BELLE NEWSLETTER

Biological Effects of Low Level Exposures

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BIOMEDICAL IMPLICATIONS OF HORMESIS – PART 2

INTRODUCTION

This issue of the BELLE Newsletter represents a continuation of the evaluation of the biomedical implications of hormesis as seen in the most recent issue of the Newsletter. Since the hormetic dose response is independent of biological model, endpoint measured and chemical agent it suggests not only widespread implications in the environmental health domain but biomedically as well. As numerous articles in the biomedical literature suggest hormetic dose responses are common and of high clinical relevance. Thus, it is important to explore the broader implications of the hormetic-biphasic dose response relationship, especially as they may impact the future of biomedical research, drug development and patient treatment. It is hoped that this issue will not only be intellectually challenging but will provide an incentive to explore this area further. If there are other areas of interest the Newsletter could be directed to explore, your suggestions would be welcomed.

TABLE OF CONTENTS

PRINCIPLES AND PRACTICE OF HORMETIC TREATMENT OF AGING AND AGE-RELATED DISEASES

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ABSTRACT

Aging is characterized by stochastic accumulation of molecular damage, progressive failure of maintenance and repair, and consequent onset of age-related diseases. Applying hormesis in aging research and therapy is based on the principle of stimulation of maintenance and repair pathways by repeated exposure to mild stress. Studies on the beneficial biological effects of repeated mild heat shock on human cells in culture, and other studies on the anti-aging and life prolonging effects of proxidants, hypergravity, irradiation, and ethanol on cells and organisms suggest that hormesis as an anti-aging and gerontomodulatory approach has a promising future. Its clinical applications include prevention and treatment of diabetes, cataract, osteoporosis, dementia and some cancers.

INTRODUCTION

The highly complex biological phenomenon of aging is now considered as being epigenetic and stochastic in origin. The three main principles of biological aging and longevity are: the life history principle, the mechanistic principle, and the non-genetic principle. Briefly, according to these principles, aging is, first and foremost, an emergent phenomenon seen primarily in protected environments which allow survival beyond the natural lifespan in the wild. Second biochemcial and molecular basis of aging reside in the mechanisms of progressive failure of homeostasis or homeodynamics, which leads to the accumulation of damage in nucleic acids, proteins and lipids. This results in the impairment in functional ability at all levels of organization thereby increasing the possibilities of a plethora of diseases and eventual death of the organism. Third, the non-genetic principle of aging rules out any genetic program for aging and the genes that do influence aging and longevity are those that have evolved in accordance with the life history of a species. Identification of genes so far have shown that these cover a wide range of biochemical pathways, such as insulin metabolism, kinases and kinase receptors, transcription factors, DNA helicases, telomerase, membrane glucosidases, GTP-binding protein coupled receptors, cholesterol metabolism, heat shock protein genes, cell cycle arrest pathways and others.[1, 2] Such genes are known as virtual gerontogenes.[3]

AGING: THERAPY OR PREVENTION?

Occurrence of aging during extended period of survival and the onset of one or more diseases before eventual death appear to be the "normal" sequence of events. This viewpoint makes modulation of aging different from the treatment of one or more specific diseases. In the case of a disease, such as a cancer of any specific kind, its therapy means the removal and elimination of the cancer cells and restoration of the affected organ/tissue to its original disease-free state. Attaining such an "age-free" state is not a realistic possibility. Similarly, although piecemeal replacement of non-functional or half-functional body parts with natural or synthetic parts made of more durable material may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying aging process as such.[2]

Scientific and rational anti-aging strategies aim to slow down aging, to prevent and/or delay the physiological decline, and to regain lost functional abilities. Hormesis offers a promising approach in aging intervention and prevention, and is based in making use of an organism's intrinsic homeodynamic property of self maintenance and repair. Since aging is characterized by a decrease in the adaptive abilities due to progressive failure of homeodynamics, it has been hypothesized that if cells and organisms are exposed to brief periods of stress so that their stress response-induced gene expression is upregulated and the related pathways of maintenance and repair are stimulated, one should observe anti-aging and longevity-promoting hormetic effects.[4]

During the last few years, research done in our labs has shown hormetic effects of mild stress. We have demonstrated the hormetic effects of repeated mild stress (RMS) on human cells undergoing aging in culture. Using a mild stress regime of exposing human skin fibroblasts to 41°C for 1 hr twice a week throughout their replicative lifespan in vitro, several beneficial and anti-aging effects have been observed. These effects include reduced accumulation of oxidized proteins, increased levels of various heat shock proteins (HSP), increased proteasome

activities, and enhanced stress resistance to other stresses, for example UV, ethanol and sugars.[5, 6]

Other chemical, physical and biological treatments have been used to unravel various pathways of maintenance and repair whose sustained activities improve the physiological performance and survival of cells and organisms. Stresses that have been reported to delay aging and prolong longevity in various systems (for example, yeast, Drosophila, nematodes, rodents and human cells) include temperature shock, irradiation (UV-, gamma- and X-rays), heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise and caloric restriction (CR).[7-9] Hormesis-like beneficial effects of chronic but mild undernutrition have been reported for human beings.[10] For example, it was reported that peripheral blood lymphocytes isolated from people with low body mass index, representing a group with natural intake of restricted food calories, had higher DNA repair capacity and higher levels of DNA polymerase-beta, which were also maintained during aging.[10] Intermittent fasting has been reported to have beneficial effects on glucose metabolism and neuronal resistance to injury.[11]

The proof of the hormetic principle being applicable to aging intervention has now been provided by experiments with a wide variety of biological systems and by using a range of physical, chemical and biological stressors. Two of the main lifestyle interventions, exercise and reduced food intake or CR, both of which bring their beneficial and anti-aging effects through hormesis,[12-16] are being widely recognized and increasingly practiced as an effective means of achieving a healthy old age. Clinical applications of exercise-mediated hormesis in the prevention or slowing down of the progress of age-related diabetes 2, osteoporosis and sarcopenia are being tested.[12, 17-19] Improvement in the biochemical response of the heart, strengthening of the immune system by biological (mild infection), physical and mental stress and challenge, and the possibility of stimulating protein turnover pathways to prevent the accumulation of abnormal proteins leading to neuronal degeneration and dementia are some of the other clinical avenues under investigation.[20-24]

From health care point of view, one can also expect the availability of certain nutriceutical and pharmacological hormetic agents to mimic the heat shock response and CR. For example, bimoclomal, a nontoxic, hydroxylamine derivative with HSP-inducing activity and cytoprotective effects is under clinical trials.[25, 26] Celastrol, a quinone methide triterpene,[27] and paeoniflorin,[28] which are active components of certain Chinese medicinal herbs are other HSP-inducing hormetic agents under test for their cytoprotective effects. Similarly, curcumin, an Indian yellow spice, has also been shown to have cytoprotective effects through its hormetic action in co-stimulating the synthesis of HSP.[29] Various chemical mimetics of CR, such as 2-deoxy-D-glucose and its analogues,[30] and resveratrol, which is a polyphenol found in red wine, are being tested for their use as anti-aging hormetic agents.[31-33] Another small molecule, N6-furfuryladenine or kinetin, has been shown to have significant anti-aging[34, 35] and anti-thrombotic[36] effects in human cells. Kinetin is considered to work both as a direct antioxidant[37, 38] and as a hormetic agent by inducing the synthesis of other protective enzymes and HSP.[35, 39, 40] Recently reported anti-aging effects of another cytokinin zeatin may also operate through hormetic pathways.[41] Although at present the use of kinetin has been limited to being a cosmeceutical ingredient in a range of cosmetics products, its usefulness as a hormetic nutriceutical agent is under investigation. In experimental hairless mice system, topical application of DNA-damage product thymidine dinucleotides (pTT) has horemtic effects in the prevention of UV-induced mutations and photocarcinogenesis by activating the expression of a tumour suppressive gene p53.[42, 43]

