Chapter 2

Death by Dose—The Most Toxic Compounds

A fun night out? Let us go to an oxygen bar. A high concentration of oxygen gas (O_2) is bubbled through bottles containing aromatic flavors (e.g., mint or lavender) and led via small tubes hooked over the ears to openings under the nostrils where it is released. You are lured into the establishment because it is advertised that the blood oxygen concentration rises which will reduce stress, increase energy and alertness, and alleviates headaches. Recreational inhalation of oxygen: A fun night out, or an appropriate beginning of a chapter on "the most toxic compounds"?

THE CHEMISTRY OF OXYGEN

In this chapter we will focus on one of the most toxic compounds in our environment, oxygen (O_2). The struggles of its discovery, its role in evolution, and its reactivity will be looked at. We will show and explain the extreme toxicity of oxygen and its involvement in many age-related diseases. Questions on the possibilities to counteract its toxicity will be discussed. Finally we will show how, during evolution, we have been adapted to oxygen toxicity and how this adaptation process can still be used to benefit our health, adaptation being the keyword here.

The English theologian and chemist Joseph Priestley (1733−1804) is credited with the discovery of oxygen. He called the gas that he isolated "dephlostigated air," which he made by focusing sun rays on a sample of mercuric oxide. To his surprise mice survived in this "dephlostigated air." His ideas were in line with the prevailing idea that all burnable material comprised of two parts. One part was called phlogisticon which was given off when the substance containing it was burned. The residual part was thought to be the real form of the material.

As with many important discoveries, others at the same time had similar ideas. In fact the Swedish pharmacist Carl Wilhelm Scheele produced oxygen gas in 1772, two years earlier than Priestley. The Frenchman Lavoisier conducted quantitative combustion experiments using oxidation in a closed environment. He reached the conclusion that air consisted of gases,

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"vital air" which is used for the combustion and for respiration and "lifeless air" or azote. The latter is still the word for nitrogen in French.

"Vital air" was renamed by Lavoisier in 1777 to oxygène. This name appeared to be wrong because Lavoisier believed that oxygen was part of all acids (*oxys* (Greek) means acid or sharp taste of acid and *−genes* (Greek) means producer, oxygen is then "acid producer").

Not only in the life of scientists with their experiments and theories of the 18th century the struggle with oxygen was clearly visible. Also in the development and organization of life on earth as a whole the fight with oxygen played a crucial role.

The estimated age of our earth is 4.6 billion (4.6×10^9) years. Intense solar radiation bombarded the surface of the earth at 3500 million (=3.5 billion) years when anaerobic life began. Blue green algae in the oceans acquired the ability to use the process of photosynthesis which uses solar energy, water, and carbon dioxide releasing oxygen at 2500 million years ago. The biological appearance of oxygen is called the Great Oxygenation Event. Obligate anaerobic organisms may have been wiped out by the appearance of oxygen. Around 1300 million years back, oxygen levels in the atmosphere reached 1%.

Recent studies suggest that interplay between biology and geology let the oxygen concentrations fluctuate periodically over the past 2 billion years. It commences with some oxygen in earth's atmosphere which reacted in the geosphere. Oxygen has a peculiar reactivity. It vividly reacts with iron in rocks or with hydrogen spewed out of volcanoes. When the earth calmed down, less geological oxygen reactions occurred. The extra oxygen fueled biological life. The abundant growth of microbes created carbon-rich rocks onto the sea floor. Later the rocks formed dry land and reacted with the oxygen out of the atmosphere and oxygen levels decreased again.

More complex cells with nuclei (eukaryotes) began to evolve and multicellular organisms emerged. The ozone (O_3) layer in the atmosphere formed and subsequently screened out much of the UV light and facilitated the emergence of life forms from the sea. Then 65 million years ago primates appeared and only 5 million years ago humans came on the scene. At that time the atmospheric oxygen concentration was 20.8%. During evolution oxygen slowly increased in the atmosphere and living organisms could gradually adapt to the apparent toxic properties of oxygen.

The unusual reactivity of oxygen necessitates its continuous formation by photosynthesis. At the same time we use its reactivity in various physiological processes. Oxygen is reduced by four electrons in a stepwise fashion (Fig. 2.1).

Oxygen has two unpaired electrons. This explains the ease by which oxygen can readily take up additional electrons. Oxygen with one additional electron is called superoxide radical. Oxygen with two electrons is called the peroxide anion; this form can lead to the formation of hydrogen peroxide.

