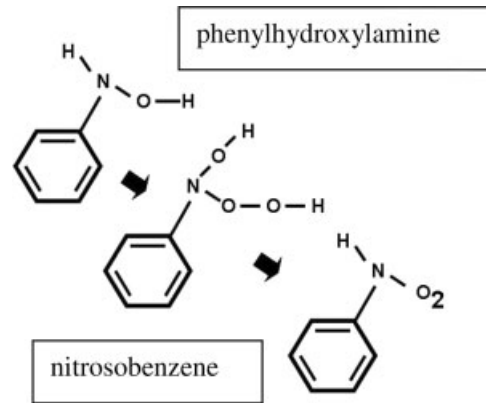
not reduced, but increased by exogenous iron [68,69]. As a side effect, triapine can cause methemoglobinemia, probably by a similar mechanism of direct transfer of an electron form the Fe(II) heme to the Fe(III) triapine to form methemoglobin (Hgb(Fe(III))), a mechanism referred to as "electron steal."

Indirect oxidation is more prevalent. Oxidation occurs with participation of both the iron and the oxygen, with superoxide and hydrogen peroxide production when the hemoglobin bound oxygen accepts electrons from ferrous iron [70].

Aniline is a potent inducer of methemoglobinemia and hemolysis, but is converted first to phenylhydroxylamine that is oxidized to nitrosobenzyene by Hgb(Fe(II)) and oxygen (Fig. 8).

The nitrosobenzene is subsequently reduced by a NADPH flavin reductase back to aniline, using NADPH derived from glucose-6-phoshate dehydrogenase [71] and the hexose monophosphate shunt. Alternatively, glutathione can be used as a source of reducing power [72,73]. Aniline and nitros-derivatives are transformed into phenylhydroxylamines by hepatic mixed function oxidases, and then can become inducers of methemoglobin [74]. Such bioactivation is important for the toxic effects of dapsone and sulfamethoxazole (the sulfa component in trimethoprim-sulfamethoxazole, Septra, or Bactrim), probably via the formation of hydroxylamines. The dapsone hydroxylamine was the more potent in forming methemoglobin and consuming glutathione compared to the sulfamethoxazole hydroxyl amine, paralleling the in vivo findings [75].

Along with the production of methemoglobin, reducing power in the cell is depleted. The depletion is explained by the recycling mechanism, and once the glutathione is depleted, the continued formation of methemoglobin should stop [76]. The hexose monophosphate shunt was also stimulated to produce more NADPH [77].
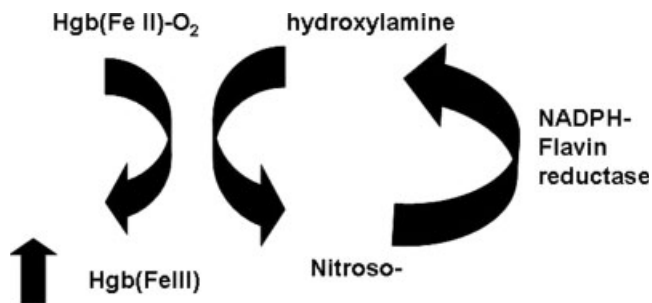
Fig. 9. The reduction of the oxidized phenylhydroxylamine results in depletion of reducing power and an accumulation of methemoglobin.

Fig. 10. The oxidation of phenylhydroxylamine by Hgb(Fe(II))O$_2$ involve an electron from iron and one from the substrate to reduce oxygen to form water.

Since in these cases the oxidized substrate can be rereduced the result is a cycle driving hemoglobin into methemoglobin (Fig. 9). The cyclic reaction is driven by NADPH flavin reductase or glutathione, which explains why a relatively small amount of drug can result in a relatively large amount of methemoglobin. In the methemoglobin formation by phenacetin metabolites, depletion of the thiol reduction molecules in the cell retarded the production of methemoglobin, since the drug could not recycle [78]. The role of defects in G6PD in induction of methemoglobinemia [79] is a topic beyond the scope of this review [80].

As in the case of nitrite oxidation (Fig. 4), the oxidation of hemoglobin in the process of oxidation of the substrate is due to the participation of oxygen (Fig. 10), and requires the oxygenated form of hemoglobin. Oxygen's reduction to water requires two electrons, one from Fe(II) and one from the substrate.