Although at present there are only a few studies performed which utilize mild stress as a modulator of aging and longevity, hormesis can be a useful experimental approach in biogerontology and geriatric medicine. However, there are a few issues that remain to be resolved before hormetic approaches can be used widely as a clinical tool to affect aging and to prevent the onset of age-related impairments and pathologies. Some of these issues are: (1) to establish biochemical and molecular criteria for determining the hormetic levels for different stresses; (2) to identify differences and similarities in stress response pathways initiated by different stressors; (3) to quantify the extent of various stress responses; (4) to determine the interactive and pleiotropic effects of various stress response pathways; (5) to adjust the levels of mild stress for age-related changes in the sensitivity to stress; (6) to determine the biological and evolutionary costs of repeated exposure to stress; and (7) to determine the biological significance of relatively small hormetic effects, which may or may not have large beneficial effects during the entire lifespan. Resolution of these issues requires much more research on hormesis than being carried out at present.

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REFERENCES

1. Rattan, SIS. Biology of aging and possibilities of gerontomodulation. Proc Indian Nat Sci Acad 2003;B69:157-164.
2. Rattan, SIS, Clark, BFC. Understanding and modulating ageing. IUBMB Life 2005;57:120-128.

3. Rattan, SIS. Gerontogenes: real or virtual? FASEB J 1995;9:284-286.
4. Rattan, SIS. Applying hormesis in aging research and therapy. Hum Exp Toxicol 2001;20:281-285.
5. Rattan, SIS, Eskildsen-Helmond, YEG, Beedholm, R. Molecular mechanisms of anti-aging hormetic effects of mild heat stress on human cells. Nonlinear Biol Toxicol Med 2003;2:105-116.
6. Rattan, SIS, Gonzales-Dosal, R, Nielsen, ER, Kraft, DC, Weibel, J, Kahns, S. Slowing down aging from within: mechanistic aspects of anti-aging hormetic effects of mild heat stress on human cells. Acta Biochimica Polonica 2004;51:481-492.
7. Minois, N. Longevity and aging: beneficial effects of exposure to mild stress. Biogerontology 2000;1:15-29.
8. Cypser, JR, Johnson, TE. Hormesis in Caenorhabditis elagans dauer-defective mutants. Biogerontology 2003;4:203-214.
9. Rattan, SIS. Aging intervention, prevention, and therapy through hormesis. J Gerontol Biol Sci 2004;59A:705-709.
10. Raji, NS, Surekha, A, Subba Rao, K. Improved DNA-repair parameters in PHA-stimulated peripheral blood lymphocytes of human subjects with low body mass index. Mech Ageing Dev 1998;104:133-148.
11. Anson, RM, Guo, Z, de Cabo, R, Lyun, T, Rios, M, Hagepanos, A, et al. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie restriction. Proc Natl Acad Sci USA 2003;100:6216-6220.
12. Singh, AMF. Exercise comes of age: rationale and recommendations for geriatric exercise prescription. J Gerontol Med Sci 2002;57A:M262-M282.
13. McArdle, A, Vasilaki, A, Jackson, M. Exercise and skeletal muscle ageing: cellular and molecular mechanisms. Ageing Res Rev 2002;1:79-93.
14. Masoro, EJ. Hormesis and the antiaging action of dietary restriction. Exp Gerontol 1998;33:61-66.
15. Masoro, EJ. Caloric restriction and aging: an update. Exp Gerontol 2000;35:299-305.
16. Yu, BP, Chung, HY. Stress resistance by caloric restriction for longevity. Ann NY Acad Sci 2001;928:39-47.
17. Hitomi, Y, Kizaki, T, Katsumura, T, Mizuno, M, Itoh, C, Esaki, K, et al. Effect of moderate acute exercise on expression of mRNA involved in the calcineurin signaling pathway in human skeletal muscle. IUBMB Life 2003;55:409-413.
18. Hooper, PL. Hot-tub therapy for type 2 diabetes mellitus. N Engl J Med 1999;341:924-925.
19. Rubin, C, Turner, AS, Bain, S, Mallinckrodt, C, McLeod, K. Low mechanical signals strengthen long bones. Nature 2001;412:603-604.
20. Benjamin, IJ, McMillan, DR. Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. Circ Res 1998;83:117-132.
21. Bierhaus, A, Wolf, J, Andrassy, M, Rohleder, N, Humpert, PM, Petrov, D, et al. A mechanism converting psychosocial stress into mononuclear cell activation. Proc Natl Acad Sci USA 2003;100:1920-1925.
22. Wakatsuki, T, Schlessinger, J, Elson, EL. The biochemical response of the heart to hypertension and exercise. Trends Biochem Sci 2004;29:609-617.
23. Beedholm, R, Clark, BFC, Rattan, SIS. Mild heat stress stimulates proteasome and its 11S activator in human fibroblasts undergoing aging in vitro. Cell Stress & Chaperones 2004;9:49-57.
24. Deocaris, CC, Taira, K, Kaul, SC, Wadhwa, R. Mimotope-hormesis ad mortalin/grp75/mthsp70: a new hypothesis on how infectious disease-associated epitope mimicry may explain low cancer burden in developing nations. FEBS Lett 2005;579:586-590.
25. Vigh, L, Literati, PN, Horváth, I, Török, Z, Balogh, G, Glatz, A, et al. Bimoclomol: a nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. Nature Medicine 1997;3:1150-1154.
26. Vigh, L, Maresca, B, Harwood, JL. Does the membrane's physical state control the expression of heat shock and other genes? TIBS 1998;23:369-374.
27. Westerheide, SD, Bosman, JD, Mbadugha, BNA, Kawahara, TLA, Matsumoto, G, Kim, S, et al. Celastrols as inducers of the heat shock response and cytoprotection. J Biol Chem 2004;279:56053-56060.
28. Yan, D, Saito, K, Ohmi, Y, Fujie, N, Ohtsuka, K. Paeoniflorin, a novel heat shock protein-inducing compound. Cell Stress & Chaperones 2004;9:378-389.
29. Dunsmore, KE, Chen, PG, Wong, HR. Curcumin, a medicinal herbal compound capable of inducing heat shock response. Crit Care Med 2001;29:2199-2204.
30. Lane, MA, Ingram, DK, Roth, GS. The serious search for an anti-aging pill. Sci Amer 2002;287:24-29.
31. Howitz, KT, Bitterman, KJ, Cohen, HY, Lamming, DW, Lavu, S, Wood, JG, et al. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 2003;425:191-196.
32. Lamming, DW, Wood, JG, Sinclair, DA. Small molecules that regulate lifespan: evidence for xenohormesis. Mol Microbiol 2004;53:1003-1009.
33. Wood, JG, Rogina, B, Lavu, S, Howitz, KT, Helfand,

SL, Tatar, M, et al. Sirtuin activators mimic caloric restricition and delay ageing in metazoans. Nature 2004;430:686-689.