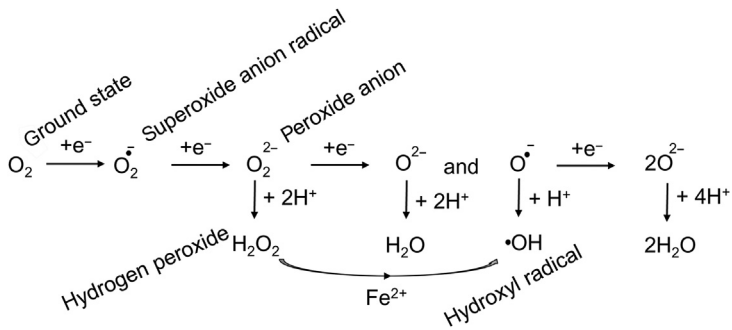

FIGURE 2.1 Scheme of stepwise reduction of O_2. In this scheme the formation of superoxide anion radicals, peroxide anions, hydrogen peroxide, and water is illustrated.

With three electrons the very reactive hydroxyl radical is generated. And finally, with four electrons oxygen is transformed into water.

So-called transition metals like iron or copper can activate the formation of reactive oxygen species (ROS). Luckily in our body the iron is generally safely stored away in proteins limiting the reactivity of the transition metal. Energy (light) may also activate oxygen, generating the so-called singlet oxygen forms.

All these reactive oxygen forms are called "ROS." In the lay press these forms are regularly indicated as oxygen radicals. This is not correct because not all of these intermediate oxygen species contain a free unpaired electron, which is the definition of a radical. They are reactive though, some more than others.

Because of its facile reaction with electrons, oxygen can be used as an electron sink in physiological processes. This is applied in mitochondria, cellular organelles which are crucial for energy, in the form of ATP production. Mitochondria are the power houses of the cell. The electron transport chain in the mitochondria is the driving force for mitochondrial ATP synthesis. The flow of electrons, through this chain, eventually goes to oxygen which is thereby transformed into water. Partial reduction of oxygen in this process may lead to ROS and may lead to damage.

Oxygen binds to iron. As mentioned, this already played a role in regulating the oxygen in the development of the earth's oxygen atmosphere. Also in the transport of oxygen in the body binding to iron plays a role. Iron is an intricate part of the protein hemoglobin which is the transporter for oxygen in red blood cells from lungs to the mitochondria. Heme (as part of hemoglobin) is a specific molecular structure that holds iron and allows iron to bind oxygen.

Another evolutionary old enzyme is the liver heme-containing protein cytochrome P450. The enzyme plays an important role in the liver (Chapter 3: The Coping Body—A Myriad of Exposures). It metabolizes both

endogenous compounds like hormones or fatty acids as well as xenobiotics (compounds which are strange to the body like drugs or exogenous toxins). It is however also found in other organs. Moreover, the enzyme can be found over the entire phylogenetic scale: mammals, plants, and bacteria. The widespread availability suggests its early presence in evolution. This has recently been corroborated by genomics informatics. Its early role could have been that it offered protection against the toxicity of oxygen in favor of the anaerobic organisms. Interestingly, under strict anaerobic conditions this mammalian cytochrome P450 enzyme displays a different function which indeed is suggestive for this other evolutionary role for this old enzyme.

Oxygen has also gained a pivotal role in immune defense. Upon stimulation immune cells can produce various ROS. They form the last line of defense against invading microorganisms. The microorganisms are taken up by these cells like macrophages and bombarded with these oxidizing species