Primaquine, an important antimalarial, is metabolized to derivatives that then induce methemoglobinemia [81]. It is not clear what the exact derivatives are, and perhaps it is a combination. The hydroxylamine is also produced in the microsomes, and would act completely analogous to the aniline-like drugs. The 6-methoxy-8-hydroxylaminoquinoline derivative of primaquine also induces methemoglobinemia, glutathione depletion, and hemolytic anemia [82]. But besides the hydroxylamine the phenolytic derivative 5-hydroxyprimaquine is produced and induces methemoglobin formation and depletion of red cell glutathione. The 5-hydroxycompounds can be spontaneously oxidized to the quinine-imine, probably producing hydrogen peroxide [83]. This reaction is analogous to the formation of toxic metabolites form acetaminophen [84]. But which is the more important in the production of hemolytic anemia and methemoglobinemia in vivo is not known, nor is the mechanism of hemoglobin oxidation by the phenolic compound.

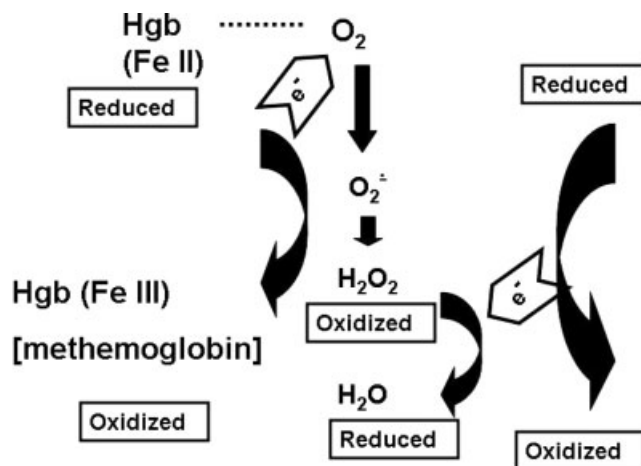How the defects lead to the hemolytic anemia is even less clear. Hemolysis is not evident in the congenital methemoglobinemias. As mentioned, the mechanism may be production of hydrogen peroxide by the oxidation reactions and whose products escape and damage the cell [85]. The production of hydrogen peroxide, whether from the conversion of Hgb(Fe(II))O$_2$ to methemoglobin, Hgb(Fe(III)), or from the formation of the hydroxyamides, could react with ferrous iron released from methemoglobin to form the hydroxyl anion radical with subsequent damage to the red cell membrane. In the related acute hemolytic anemia due to divicine, an active ingredient in favism, the hexose monophosphate shunt is activated, and depletion of glutathione, and possible oxidation of membrane skeletal proteins results. This is likely due to disulfide linking of the proteins since it was reversed by dithiothreitol [86]. Oxidation by integration of transferred ferric heme from methemoglobin accretion to the membrane, analogous to the oxidation of LDL is also reasonable. Induction of methemoglobinemia with hydroxylated products from dapsone or anilines causes the release of iron, resulting in iron overload in the spleen and liver Kupffer cells [87].

It is possible that the hemolysis is due to the free iron. Iron results in oxidative damage to band 3, resulting in the production of senescent cell antigen (SCA) [88,89]. Oxidized band 3 (SCA) is bound by endogenous, autologous IgG, which attracts the cell to macrophages and leads to cell destruction. This is the natural process for aged RBC but would be accelerated in methemoglobinemia [90], especially if iron is released, or if free radical induction is increased by the oxidation of the offending drug.

