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Oxygen in Space Radiation Biology

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Introduction

This brief review summarizes selected information relevant to the role of molecular oxygen in space radiation biology from the standpoint of the classical literature on the radiobiological effect of oxygen – the "Oxygen Enhancement Ratio" (OER), mainly as measured using cell killing, and from the standpoint of considerations of the significance of Reactive Oxygen Species (ROS).

The Oxygen Enhancement Ratio (OER)

Ionizing radiation at low LET is more effective in the presence of oxygen than in its absence in producing most biological effects. Most experimental radiobiological irradiations are conducted in the presence of oxygen at sufficient concentration to produce a "full oxygen effect". To understand the full oxygen effect it is helpful to review the traditional definition of OER, which is the ratio of dose at reduced oxygen level to the dose in the presence of air to produce the same biological effect:

$$OER = D_e(red.O_2)/ D_e(air) \qquad (1)$$

where "reduced" means any O_2 concentration below normal; "normal" means the O_2 concentration found in cells in medium in equilibrium with air or tissues in animals breathing air, D_e means end-point dose, such as a mean lethal dose of organisms, D_{37} for 1/e survival dose, D_{10} for 90% inactivation dose, etc. It is usually found that D_e(air) differs very little from $D_e(100\%O_2)$. The reciprocal of OER is *Relative Radiosensitivity*, defined as 1.0 at 0% O_2. When this is plotted against oxygen concentration the graph typically looks like that shown in Figure 1.

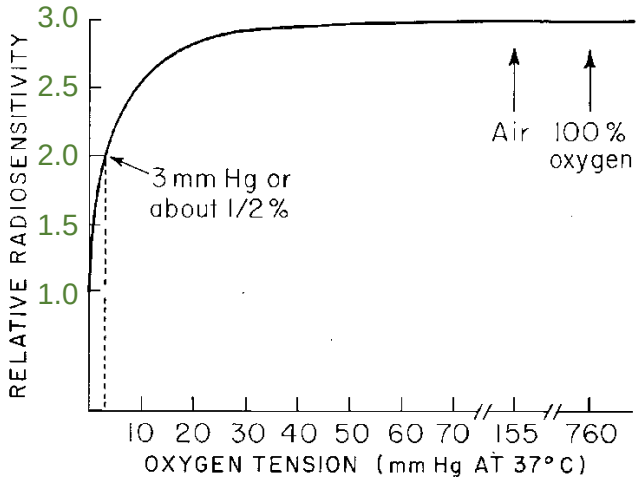

Figure 1. Plot of Relative Radiosensitivity vs. oxygen concentration showing very low concentration at which cells or organisms are protected by, at most, a factor of 3 by the absence of O_2. Note that the value does not change significantly between air and 100% O_2 and that the abscissa is broken twice. Figure from (Hall, 1973).

To get OER = 3.0 requires very low O_2 levels in tissues and cells. In simple terms, the concentration of oxygen molecules in cells in tissues is generally high compared to the concentration of ionizations required to lead to molecular and cellular radiation damage.

As Linear Energy Transfer (LET) is increased the OER for mammalian cell killing decreases, as seen in the various experiments performed a few decades ago represented by Figure 2 and explicitly in the Figure 3 graph.