34. Rattan, SIS, Clark, BFC. Kinetin delays the onset of ageing characteristics in human fibroblasts. Biochem Biophys Res Commun 1994;201:665-672.

35. Rattan, SIS. N6-furfuryladenine (kinetin) as a potential anti-aging molecule. J Anti-aging Med 2002;5:113-116.

36. Hsiao, G, Shen, MY, Lin, KH, Chou, CY, Tzu, NH, Lin, CH, et al. Inhibitory activity of kinetin on free radical formation of activated platelets in vitro and on thrombus formation in vivo. Eur J Pharmacol 2003;465:281-287.

37. Olsen, A, Siboska, GE, Clark, BFC, Rattan, SIS. N6-furfuryladenine, kinetin, protects against Fenton reaction-mediated oxidative damage to DNA. Biochem Biophys Res Commun 1999;265:499-502.

38. Verbeke, P, Siboska, GE, Clark, BFC, Rattan, SIS. Kinetin inhibits protein oxidation and glyoxidation in vitro. Biochem Biophys Res Commun 2000;276:1265-1267.

39. Barciszewski, J, Rattan, SIS, Siboska, G, Clark, BFC. Kinetin - 45 years on. Plant Sci 1999;148:37-45.

40. Holmes-Davis, R, Payne, SR, Comai, L. The effects of kinetin and hydroxyurea on the expression of the endogeneous and transgenic Heat Shock Cognate 80 (HSC80). Plant Cell rep 2001;20:744-748.

41. Rattan, SIS, Sodagam, L. Gerontomodulatory and youth-preserving effects of zeatin on human skin fibroblasts undergoing aging in vitro. Rejuven Res 2005;8:46-57.

42. Goukassian, DA, Helms, E, Van Steeg, H, van Oostrom, C, Bhawan, J, Gilchrest, BA. Topical DNA oligonucleotide therapy reduces UV-induced mutations and photocarcinogenesis in hairless mice. Proc Natl Acad Sci USA 2004;101:3933-3938.

43. Goukassian, DA, Gilchrest, BA. The interdependence of skin aging, skin cancer, and DNA repair capacity: a novel perspective with therapeutic implications. Rejuven Res 2004;7:175-185.

HORMESIS AND DISEASE RESISTANCE: ACTIVATION OF CELLULAR STRESS RESPONSE PATHWAYS

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ABSTRACT

The survival of all organisms depends upon their ability to overcome stressful conditions, an ability that involves adaptive changes in cells and molecules. Findings from studies of animal models and human populations suggest that hormesis (beneficial effects of low levels of stress) is an effective means of protecting against many different diseases including diabetes, cardiovascular disease, cancers and neurodegenerative disorders. Such stress resistance mechanisms can be bolstered by diverse environmental factors including exercise, dietary restriction, cognitive stimulation and exposure to low levels of toxins. Some commonly used vitamins and dietary supplements may also induce beneficial stress responses. Several interrelated cellular signaling molecules are involved in the process of hormesis. Examples include the gases oxygen, carbon monoxide and nitric oxide, the neurotransmitter glutamate, the calcium ion and tumor necrosis factor. In each case low levels of these signaling molecules are beneficial and protect against disease, whereas high levels can cause the dysfunction and/or death of cells. The cellular and molecular mechanisms of hormesis are being revealed and include activation of growth factor signaling pathways, protein chaperones, cell survival genes and enzymes called sirtuins. Knowledge of hormesis mechanisms is leading to novel approaches for preventing and treating a range of human diseases.

INTRODUCTION

Renewed interest in hormesis has developed as the result of mounting evidence that low doses of toxins can have beneficial effects ranging from growth stimulation in plants to protection against cancers and other diseases in humans (Calabrese, 2002). However, despite the fact that doctors routinely prescribe low doses of toxins (i.e., medicines) for the treatment of many different health problems, this knowledge has not led to the acceptance of exposure to low doses of environmental toxins that have been proven safe. Developing in parallel with the literature on toxin-induced hormesis is evidence that dietary and behavioral factors that are known to reduce the risk of disease may act by stimulating hormesis mechanisms. Dietary restriction, regular exercise and cognitive stimulation are three such beneficial "exposures" described below. The latter stimuli may exert their beneficial affects by the same or similar mechanisms as low doses of toxins and, indeed, dietary restriction and exercise can protect against exposures to high doses of toxins. In this article I present examples of cellular and molecular pathways that mediate hormesis in response to a wide range of stimuli, and consider ways in which such adaptive responses may be used to prevent and treat disease. The emerging findings concerning hormesis mechanisms in the context of health and disease suggest that attempts to completely eliminate toxins/stressors from the environment may be misguided or even deterimental.

HORMESIS IS A FUNDAMENTAL PROCESS IN EVOLUTION

To survive in the hostile environment of primitive earth, cells had to acquire mechanisms of protecting themselves against free radicals, ultraviolet light and extreme changes in temperature, pH and osmolarity. The fitness of an organism was therefore determined, in large part, by its ability to avoid or resist mild to moderate levels of stress. Many different genes and their encoding proteins evolved to mediate adaptive responses to stress; these molecular mechanisms protect single-cell organisms and mammals alike against a range of metabolic, oxidative and chemical stresses. The nonlinear nature of the effects of many environmental stresses on survival is therefore a consequence of the evolutionary process (Parsons, 2003). For example, a family of histone deacetylases called sirtuins played pivotal roles in stress resistance through a vast expanse of evolutionary history (Lamming et al., 2004). The sirtuins are activated by various types of stress, and can protect cells against energy deprivation and oxidative stress. Two other types of stress resistance proteins are chaperones such as heat-shock protein 70 and glucose-regulated protein 78, and antioxidant enzymes such as Mn-superoxide dismutase and glutathione peroxidase (Macario and de Macario, 1999; Benzie, 2000).

At the core of evolutionary theory is the concept that mutations in DNA are the substrate for adaptive changes in an organism's phenotype. The dogma that mutations

are random rather than regulated has recently been challenged by data showing that some mutations that are induced by stress allow adaptation to the stress (Rosenberg, 2001). In this context, the stressor itself was a signal that served important roles in the orchestration of hormesis. Examples of such stressor/signaling molecules are oxygen, calcium, and carbon monoxide. During the evolution of higher organisms, including mammals, hormesis mechanisms played important roles in resistance to and recovery from injury, infection and starvation. The recent extension of human lifespan well beyond reproductive years has been accompanied by changes in the major causes of death to age-related diseases such as cardiovascular disease, diabetes, cancers and neurodegenerative disorders. From studies of humans and of animal models of age-related diseases, has come considerable evidence suggesting that activation of hormesis pathways can protect against such diseases. The remainder of this article is devoted to a consideration of different endogenous inter- and intra-cellular signaling molecules that protect against disease via hormesis.

HORMESIS AND HEALTH: IMPETUS FOR REEMERGENCE OF THE SPARTAN LIFESTYLE?