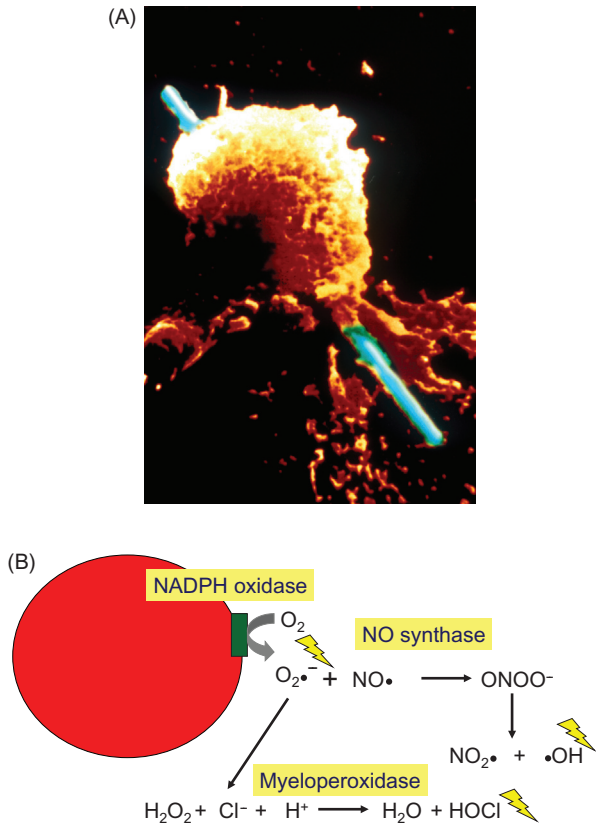

FIGURE 2.2 **(A)** Picture of macrophage trying to destroy asbestos particle. http://www.alamy.com/stock-photo-asbestos-49168810.html. **(B)** Illustration of various oxidizing species formed by phagocytic cells. *(A) From BSIP SA/Alamy Stock Photo.*

and destroyed. People who do not have this capability because they miss the enzyme producing these ROS in the immune cells cannot defend themselves properly and will die at an early age. This genetic disease is called chronic granulomatous disease. Sometimes macrophages can engulf a particle but cannot destroy it. This is the case with some asbestos fibers. The cells begin to produce the oxidizing species but the only result is that the surrounding tissue is affected by them (Fig. 2.2).

THE TOXICITY OF OXYGEN

The health risk of a compound is determined by its hazard and by the exposure to the compound. This sounds quite logical. In order to be a risk, the compound should have some hazardous properties and you at least should be exposed to the substance. In lay press this is frequently forgotten. News items on the health risk of flame retardants in the computer or plasticizers in a rain coat are just examples out of many. This type of news that is brought to us seems rather worrying but if there is no exposure to the compounds there is no risk.

$$\text{Risk} = \text{hazard} \times \text{exposure}$$

It would put news items into perspective if this general rule would be taken into account more often.

But what about oxygen? Yes, the gas is hazardous because of the ROS that can be generated from it. On top of that the exposure is immense. A relative high concentration to which we are enduringly exposed 24/7. In fact most of us die as a consequence of oxygen toxicity!

The theory that aging is caused by ROS is already in vogue for quite some time. ROS cause wear and tear phenomena in cells. Proteins are damaged by oxidation, cross link, and lose their function. Oxidation breaks down fatty acids and the resulting products interact with proteins.

This process can be observed by fluorescence microscopy of senescent tissues. The light produced under the fluorescent microscope lead an old researcher sigh “the older you grow the more you glow.” Also the DNA is damaged continuously in living cells. It has been estimated that DNA is oxidatively damaged a thousand times per cell per day. An extensive repair system as the DNA polymerases and ligases correct these defects. Enzymes like poly-ADPribose polymerase (PARP) are employed by the cell to loosen the DNA enabling the repair machinery to function.

PARP is dependent for its function on ATP. Extensive DNA repair consumes intracellular ATP. Not only damage to nuclear DNA but also to mitochondrial DNA affects the genetic integrity. One of the consequences is that the risk for cancer increases with age. At the end of the maximal life span of various species (including humans) there is a cumulative risk of cancer of 30%. Interestingly the higher the basal metabolism (and thus more formation

of ROS) of an organism (used calories per kg body weight per day) the lower the maximal life span.

Aging is a complex process and other explanations have also been brought to the fore like various programming theories, like sequential switching on and off of genes or hormonal control of aging or the programmed decline of the immune system. Even in these theories, ROS are involved. Overall, the paradox of aerobic life is that we need oxygen and die as a result of oxygen exposure (Fig. 2.3).

ROS are necessary for normal physiological functions. Their toxicity is restrained by the so-called antioxidants, compounds that prevent the oxidation of other compounds. A good example is butter. Oxygen will make the butter rancid. The polyunsaturated fatty acids in the butter oxidize. Light and heat augment the oxidation process. The butter is therefore kept in the refrigerator. Antioxidants are also added to the butter to protect it from deterioration. Vitamin E (also called alfa-tocopherol) is a fat-soluble antioxidant vitamin and inhibits the oxidation process. Also in our membranes, which consist of fatty acids, vitamin E inhibits the same oxidative damage.