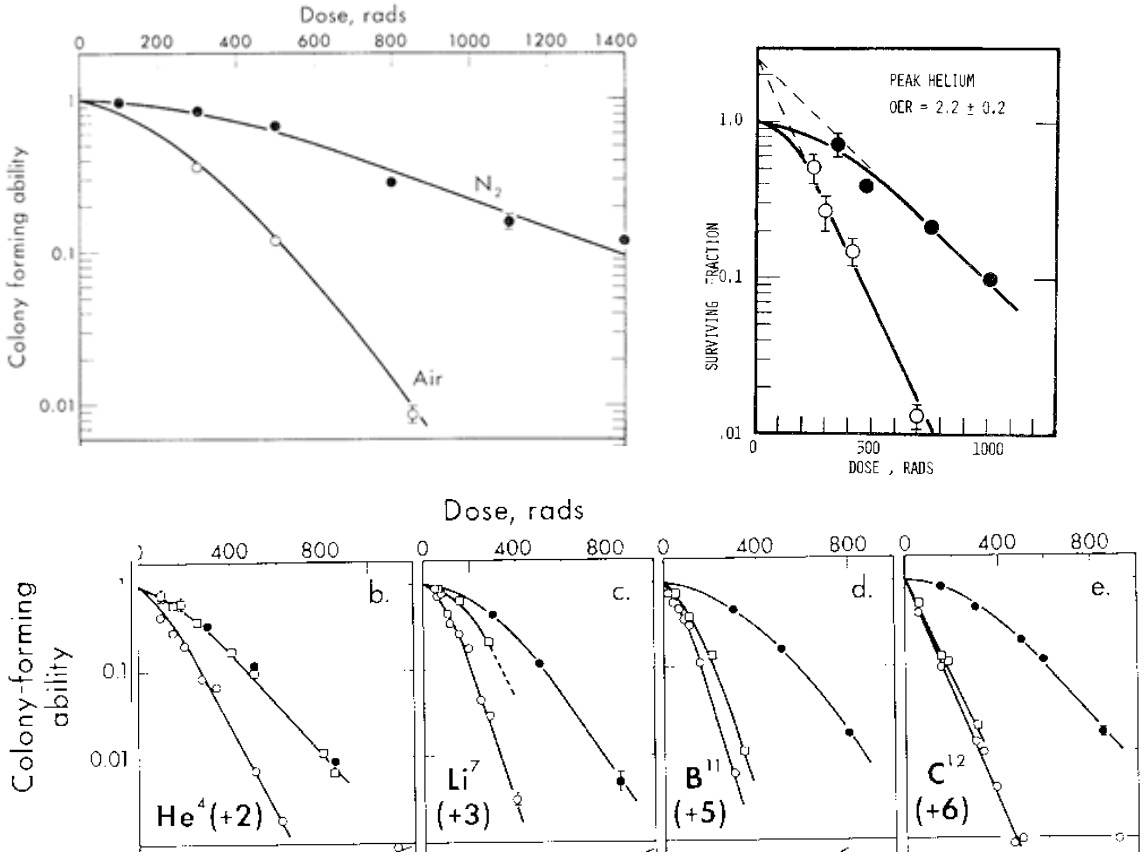

Figure 2. Published dose-effect curves for mammalian cells irradiated in air-saturated medium and in hypoxic medium. Upper left: 60 kVp x-rays (Todd, 1966, 1967); upper right: therapeutic modulated Bragg peak helium ion beam (Todd et al., 1986); lower panel: four ion species at 6.6 MeV/amu (Todd, 1966, 1967). Original notation preserved; 100 rads = 1 Gy.

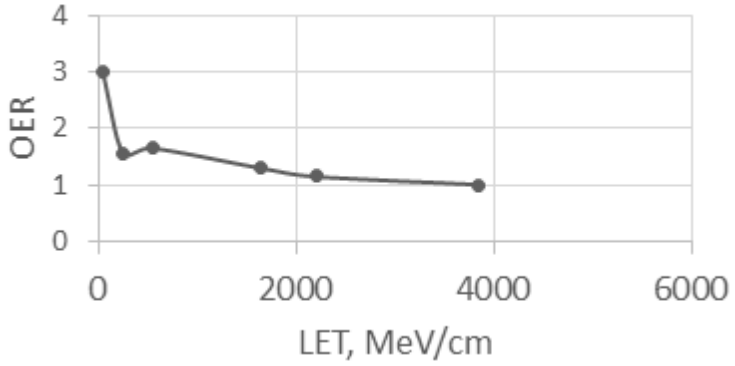

Figure 3. OER as a function of LET up to 3,850 MeV/cm. OER = 1.00 at 19,400 MeV/cm (5.2 MeV/amu Ar ions).