The Spartans developed a prominent city-state during the period of 1000 – 600 B.C. in what is now Greece. They lived a highly regimented lifestyle characterized by frugal eating and regular vigorous exercise. Today we know that health can be improved and many diseases forestalled by regimens of exercise, and by diets low in calories and rich in vegetables and fruits. Regular exercise reduces the risk of cardio- and cerebro-vascular diseases, diabetes, osteoporosis and some types of cancer (see Melzer et al., 2004 for review). Studies of rodents and monkeys have shown that dietary restriction (long-term caloric restriction or intermittent fasting) can increase lifespan and reduce the incidence of cancers, diabetes, and cardiovascular and kidney disease (see Heilbronn and Ravussin, 2003; Mattison et al., 2003; Mattson, 2003 for review). These factors exert their effects, in large part, by stimulating hormesis responses in cells and organs. During physical exercise, the cells of the cardiovascular and musculoskeletal systems are subjected to stress. At the cellular level the stresses during exercise include increased energy demand, free radical production, and physical strain (Murphy and Carroll, 2003). The muscle and bone cells respond to such stress by increasing their ability to cope with more severe stress. A similar scenario occurs in response to dietary restriction where cells throughout the body exhibit enhanced stress resistance (Mattson, 2003a).

A hormesis mechanism appears to be responsible for many of the beneficial effects of dietary restriction (Fig. 1). When rodents are subjected to caloric restriction (CR) and/or intermittent fasting (IF) dietary restriction regimens, they exhibit increased resistance to a variety of stressors including heat, cardiovascular stress and exposure to environmental toxins (Masoro, 2003; Mattson, 2003a). CR and IF have been shown to protect against a range of diseases in mouse and rat models of cancers, diabetes, ischemic heart disease, stroke, and Alzheimer's, Parkinson's and Huntington's diseases (Hursting et al., 2003; Mattson et al., 2004a). Such Spartan diets elicit a hormesis response in cells throughout the body. The response is often robust such that cells exhibit increased resistance to a range of toxins including neurotoxins (Mattson, 2003a) carcinogens (Hursting et al., 2003), mercury (Usuki et al., 2004) and ionizing radiation (Yoshida et al., 1997). These findings are consistent with evolutionary theory and the existence of powerful adaptive stress response mechanisms activated by exposure to harsh environments.

A TOXIN BY ANY OTHER NAME IS – OFTEN - THE STUFF OF LIFE

In biomedical research, the reputation of a molecule is established at the time of discovery of a function/action of that molecule. However, further investigation almost always reveals a broader range of actions of the molecule that depend upon its concentration and location (within cells and organs), and the physiological state of the organism. There are an increasing number of examples of molecules that were initially discovered because of their physiological function and were later shown to be toxins at high concentrations and/or under particular pathological conditions. Conversely, some molecules that were initially known for their toxic actions were subsequently shown to be important signaling molecules in cells. Several intriguing examples are considered below.

Oxygen

Oxygen is a component of many organic molecules, is required for energy production in mitochondria, and is a key component of several important signaling pathways. However, oxygen can be highly toxic to cells because of its ability to form various types of oxygen free radicals including superoxide anion radical, hydroxyl radical and peroxynitrite (Lane, 2002). Cells have evolved multiple mechanisms that help them cope with oxygen radicals including: antioxidant enzymes such as Mn- and Cu/Zn-superoxide dismutases, catalase and glutathione peroxidase; oxygen radical scavengers such as vitamin E, glutathione and uric acid; and enzymes that can remove or repair oxidatively damaged DNA, proteins and lipids. The amount of oxygen in the atmosphere is tightly regulated and is typically 21% at sea level, and excursions of the oxygen concentration below or above this level can result in tissue damage and death (Lane, 2002). Hormesis responses can be induced in cells and organisms as a response to either hypoxic or hyperoxic conditions. For example, hyperoxia can increase the lifespan of the roundworm *C. elegans*, and can induce cross-tolerance to other types of stress (Cypser and Johnson, 2002). Transient increases in oxygen consumption, as occur with vigorous physical exercise, are associated with a hormesis response in cells of the musculoskeletal and cardiovascular systems (Mi-

chaelides et al., 2003). Transient exposure of the brain or heart to low levels of oxygen can protect those organs against more severe hypoxia that occurs after a stroke or myocardial infarction (Hawaleshka and Jacosohn, 1998).

Carbon Monoxide

Life evolved in an atmosphere that contained carbon monoxide (CO); our exposure to this gas has increased considerably as a pollutant generated by combustion of coal, gasoline and tobacco. CO can displace oxygen in blood and cause hypoxia and death. Exposure to high concentrations of CO can be lethal, and exposures to somewhat lower concentrations of CO can cause damage to nerve cells in the brain. While the toxicity of CO is well known, it has recently been shown that CO can be produced by many different types of cells in the body during the process of heme degradation by heme oxygenase (Ryter and Otterbein, 2004). Mammals posses two different forms of heme oxygenase, and inducible form called HO-1 and a constitutively expressed form (HO-2). HO-1 levels are normally very low, but its expression can be dramatically increased in many types of cells under conditions of stress. On the other hand, the expression of HO-2 is present at much higher levels in the nervous and cardiovascular system compared to other tissues.

CO is generated during normal physiological process such as synaptic transmission and smooth muscle contraction (Wang et al., 1997; Zhuo et al., 1998). Low concentrations of CO (below 200 ppm) can inhibit apoptosis and inflammation, and can be protective in animal models of ischemia-reperfusion injury, hepatitis and vascular injury. In excitable cells such as neurons and smooth muscle cells CO hyperpolarizes the plasma membrane by activating calcium-dependent potassium channels. CO may exert such biological effects by stimulating soluble guanylate cylase and by more directly activating potassium channels (Kaide et al., 2001). The involvement of CO in regulating blood pressure and its production in response to ischemic and metabolic stress suggest roles for CO in the pathogenesis of cardiovascular disease. Similarly, the emerging evidence that CO plays roles in learning and memory and other behaviors (Zhuo et al., 1998), has led to investigations suggesting roles for CO in neurological disorders such as stroke and Alzheimer's disease (Schipper, 2004).

The increased production of CO under stressful conditions suggests the possibility that it functions in stress adaptation. In this regard, it has been shown that administration of CO can protect endothelial cells, smooth muscle cells and neurons against apoptosis in models of ischemic injury (Dore et al., 2000; Neto et al., 2004). Conversely, blockade of HO activity can exacerbate injury in such animal models. Thus, beneficial actions of CO appear to be mediated by a hormesis response. The signaling pathways of CO-mediated hormesis are beginning to be elucidated and may involve cyclic GMP production and activation of the transcription factor NF-KB (Brouard et al., 2002). NF-KB is known to induce the expression of genes that help cells resist stress including Bcl-2 family members and antioxidant enzymes (Mattson and Camandola, 2001).

Glutamate

The amino acid glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) of mammals. As such it is essential for most behaviors including sensory-motor functions and learning and memory (Mattson, 1988; Lessmann, 1998). Glutamate exerts these actions at synapses by binding to receptors that cause membrane depolarization and calcium influx through ligand-gated and voltage-dependent calcium channels (Mattson and Chan, 2003). While glutamate signaling is normally beneficial, excessive activation of glutamate receptors can cause the degeneration and death of neurons. This "excitotoxic" process is believed to play a role in several disorders including epilepsy, stroke, and Alzheimer's and Parkinson's diseases (Mattson, 2003b). Glutamate kills neurons by causing sustained increases in the intracellular calcium concentration which results in activation of proteases such as calpains and caspases, and impairment of mitochondrial function.