Other antioxidants are vitamin C (ascorbic acid), beta-carotene, and so-called polyphenols. These compounds are abundantly found in fruits and vegetables. The antioxidants work in conjunction and form a network that protects against oxidation.

We are also equipped with an intricate system of antioxidant enzymes, which are, not surprisingly, evolutionary early proteins. Aerobic metabolism is regarded as a system that should be in balance. Oxygen and oxygen-derived reactive species are at the same time a necessity and a danger. In the 1985 the term "oxidative stress" was coined in a book by H. Sies. This expression "oxidative stress" was defined as a disturbance in oxidant−antioxidant balance in favor of the former. It gives rise to the feeling that the stress (which has a negative connotation) should be resisted. The strategy seems simple: antioxidants should do the job. And the marketing for antioxidants is easy: oxidative stress

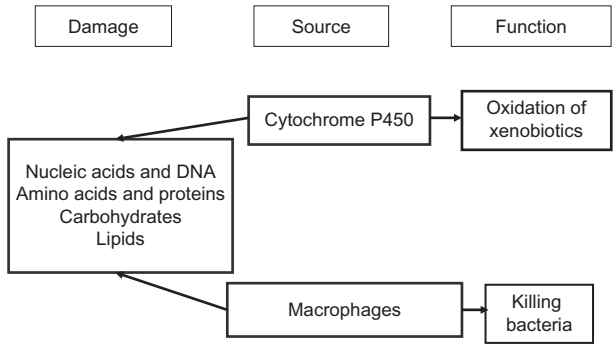

FIGURE 2.3 Function versus toxicity of reactive oxygen species.

is bad, antioxidants protect against oxidative stress, take antioxidants, and health effects are guaranteed.

It could have been anticipated that it is not that simple because it was known already at that time that normal physiology also needs oxidants. However, oxidative stress has been associated with many chronic, frequently age-related, diseases.

Quite a few chemicals are toxic via the formation of ROS in a process called "redox cycling." Compounds first take up an electron and shuttle this to oxygen, whereby oxygen becomes activated. In this way the toxicity of the herbicide paraquat is explained. Paraquat is banned in Europe but still heavily used in Brazil. Human exposure leads to lung toxicity via this mechanism. Adequate treatment is cumbersome.

The cardiotoxicity of the antitumor drug doxorubicin (Chapter 1: From Pretaster to Toxicologist) also occurs via this process of redox cycling. The process is dose limiting on the use of doxorubicin and forms one of the major drawbacks of this effective cytostatic.

The antibiotic nitrofurantoin, frequently used in the treatment of urinary tract infections, can display toxic side effects, for example in the lungs, via redox cycling. The same process has been described for cocaine. In some cases, where the endogenous antioxidant protection is substandard, nitrofurantoin, or cocaine toxicity may be brought to the fore.

ADAPTATION PROCESSES

Not only in science but also in marketing the term "ROS" is used quite frequently. As shown in Fig. 2.1 these species in fact differ and their chemical properties are very diverse. The more detailed knowledge is obtained on the subject, the more differentiation between all these forms of oxygen is required.

Questions as to their source, their reactivity toward all kind of biomolecules, and their interaction with antioxidants are all of biological importance. As a matter of fact, the terminology "antioxidants" is much too broad. Each antioxidant has its own reactivity. This might be an important factor in understanding their action.

People use antioxidants in general but in fact every compound with an antioxidant profile has its own characteristic. Also, the use of antioxidants should be refined. An interesting example in this respect is the use of vitamin E in persons that experienced an ischemic heart disease in two groups, a British and an Italian high-risk patient population. The British group (Cambridge to be precise) profited more than the Italian group of the supplementation. It was explained that unlike the British group the Italian population already had the benefit of an antioxidant-rich Mediterranean diet, and additional supplementation was less effective. A form of what we would call personalized nutrition.

As said, the extensive use of the term "oxidative stress" set in motion a certain type of research that was directed to counteract the stress. We now

realize that in cells several oxidation and reduction processes play a role. So-called redox processes and not only oxidation. The redox processes regulate all kinds of cellular reactions.