In the context of risk cross-section it is found that the increased cell killing cross section is increased by O_2, in a diminishing fashion, up to about 2200 MeV/cm (Figure 4). Also the cross section saturates at an area roughly equal to the cell nuclear area, some 90 μm^2.

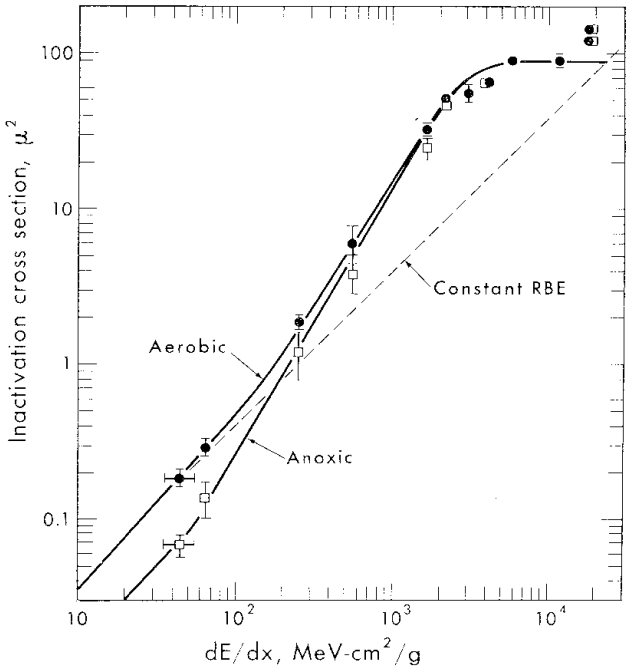

Figure 4. Human cell killing cross section as a function of LET for cells irradiated with low-energy heavy ions in the presence and absence of O_2 (Todd, 1966, 1967).

Various chemical explanations of the oxygen effect have been offered. At low LET oxygen is considered to react with radiation induced free radicals in target molecules and other molecules within the cell (Hall and Giaccia, 2012) with the result that incipient damage becomes "fixed" when the radical has reacted with O_2, typically in less than 10 ms. There are intrinsic reactive species within the cell, such as organic thiol compounds, that react with target radicals in competition with O_2 (Denekamp et al., 1974) (see below). At high LET radiation chemistry experiments (Frankenberg et al., 1990) indicate that ionized molecules adjacent to one another in the particle path are proximate enough to react mainly with one another as soon as they become free radicals (< 1 ns) and before freely diffusing competing O_2 molecules reach them (>1 ms) (Michael & Prise, 1996). Similarly, water radicals, which are formed in great abundance, if formed within a few nm of target molecules, may react on the <1 ns timeframe. This interpretation may be considered consistent with the notion that the LET at which OER = 1.0 corresponds to about 1 Ångstrom unit between ions inferring that nearly every atom in the path of the fast particle has been ionized.

At the highest LET in the Figure 4 illustration, 19,400 MeV/cm, there is an increase in cross section above the saturation value, and this was subjected to further testing in the 1980's. At high primary ion charge and/or high ion energy delta electrons (low LET) have considerable range and, at high ion charge, deliver a significant fraction of the dose at low LET – the "penumbra" effect. In this penumbra O_2 molecules can react with the sparsely distributed free radicals. These principles are demonstrated graphically in Figure 5, which illustrates one cell being traversed by the primary ion and two cells being struck by secondary ("delta") electrons

only in the track penumbra. Those cells accumulating enough damage to be killed by these electrons are irradiated at low LET, for which OER could be as high as 3.0.