Glutamate is an excellent example of an endogenous mediator of hormesis (Fig. 2). Moderately high levels of glutamate receptor activation, such as occurs during cognitive stimulation, activate signaling pathways that enhance the ability of the nerve cells to resist stress. A similar process occurs when the brain is subjected to a mild ischemia; having experienced the ischemic stress, neurons become resistant to being killed after a stroke. This adaptive process is called "preconditioning" and is mediated at least in part by activation of glutamate receptors (Lipsky et al., 2001). Glutamate exerts its neuroprotective effect by inducing calcium influx which activates transcription factors such as NF-KB and CREB (cyclic AMP response element binding protein) that induce the expression of genes that encode neuroprotective proteins such as brain-derived neurotrophic factor (BDNF) and the anti-apoptotic protein Bcl-2 (Mabuchi et al., 2001; Mattson and Camandola, 2001) (Fig. 2).

Several naturally occurring neurotoxins have been identified that act by binding with high affinity to certain types of glutamate receptors. For example, the excitotoxin domoic acid was identified as the toxin responsible for a seizure/dementia syndrome caused by consumption of shellfish in a Canadian restaurant (Jeffery et al., 2004). Domoic acid is produced by algae and the algae are eaten by shellfish. A related excitotoxin called kainic acid is widely used as a research tool in animal models of epilepsy and Alzheimer's disease. Domoic acid and kainic acid activate particular types of glutamate receptors called AMPA/kainate receptors. Other environmental excitotoxins exist and some have been implicated in neurodegenerative disorders such as amyotrophic lateral sclerosis (Kisby et al., 1992). On the other hand, low concentra-

tions of such neurotoxins can have beneficial effects on neurons as the result of stimulation of glutamate receptor-mediated stress resistance mechanisms (Kerr et al., 2002; Nvue et al., 2004). As is the case with other environmental toxins, the possible therapeutic value of glutamate receptor agonists has not been pursued due to concern that high doses can be toxic. However, because of the well-established and highly specific mechanism of action of excitotoxins, they would seem to be a class of toxins that might prove effective in hormesis-based therapeutic approaches for neurological disorders.

Nitric oxide

Nitric oxide is a gas produced by many different types of cells in the body including vascular endothelial cells, macrophages and neurons (Lowenstein et al., 1994). It is produced during the conversion of L-arginine to L-citrulline which is catalyzed by nitric oxide synthase (NOS). NOS is activated by calcium influx and/or release from inracellular strores. Because of its abilities to rapidly spread from its site of production to adjacent cells, and to activate a signal transduction pathway involving cyclic GMP, nitric oxide plays important roles in many different physiological processes. Examples include, relaxation of blood vessels, regulation of reproductive functions, learning and memory and immune responses to pathogens. On the other hand, excessive production of nitric oxide is implicated in the pathogenesis of major diseases including cardiovascular disease, diabetes, cancer, stroke and neurodegenerative disorders (Hofseth et al., 2003; Duncan et al., 2005; Pacher et al., 2005). At least some of the beneficial effects of subtoxic levels of nitric oxide are consistent with a hormesis mechanism. For example, nitric oxide plays a role in ischemic preconditioning (Huang, 2004). Nitric oxide may protect cells against severe stress by stabilizing hypoxia inducible factor-1, an important mediator of adaptive responses of cells to stress (Brune and Zhou, 2003).

Calcium

The Ca^{2+} concentration outside of cells (1-2 mM) is approximately 10,000-fold greater than the concentration in the cytoplasm (100-200 nM). This concentration gradient is maintained by the continuous activity of ATP-dependent Ca^{2+} pumps located in the plasma membrane. Numerous signals that affect cell function do so by stimulating Ca^{2+} influx through ligand-gated and/or voltage-dependent Ca^{2+} channels in the plasma membrane, and/or release of Ca^{2+} from the endoplasmic reticulum (ER). For example, processes as diverse as proliferation of fibroblasts, contraction of muscles and learning and memory are mediated by Ca^{2+} influx induced by growth factors or neurotransmitters (Gnegy, 2000; Agell et al., 2002; Shannon and Bers, 2004). The rapid restoration of the Ca^{2+} concentration following cell stimulation is important for proper regulation of the cellular response, and is also critical for cell survival as a sustained elevation of the intracellular Ca^{2+} concentration can be cytotoxic (Trump and Berezesky, 1995). Excessive accumulation of Ca^{2+} in cells may damage and kill them by causing the activation of proteases, the production of oxygen radicals and energy (ATP) depletion (Paschen, 2000). On the other hand, a more moderate and transient increase in the intracellular Ca^{2+} concentration can induce adaptive stress responses in cells. For example, Ca^{2+} influx mediates the hormetic effects of some endogenous growth factors and neurotransmitters, as well as some environmental agents including excitotoxins and caffeine (Furst, 1987; Kerr et al., 2002; Tauskela et al., 2003).

Tumor Necrosis Factor

In response to injury, infection and degenerative disease cells in produce the cytokine tumor necrosis factor-alpha (TNF). TNF activates cell surface receptors that are coupled to multiple signal transduction cascades that can either prevent or promote cell death (Gaur and Aggarwal, 2003). TNF plays a key role in the killing and removal of infectious agents and damaged cells within the affected tissue. At the same time, TNF can prevent the death of cells that are not severely damaged. The ability of TNF to protect cells against injury and disease is mediated by a stress response pathway involving the transcription factor NF-KB (Mattson and Camandola, 2001). NF-KB may be activated by TNF receptor-induced hydrolysis of sphingomyelin resulting in the production of ceramide (Fig. 3). Ceramide has been demonstrated to have a biphasic effect on cell survival, consistent with a hormesis mechanism (Goodman and Mattson, 1996).

CHEMICALS IN VEGETABLES AND FRUITS

Numerous studies have led to the conclusion that diets with high amounts of vegetables and fruits can reduce the risk of cardiovascular disease, cancers, diabetes and neurodegenerative disorders (Heber, 2004). Because vegetables and fruits have high concentrations of antioxidants such as vitamins E and C, it is believed that these and other beneficial chemicals in these foods act by decreasing oxidative stress in cells. On the other hand, many phytochemicals are toxins that serve the function of protecting the plants against insects and other predators (Groot and Dicke, 2002; Trewavas and Stewart, 2003). Emerging findings suggest that some of the beneficial chemicals in vegetables and fruits may actually induce a mild stress response in cells. In this regard, several phytochemicals that are highly touted for their health benefits have been shown to induce hormesis effects. For example, resveratrol, a chemical in red wine that may protect against cardiovascular disease, has been shown to exert its beneficial effects on cells by inducing a stress resistance response mediated by a protein called Sir-2 (Tissenbaum and Guarente, 2001; Howitz et al., 2003). Other examples of phytochemicals that may act via a hormesis mechanism include isothiocyanates which are present at high levels in broccoli (Faulkner et al., 1998) and quercetin which is found in red grape seeds (Blardi et al., 1999). Therefore,

from both evolutionary (phytochemicals protect plants against predators) and mechanistic (phytochemicals induce a cellular stress response) perspectives, at least some beneficial effects of phytochemicals in the human diet are mediated by stimulation of stress resistance mechanisms.