There are long lists of drugs that can induce methemoglobinemia [91–93]. Among the more recent reports are methemoglobinemia due to prolonged exposure to prilocaine for liposuction [94], topical benzocaine [95–97], and celecoxib [98]. All these substrates have an ar-
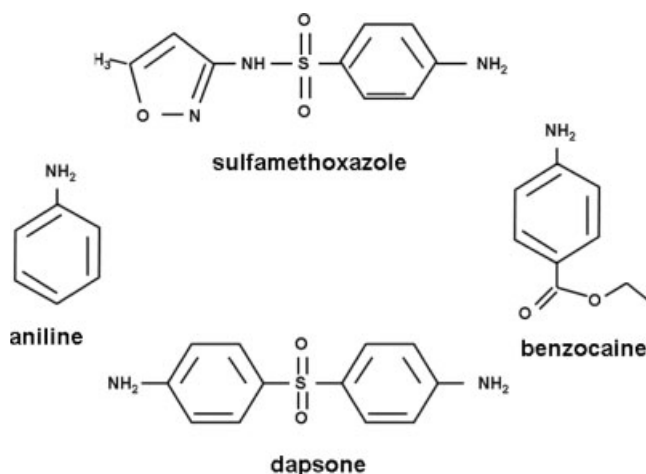
Fig. 11. Some of the common compounds that induce methemoglobinemia probably via the hydroxylamines and Hgb(Fe(II))O$_2$. There is common aniline like moiety in each of these drugs.
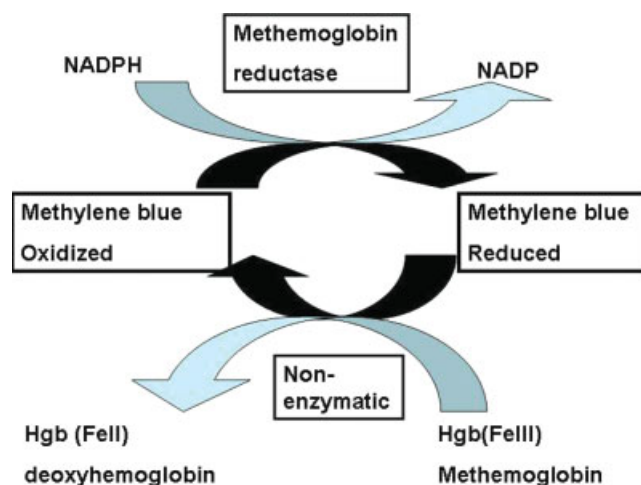

Fig. 12. Rereduction of methemoglobin by methylene blue. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

omatic amine that could be converted to a phenylhydroxylamine (Fig. 11). Benzocaine spray has become an increasing problem, and spray directed to the mucosa can gain rapid entry to the blood and result in severe levels of methemoglobin. There are a wide variety of case reports [99], but no systematic study. In one review from a transesophageal echocardiology laboratory the incidence was estimated at 0.115% [100].

METHYLENE BLUE

The treatment for symptomatic methemoglobinemia is infusion of methylene blue.

Methylene blue acts as an electron donor for the nonenyzmatic reduction of methemoglobin. A distinct enzyme, NADPH methemoglobin reductase, converts methylene blue (the oxidized form of the dye) to leukomethylene blue (the reduced form), using NADPH (Fig. 12). The reduced form then chemically reduces methemoglobin (Hgb(Fe(III))) to hemoglobin Hgb(Fe(II)). The dye is then recycled. The enzyme responsible, NADPH methemoglobin reductase, is not the same enzyme involved in the physiological reduction of methemoglobin (the NADH-cytochrome b5 reductase), and in the absence of methylene blue this enzyme is responsible for less than 6% of the restoration of hemoglobin from methemoglobin.

Since symptoms do not occur at levels less than 20–30%, treatment is not needed below that level, unless other conditions (such as anemia, respiratory distress, and cardiac disease) contribute to the clinical scenario. It should be noted that methylene blue interferes with oximeter readings of oxygen saturation [101].

Individuals with glucose-6-phosphate dehydrogenase deficiency lack the ability to produce NADPH. They are more susceptible to oxidative stress because NADPH is needed to maintain glutathione (not NADH-cytochorme b5 reductase). Since the methylene blue systems require NADPH as the reductase, methylene blue will be ineffective in these individuals, but by further reducing levels of NADPH and preventing reduction of glutathione, the use of methylene blue may actually worsen the methemoglobinemia [102]. If there is extensive hemolysis, such as would be seen with the chemically induced methemoglobinemias due to chlorates or with aniline, then the enzymes needed to reduce methylene blue are released from red cells and the drug is ineffective. If methylene blue cannot be reduced, it can function as an oxidizing agent and convert hemoglobin to methemoglobin exacerbating the condition it was used to treat. This can occur in individuals with G6PD deficiency as well. Patients exposed to anilines may also block the uptake of methylene blue by the production of phenylhydroxylamine [103,104]. Methylene blue can worsen the hemolytic anemia due to dapsone via the formation of hydroxylamine that oxidizes the hemoglobin, a complication that can be delayed for several days [105,106].

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