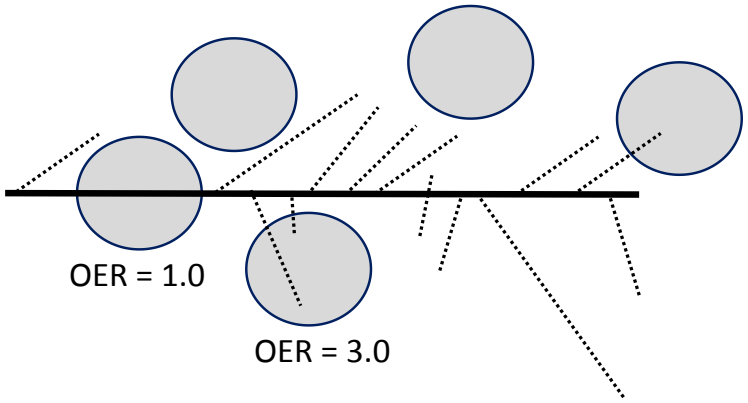

Figure 5. Illustration of cells being directly hit by a high-LET ion track (OER = 1.0) or by its long-range low-LET delta-electrons (OER = 3.0).

These principles have been demonstrated in, for example, experiments in which yeast cells were either directly hit or grazed by ion tracks. Directly hit cells had no O_2 effect, and grazed cells were more sensitive in the presence of O_2 . When yeast cells were irradiated with ions heavier than Ar – higher LET than shown in Figure 4 – it was found that the inactivation cross section increased beyond that of the target geometry and that OER returned to levels greater than 1.0, up to 1.8 at the highest LET studied, ca. 140,000 MeV/cm Pb ions at 7.7 MeV/amu. (Schneider et al., 1986).

It was at one time believed that radiation delivered at a very high dose rate (order of kGy/s) would consume O_2 faster than O_2 molecules could diffuse to ionized molecules and react. However, the end-point doses (usually for cell killing) are actually far too low to deplete significant dissolved O_2. When dose rates exceeding 10^{10} Gy/min were used in mammalian-cell killing experiments no protective effect due to high dose rate was observed (Todd et al., 1968). Thus the dose-effect curves for cell killing at this high and at standard dose rate (Figure 6) did not meaningfully differ, and oxygen removal did reduce the effectiveness of this radiation (Todd et al., 1968).

In this demonstration extremely high dose rate did not reduce the importance of O_2 in the action of low LET radiation, and this is a hint that the O_2-independence of cellular effects of high LET is not related to track-core reaction rates but the production of molecular, vs. radical, products in the track core, as radiation chemical experiments demonstrate. Although it is likely that reactive oxygen species (ROS, see below) are produced in the track core (LaVerne, 2000), lethal damage at high LET does not seem to depend on them (Figure 3, Figure 4).