CELLULAR AND MOLECULAR MECHANISMS OF HORMESIS

How do low levels of environmental and endogenous toxins protect cells and organisms against disease? In many cases a cellular stress response is induced that involves the activation of genes that encode stress resistance proteins such as heat-shock proteins, endoplasmic reticulum protein chaperones, antioxidant enzymes and/or Bcl-2 family members (Bartling et al., 2003; Mattson et al., 2004a). In addition, the production and release of certain growth factors and cytokines appear to be important mediators of hormesis responses to low dose toxins. As noted above, such mechanisms may be responsible for the beneficial effects of moderate physical exercise, cognitive stimulation and dietary restriction (Fig. 1). Several signal transduction pathways have been identified that mediate hormesis responses of cells in many different types of organisms including humans. One pathway involves membrane receptors coupled to phosphatidyl inositol-3 kinase (PI3K) and Akt kinase; ligands that activate this pathway include insulin, insulin-like growth factors and BDNF. Examples of mild stressors that activate the latter pathway include physical exercise, dietary restriction and low doses of neurotoxins. Another hormetic pathway involves the transcription factor Hif-1 (hypoxia inducible factor-1) which is activated during metabolic and oxidative stress and plays a pivotal role in protecting cells against subsequent and more severe stress (Williams et al., 2002). The latter mechanism may play a key role in the hormesis effects of low doses of cyanide and arsenic. One final example involved in a hormesis response to the toxin diazoxide is activation of mitochondrial potassium channels and the transcription factor CREB (Eliseev et al., 2004).

The nervous system is the master regulator of responses to environmental stressors and toxins. The mechanisms by which the nervous system mediates hormesis are beginning to be revealed and involve multiple signaling pathways including those activated by neurotransmitters, neuroendocrine peptides and neurotrophic factors (Mattson et al., 2004b). In particular, the neurotransmitters serotonin and norepinephrine, the neuropeptide CRH (corticotrophin-releasing hormone), and the neurotrophic factor BDNF have major influences on the nerve cell circuits that regulate stress responses. In response to physical exercise, cognitive stimulation and dietary restriction levels of BDNF and CRH are increased in multiple regions of the brain. The serotonin signaling pathway is also activated in response to mild stressors and, in turn, stimulates production of BDNF. Recent findings suggest that these signaling pathways in the brain can increase the resistance of many different organ systems to disease; this may occur by improvements in energy metabolism and adaptive changes in the cardiovascular system, for example (Mattson et al., 2004b).

CONCLUSIONS

-Evolution favored organisms that used toxic agents in their environment to their advantage, often as signaling molecules that trigger adaptive stress responses. Examples include gases such as NO and CO, amino acids such as glutamate, and ions such as Ca^{2+} and K^{+}.

-The health benefits of exercise, dietary restriction and cognitive stimulation are mediated in large part by a hormesis mechanism involving activation of stress resistance cellular signaling pathways.

-Many of the phytochemicals responsible for health benefits of vegetable and fruit consumption may exert their effects by inducing a hormesis response in cells.

-Stimulating stress resistance mechanisms by dietary, behavioral and toxin-based regimens holds great promise for the prevention and treatment of a range of diseases including cardiovascular disease, cancers, diabetes and neurodegenerative disorders.

-The brain mediates adaptive responses of organisms to a range of environmental stressors. A better understanding of the cellular and molecular mechanisms by which the brain mediates hormesis responses may lead to novel approaches to improving the health of humans.

REFERENCES

Agell N, Bachs O, Rocamora N, Villalonga P. 2002. Modulation of the Ras/Raf/MEK/ERK pathway by Ca(2+), and calmodulin. Cell Signal 14:649-654.

Bartling B, Hilgefort C, Friedrich I, Silber RE, Simm A. 2003. Cardio-protective determinants are conserved in aged human myocardium after ischemic preconditioning. FEBS Lett. 555:539-544.

Benzie IF (2000) Evolution of antioxidant defence mechanisms. Eur. J. Nutr. 39:53-61.

Blardi P, De Lalla A, Volpi L, Di Perri T. 1999. Stimulation of endogenous adenosine release by oral administration of quercetin and resveratrol in man. Drugs Exp. Clin. Res. 25:105-110.

Brouard S, Berberat PO, Tobiasch E, Seldon MP, Bach FH, Soares MP (2002) Heme oxygenase-1-derived carbon monoxide requires the activation of transcription factor NF-kappa B to protect endothelial cells from tumor necrosis factor-alpha-mediated apoptosis. J. Biol. Chem. 277:17950-17961.

Brune B, Zhou J (2003) The role of nitric oxide (NO) in stability regulation of hypoxia inducible factor-1alpha (HIF-1alpha). Curr. Med. Chem. 10:845-855.

Cypser JR, Johnson TE (2002) Multiple stressors in Caenorhabditis elegans induce stress hormesis and extended longevity. J. Gerontol. A Biol. Sci. Med. Sci. 57:B109-114.

Dore S, Goto S, Sampei K, Blackshaw S, Hester LD, Ingi T, Sawa A, Traystman RJ, Koehler RC, Snyder SH (2000) Heme oxygenase-2 acts to prevent neuronal death in brain cultures and following transient cerebral ischemia. Neuroscience 99:587-592.

Duncan AJ, Heales SJ (2005) Nitric oxide and neurological disorders. Mol. Aspects Med. 26:67-96.

Eliseev RA, Vanwinkle B, Rosier RN, Gunter TE. 2004. Diazoxide-mediated preconditioning against apoptosis involves activation of cAMP-response element-binding protein (CREB) and NFkappaB. J. Biol. Chem. 279:46748-46754.

Faulkner K, Mithen R, Williamson G. 1998. Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. Carcinogenesis 19:605-609.

Furst, A. (1987) Hormetic effects in pharmacology: pharmacological inversions as prototypes for hormesis. Health Phys. 52:527-530.

Gnegy ME (2000) Ca2+/calmodulin signaling in NMDA-induced synaptic plasticity. Crit. Rev. Neurobiol. 14:91-129.

Goodman Y, Mattson MP. 1996. Ceramide protects hippocampal neurons against excitotoxic and oxidative insults, and amyloid beta-peptide toxicity. J. Neurochem. 66:869-872.

Groot, A. T., Dicke, M. (2002) Insect-resistant transgenic plants in a multi-trophic context.Plant J. 31:387-406.

Guar, U., Aggarwal, B. B. (2003) Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. Biochem. Pharmacol. 66:1403-1408.

Hawaleshka A, Jacobsohn E (1998) Ischaemic preconditioning: mechanisms and potential clinical applications. Can. J. Anaesth. 45:670-682.

Heber D. 2004. Vegetables, fruits and phytoestrogens in the prevention of diseases. J. Postgrad. Med. 50:145-149.

Heilbronn LK, Ravussin E (2003) Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr. 78:361-369.

Hofseth LJ, Hussain SP, Wogan GN, Harris CC (2003) Nitric oxide in cancer and chemoprevention. Free Radic. Biol. Med. 34:955-968.

Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung, P., Kisielewski, A., Zhang, L. L., Scherer, B., Sinclair, D. A. (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425:191-196.

Huang PL (2004) Nitric oxide and cerebral ischemic preconditioning. Cell Calcium 36:323-329.

Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Barrett JC. 2003. Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. Annu. Rev. Med. 54:131-152.

Jeffery B, Barlow T, Moizer K, Paul S, Boyle C (2004) Amnesic shellfish poison. Food Chem Toxicol. 42:545-557.

Kaide JI, Zhang F, Wei Y, Jiang H, Yu C, Wang WH, Balazy M, Abraham NG, Nasjletti A (2001) Carbon monoxide of vascular origin attenuates the sensitivity of renal arterial vessels to vasoconstrictors. J. Clin. Invest. 107:1163-1171.