In fact, gene regulation and thereby protein modulation is steered by redox processes. Several cellular "master switches" that regulate gene expression are under redox control. These master switches are NF-κB and Nrf2. Oxidative damage activates these switches. NF-κB activation leads to inflammation and activation of Nrf2 tunes-up gene expression leading to endogenous antioxidant enzymes. In this way adaptation to oxidative damage occurs (Fig. 2.4).

Oxidative stress and inflammation are intricately coupled. Oxidative stress leads to inflammation (via NF-κB) and activation of inflammatory cells cause oxidative stress. Table 2.1 presenting diseases associated with oxidative stress might also be read as illnesses associated with inflammation.

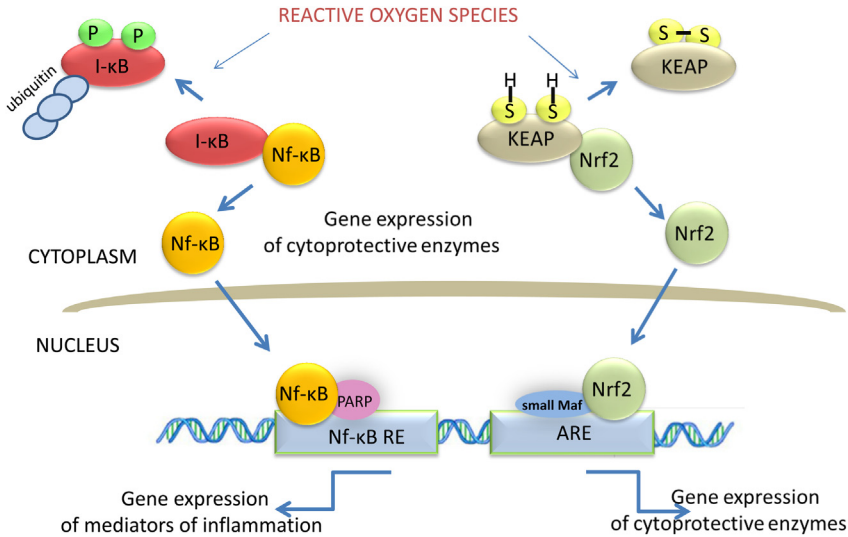

FIGURE 2.4 Figure explaining the activation of the transcription factors by oxidative stress and their cellular consequences.

TABLE 2.1 Examples of oxidative stress associated diseases

Several disorders in the lungs and the cardiovascular system associated with oxidative stress
• Lung: COPD, idiopathic pulmonary fibrosis, bronchopulmonary dysplasia, inhaled oxidants (like ozone), cigarette smoke, chemicals (e.g., paraquat and bleomycin), and adult respiratory distress syndrome, sarcoidosis • Cardiovascular system: Myocardial infarction, atherosclerosis, (pre-)eclampsia, and chemicals (doxorubicin)

Mild damage of the Nrf2 system gives activation of this gene expression system and upregulates the endogenous antioxidant system. In other words a mild oxygen stress (not enough to kill a cell of course) enhances protective mechanism. In the redox field this is now widely accepted. This process is called eustress (good stress) or hormesis. From an evolutionary point of view this process of adaptation makes perfect sense. A continuous adaptation to oxygen took place in evolution. This process has of course an enormous impact on the way we perceive risks. A little oxygen toxicity might even be good for you.

Physical exercise also leads to oxidative stress. Increased energy demand during exercise activates mitochondrial activity which leads to ROS. Even a single bout of exercise has thus the capability to induce adaptation. This explains the training effect of mild exercise. Exhaustive exercise though might lead to damage. A delicate balance between adaptation and damage exists.

Discussion is ongoing whether antioxidants could be used to protect against the damage part without affecting the training result.

Many food-derived antioxidants like polyphenols are good activators of Nrf2. In fact polyphenols after being oxidized (that is after they have already acted as an antioxidant) activate Nrf2. In this way they further enhance the endogenous antioxidant system.

The paradox of aerobic life becomes even more intricate. We need oxygen to survive (the ATP synthesis relies on it), but at the same time oxygen offers an extreme health risk and indeed many diseases of aging have been related to oxygen toxicity (oxidative stress). Concurrent with a mild oxygen toxicity is the hormetic response, that is an induction of endogenous antioxidant systems.

The adventure in the oxygen bar definitely needs further scrutiny; which process prevails?

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