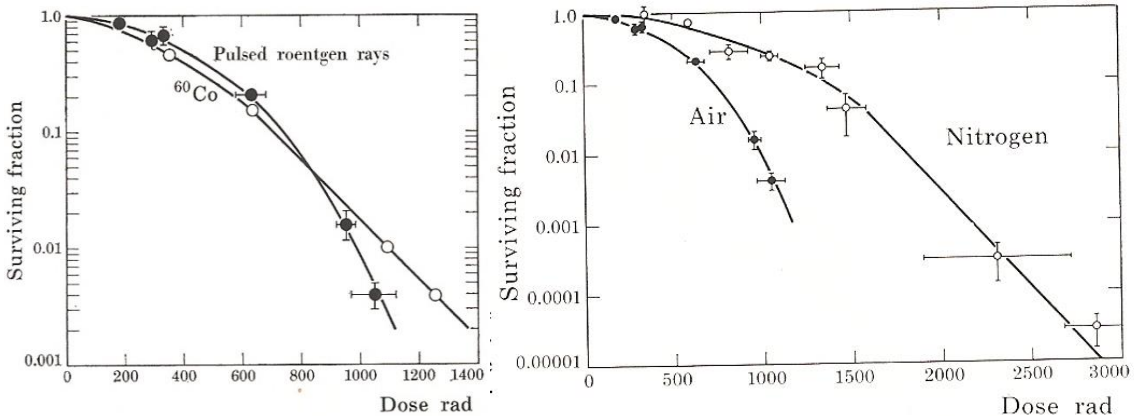

Figure 6. Cell killing dose response curves for 10 MV x rays delivered at $>10^{10}$ Gy/min. Left: compared with ^{60}Co gamma radiation delivered at 1.5 Gy/min. Right: compared with the same pulsed radiation delivered in an atmosphere of flowing nitrogen (Winchell et al., 1968).

In a typical chemical competition model intrinsic thiols and O_2 compete to react with target-macromolecule radicals (Todd et al., 1986). As molecular products increase as LET increases there are fewer target radicals with which thiols and O_2 can react, and their presence has less impact on the radiation damage at the molecular level. It is assumed that ionizing radiations induce primary radicals $R^{\cdot}$ and target radicals $T^{\cdot}$ in direct proportion to dose in the presence of a radical scavenging species Sa:

$$R \rightarrow R^{\cdot} \qquad \text{(I)}$$

$$R^{\cdot} + Sa \rightarrow RSa \qquad \text{(II)}$$

$$R^{\cdot} + T \rightarrow T^{\cdot} + RH$$

$$T \rightarrow T^{\cdot}$$

which indicates two means of starting with the target radical $T^{\cdot}$ (such as the initial chemical event in DNA), so the following target-radical reactions are considered:

$$T^{\cdot} + O_2 \rightarrow TO_2 \quad \text{unfixed damage, lethal or repaired} \qquad \text{(III)}$$

$$T^{\cdot} + F \rightarrow TF \quad \text{unfixed damage, lethal or repaired} \qquad \text{(IV)}$$

$$T^{\cdot} + S \rightarrow TS \quad \text{normal} \qquad \text{(V)}$$

$$T \rightarrow T^{*} \quad \text{unreactive and unrepairable} \qquad \text{(VI)}$$

in which F is a sensitizer (intrinsic and/or added) and S is a radioprotector (intrinsic and/or added, considered to be thiol(s)). Note that the products of reactions (III) and (IV) could include free radicals. From the rate constants and concentrations of reactants in reactions (III), (IV) and (V) the ratio of uncommitted to total damage in the presence and absence of O_2 can be in turn ratioed to calculate predicted values of OER as a function of [F] and [S] and [O_2]. Early attempts to do this using published rate constants produced reasonable agreement with observed results (Todd et al., 1986). Radiation chemical studies at increased LET have shown that molecular products (vs. primary radicals as in reaction (I)) dominate with increasing LET (Kuppermann, 1961; Appleby and Schwarz, 1969; Chatterjee and Magee, 1980). This is considered as due to instantaneous (< 1 ps) reactions between radicals formed in the ion track. In the above scheme, in the case of DNA molecules reaction (VI) is of increasing importance at high LET, and reactions (III), (IV) and (V) become less and less significant, which means effects

of S, F and O_2 become less significant, as shown for reaction (III) in Figure 4 and for reaction(V) (thiol radioprotection) in Figure 7. In very early experiments with human cells it was shown that the effectiveness of thiol radioprotection was lost at high LET. For example the final ($t > 1$ ns) yield of ·OH following heavy ion radiolysis of water decreases several fold, owing to immediate reaction in the track, relative to that following gamma irradiation.