Kerr DS, Razak A, Crawford N (2002) Age-related changes in tolerance to the marine algal excitotoxin domoic acid. Neuropharmacology 43:357-366.

Kisby GE, Ellison M, Spencer PS (1992) Content of the neurotoxins cycasin (methylazoxymethanol beta-D-glucoside) and BMAA (beta-N-methylamino L-alanine) in cycad flour prepared by Guam Chamorros. Neurology 42:1336-1340.

Lamming DW, Wood JG, Sinclair DA (2004) Small molecules that regulate lifespan: evidence for xenohormesis. Mol. Microbiol. 53:1003-1009.

Lane, N. (2002) Oxygen: The Molecule that made the World. Oxford University Press, 374 pp.

Lessmann V (1998) Neurotrophin-dependent modulation of glutamatergic synaptic transmission in the mammalian CNS. Gen. Pharmacol. 31:667-674.

Lowenstein CJ, Dinerman JL, Snyder SH (1994) Nitric oxide: a physiologic messenger.Ann. Intern. Med. 120:227-237.

Lipsky RH, Xu K, Zhu D, Kelly C, Terhakopian A, Movelli A, Marini AM (2001) Nuclear factor kappaB is a critical determinant in N-methyl-D-aspartate receptor-mediated neuroprotection. J Neurochem. 78:254-264.

Mabuchi T, Kitagawa K, Kuwabara K, Takasawa K, Ohtsuki T, Xia Z, Storm D, Yanagihara T, Hori M, Matsumoto M (2001) Phosphorylation of cAMP response element-binding protein in hippocampal neurons as a protective response after exposure to glutamate in vitro and ischemia in vivo. J. Neurosci. 21:9204-9213.

Macario AJ, de Macario EC (1999) The archaeal molecular chaperone machine: peculiarities and paradoxes. Genetics 152:1277-1283.

Masoro EJ. 2003. Subfield history: caloric restriction, slowing aging, and extending life. Sci. Aging Knowledge Environ. Feb 26; 2003:RE2.

Mattson MP (1988) Neurotransmitters in the regulation of neuronal cytoarchitecture.Brain Res. 472:179-

212.

Mattson MP, Camandola S (2001) NF-kappaB in neuronal plasticity and neurodegenerative disorders. J. Clin. Invest. 107:247-254.

Mattson MP (2003a) Gene-diet interactions in brain aging and neurodegenerative disorders.Ann Intern Med. 139:441-444.

Mattson MP (2003b) Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. Neuromolecular Med. 3:65-94.

Mattson MP, Chan SL (2003) Neuronal and glial calcium signaling in Alzheimer's disease.Cell Calcium 34:385-397.

Mattson MP, Duan W, Wan R, Guo Z. 2004a. Prophylactic activation of neuroprotective stress response pathways by dietary and behavioral manipulations. NeuroRx 1:111-116.

Mattson MP, Maudsley S, Martin B. 2004b. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci. 27:589-594.

Melzer K, Kayser B, Pichard C (2004) Physical activity: the health benefits outweigh the risks.Curr Opin Clin Nutr Metab Care. 7:641-647.

Michaelides, A. P., Andrikopoulos, G. K., Oikonomou, E. V., Psomadaki, Z. D., Richter, D. J., Dilaveris, P. E., Exadaktylos, N. I., Stefanadis, C. I., Toutozas, P. K. (2003) Improved myocardial performance during repetitive exercise testing: the role of extracellular superoxide dismutase activity in a model of exercise-induced myocardial preconditioning.Am. Heart J. 146:160-167.

Murphy NM, Carroll P (2003) The effect of physical activity and its interaction with nutrition on bone health. Proc Nutr Soc. 62:829-838.

Neto JS, Nakao A, Kimizuka K, Romanosky AJ, Stolz DB, Uchiyama T, Nalesnik MA, Otterbein LE, Murase N (2004) Protection of transplant-induced renal ischemia-reperfusion injury with carbon monoxide. Am. J. Physiol. Renal Physiol. 287:F979-989.

Nvue R, Gorianov V, Best N, Sundstrom LE, Pringle AK (2004) Time window and pharmacological characterisation of kainate-mediated preconditioning in organotypic rat hippocampal slice cultures. Neurosci. Lett. 367:365-368.

Pacher P, Obrosova IG, Mabley JG, Szabo C (2005) Role of nitrosative stress and peroxynitrite in the pathogenesis of diabetic complications. Emerging new therapeutical strategies. Curr. Med. Chem. 12:267-275.

Parsons PA (2003) Metabolic efficiency in response to environmental agents predicts hormesis and invalidates the linear no-threshold premise: ionizing radiation as a case study. Crit. Rev. Toxicol. 33:443-449.

Paschen W (2000) Role of calcium in neuronal cell injury: which subcellular compartment is involved? Brain Res. Bull. 53:409-413.

Rosenberg SM (2001) Evolving responsively: adaptive mutation. Nat. Rev. Genet. 2:504-515.

Ryter SW, Otterbein LE (2004) Carbon monoxide in biology and medicine. Bioessays 26:270-280.

Schipper HM (2004) Heme oxygenase expression in human central nervous system disorders. Free Radic. Biol. Med. 37:1995-2011.

Shannon TR, Bers DM (2004) Integrated Ca2+ management in cardiac myocytes. Ann. N. Y. Acad. Sci. 1015:28-38.

Tauskela JS, Brunette E, Monette R, Comas , Morley P. 2003. Preconditioning of cortical neurons by oxygen-glucose deprivation: tolerance induction through abbreviated neurotoxic signaling. Am. J. Physiol. Cell Physiol. 285:C899-911.

Tissenbaum, H. A., Guarente, L. (2001) Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature 410:227-230.

Trewavas, A., Stewart, D. (2003) Paradoxical effects of chemicals in the diet on health. Curr. Opin. Plant Biol. 6:185-190.

Trump BF, Berezesky IK (1995) Calcium-mediated cell injury and cell death. FASEB J. 9:219-228.

Usuki F, Yasutake A, Umehara F, Higuchi I. 2004. Beneficial effects of mild lifelong dietary restriction on skeletal muscle: prevention of age-related mitochondrial damage, morphological changes, and vulnerability to a chemical toxin. Acta Neuropathol. (Berl). 108:1-9.

Wan R, Camandola S, Mattson MP. Intermittent food deprivation improves cardiovascular and neuroendocrine responses to stress in rats. J Nutr 2003; 133:1921-1929.

Wang R, Wang Z, Wu L (1997) Carbon monoxide-induced vasorelaxation and the underlying mechanisms. Br. J. Pharmacol. 121:927-934.

Williams KJ, Telfer BA, Airley RE, Peters HP, Sheridan MR, van der Kogel AJ, Harris AL, Stratford IJ. 2002. A protective role for HIF-1 in response to redox manipulation and glucose deprivation: implications for tumorigenesis. Oncogene 21:282-290.

Yoshida K, Inoue T, Nojima K, Hirabayashi Y, Sado T. 1997. Calorie restriction reduces the incidence of myeloid leukemia induced by a single whole-body radiation in C3H/He mice. Proc. Natl. Acad. Sci. U. S. A. 94:2615-2619.

Zhuo M, Laitinen JT, Li XC, Hawkins RD (1998) On the respective roles of nitric oxide and carbon monoxide in long-term potentiation in the hippocampus. Learn. Mem. 5:467-480.