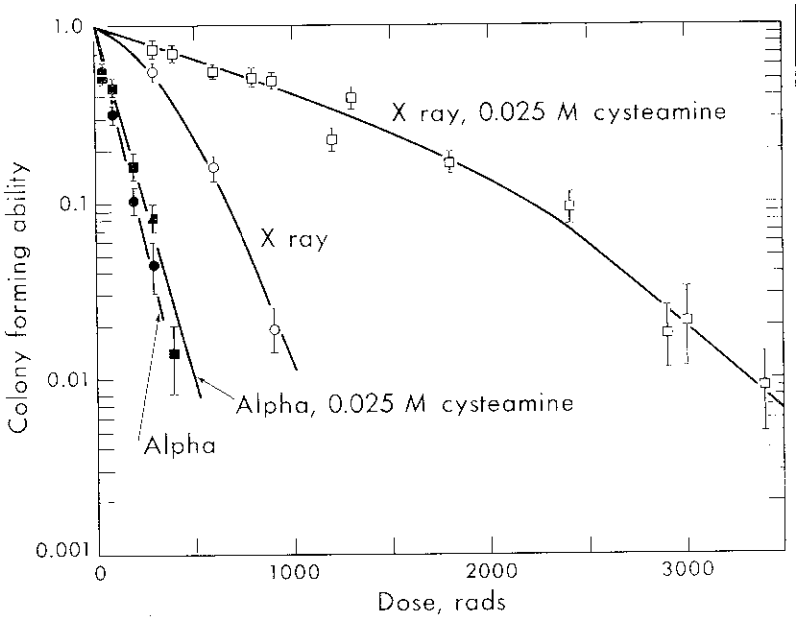

Figure 7. Loss of thiol radioprotection at increased LET (redrawn from Barendsen and Walter, 1964).

Implications for Space Radiation Health

Figure 1 is a reminder that radiation sensitivity at very low $[O_2]$ is a very steep function of $[O_2]$. Tissues this low in oxygen (<< 2%) would not be healthy and would not be expected to exist in the space environment to which healthy crew members could be exposed. At most O_2 levels encountered the dependence of radiation effect on $[O_2]$ is very flat, and it is predicted that crew members breathing an increased per cent O_2 during EVA would not experience any modification of radiation sensitivity. One concern might be cognitive impairment, so research has been done in a rodent cognitive testing study (Wheeler et al., 2014) to determine if astronauts may have a greater risk of developing radiation-induced cognitive impairment during an EVA. In this study rats were whole-body irradiated with 18 MV X-rays at a low dose rate while breathing air or 100% oxygen. At all doses tested the rats' cognitive function increased rather than decreased in irradiated O_2 breathing rats when compared to irradiated air-breathing rats. It was concluded that "astronauts are not likely to be at a greater risk of developing cognitive impairment when

exposed to space radiations while breathing 100% oxygen during an EVA." This conclusion is consistent with the fundamentals of radiobiology, although, for various reasons, crew members would not be exposed to 100% O_2.

Reactive Oxygen Species (ROS)

After the damage has been done there are biological processes that produce and are sensitive to reactive oxygen species. The spaceflight environment has for several decades been viewed as a potential source of oxidative damage. After a cell or tissue has been exposed to ionizing radiation a non-deterministic cascade of events leads to modified biochemistry that sometimes results in the metabolic generation of Reactive Oxygen Species (ROS), which may also be considered a change in homeostasis. These are considered to play a role in continuing damaging events such as mutation, cancer and abnormal growth and differentiation.

The ROS species of biological consequence are considered to be (O'Neill, 2012)

$O_2^{\cdot-}$ superoxide, generated naturally in the mitochondrial electron transport chain

H_2O_2 hydrogen peroxide, product of dismutation of superoxide

$^{\cdot}OH$ hydroxyl radical, product of iron-based Fenton reaction, radiolysis of water

HOCl hypochlorous acid, product of myeloperoxidase acting on hydrogen peroxide

$ONOO^-$ peroxynitrite, reaction product of superoxide with NO (NO is a metabolic messenger)

All of these products are actually generated by living cells performing metabolic processes, and some are also products of the radiolysis of water. For example, the critical immune process of phagocytosis by macrophages has been found to generate all of the above products. Most types of cells are highly prepared to deal with $O_2^{\bullet-}$ and H_2O_2 with high-rate enzymes superoxide dismutase (SOD) and catalase (CAT), respectively. One of the biologically significant effects of oxidizing species is the formation of disulfides, especially of protein molecules with cysteine residues whose reduced state is required for catalytic action. Cells have important enzymes to maintain a proper balance of protein thiols and disulfides (redox homeostasis), such as protein disulfide isomerase, glutaredoxin (which utilizes the reducing power of glutathione, a tripeptide that serves as the cell's reducing currency), and glutathione reductase.

Subsequent to their possible role in the production of DNA lesions (Georgakilas et al., 2013), cellular increases in ROS can be grouped into three categories, early, delayed and chronic, occurring in minutes, hours and weeks or months, respectively (Sridharan et al., 2015). This cited review serves as the basis for the following discussion: A typical cascade of ROS-related events begins with a metabolic response to DNA damage in which an early wave of increased cellular ROS, probably attributable to NADPH oxidase activity, is observed. There is evidence that a diffusible ROS product(s) is responsible for transmitting damage from cells irradiated in vitro to non-irradiated "bystander" cells, causing increased micronuclei and chromosomal translocations. It appears that early-onset ROS molecules or related signaling molecules can be transmitted via culture medium, gap junctions or exocytosis. It is known that cellular ROS can

produce DNA damage, so early post-irradiation ROS production is expected to lead to further DNA damage that is chemically distinct from that induced by high-LET radiation. Several hours after the initial ROS wave a second increase in ROS is observed in neural progenitor cells in vitro, especially after heavy-ion exposure, and evidence has been produced that mitochondrial activity is involved. This ROS wave appears to play a role in cell death in various human cells in vitro and consequences such as cell death and neoplastic transformation can be counteracted by SOD overexpression (St. Clair et al., 1992). Persistent phenotypes observed in the progeny of irradiated and bystander cells in vitro and in vivo include elevations of ROS for months or years, usually correlated with DNA damage and/or genome instability. Multiple consequences have been observed in mouse brain: increased cell death, chromic DNA damage response, senescence, cell layer thinning, volume reduction and compromised cognitive function (Suman et al., 2013). Modifications of mitochondrial structure and function have been noted and implicated in sustained ROS levels, and generations of cells derived from irradiated parent populations with genome instability had reduced electron-transport chain function (Thomas et al., 2012), reminiscent of the heritable loss of respiratory function noted decades ago (Todd, 1969). Thus, it appears that there are at least three categories of ROS function in cellular radiation effects. In the case of high-LET radiation damage, such as reaction (VI), in which cells neither recover nor die, it has been proposed that cells adopt a state of persistent oxidative stress characterized by compromised accuracy of DNA repair and genome instability leading to signaling mechanisms resulting in additional ROS release in a perpetuating cycle (Sridharan et al., 2015). These conditions may lead to malignant transformation and somatic mutations, but the probability is as yet unknown.

Implications for Space Radiation Health

Concerns about the impacts of ROS on crew health are decades old. For example, Mao et al. (2013) observed mitochondrial oxidative damage in ocular tissue. Searches for countermeasures (anti-oxidants) have been conducted in cell and animal research laboratories. Protection against post-irradiation late ROSs in vivo and at the cellular level by well-known anti-oxidant biochemical substances has been proposed and tested, and examples have been presented to space biology audiences. Specifically, various antioxidants have been found to ameliorate post-irradiation damage development in cells (Kennedy and Todd, 2003), and anthocyanins in the form of blueberry and strawberry extracts have been found to ameliorate post-heavy-ion irradiation performance decrements in rats exposed to high doses of high-energy Fe ions (Rabin et al., 2005). Crew diets high in natural anti-oxidants are recommended as protective countermeasures in space radiation health and for crew health in general.

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