FIGURES

Figure 1. Mechanism by which exercise, dietary restriction and cognitive stimulation may exert their health benefits. Each of these environmental factors induce a mild stress response in nerve cells in the brain resulting in an increase in the expression of stress resistance proteins such as heat-shock protein 70 (HSP-70) and glucose-regulated protein 78 (GRP-78), as well as nerve cell growth factors such as BDNF. This hormesis mechanism may prevent degeneration of neurons during aging and disease, and may enhance learning and memory and increase the production of new nerve cells from stem cells. Moreover, this hormesis response exerts beneficial effects on many different organ systems including the cardiovascular system and glucose-regulating systems. This hormesis mechanism may also be activated by low dose toxins such as the serotonin reuptake inhibitors (SSRI) used to treat depression and related psychiatric disorders.

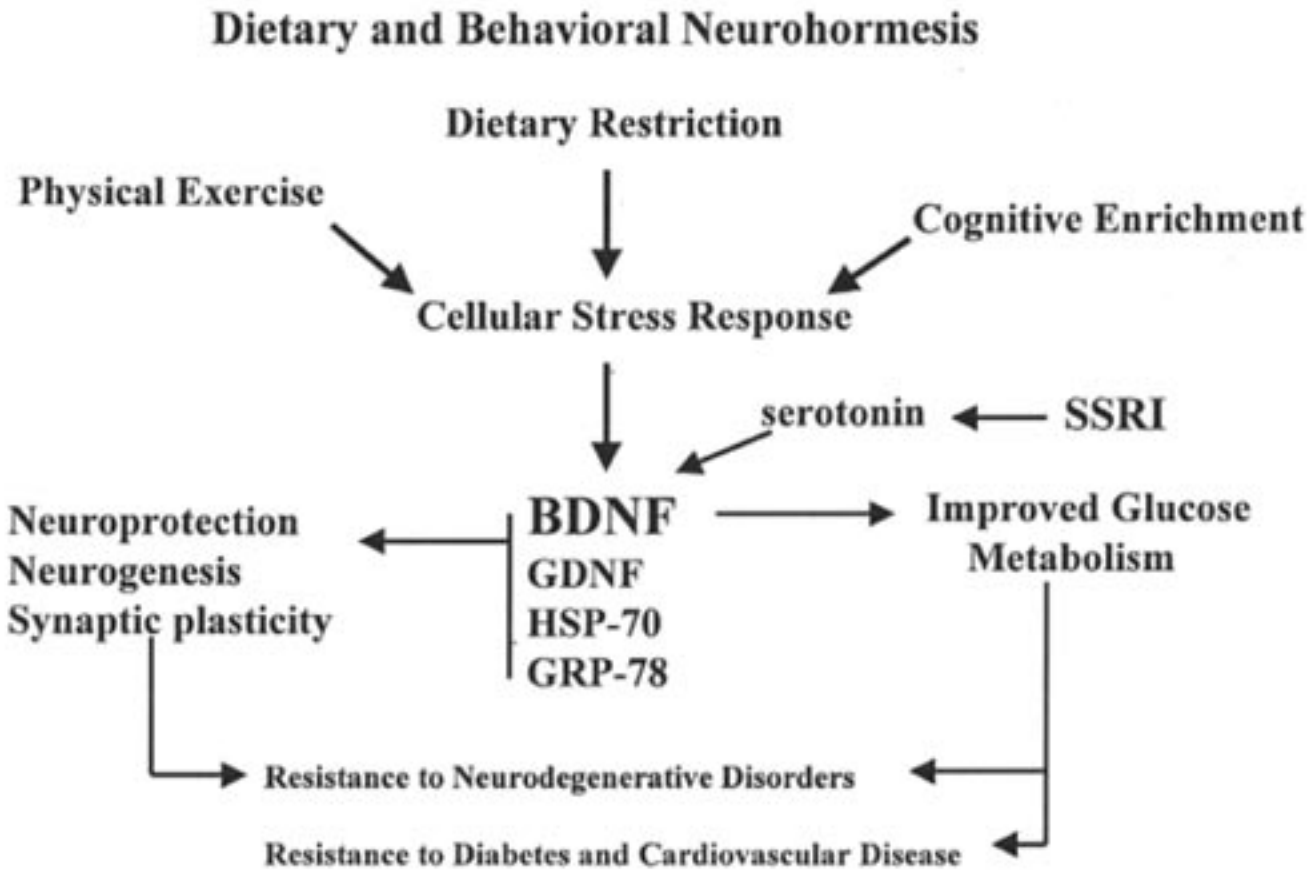


Figure 2. The excitatory amino acid neurotransmitter glutamate: a typical nonlinear dose-response hormesis mechanism.

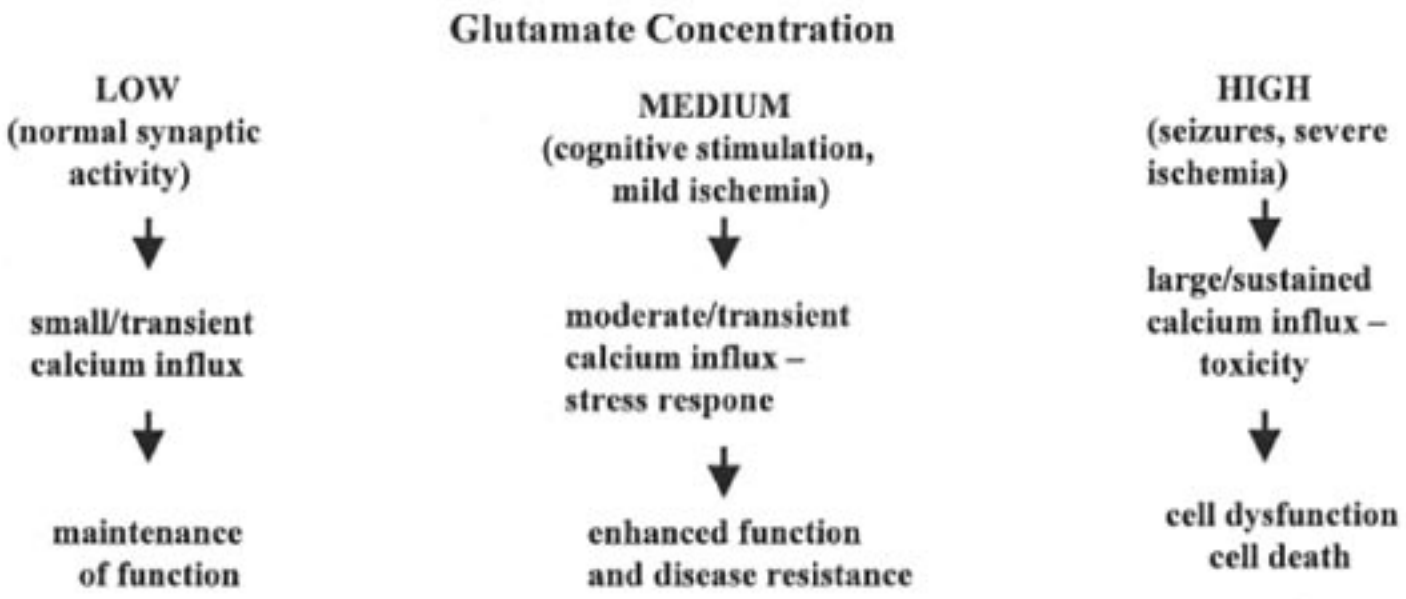
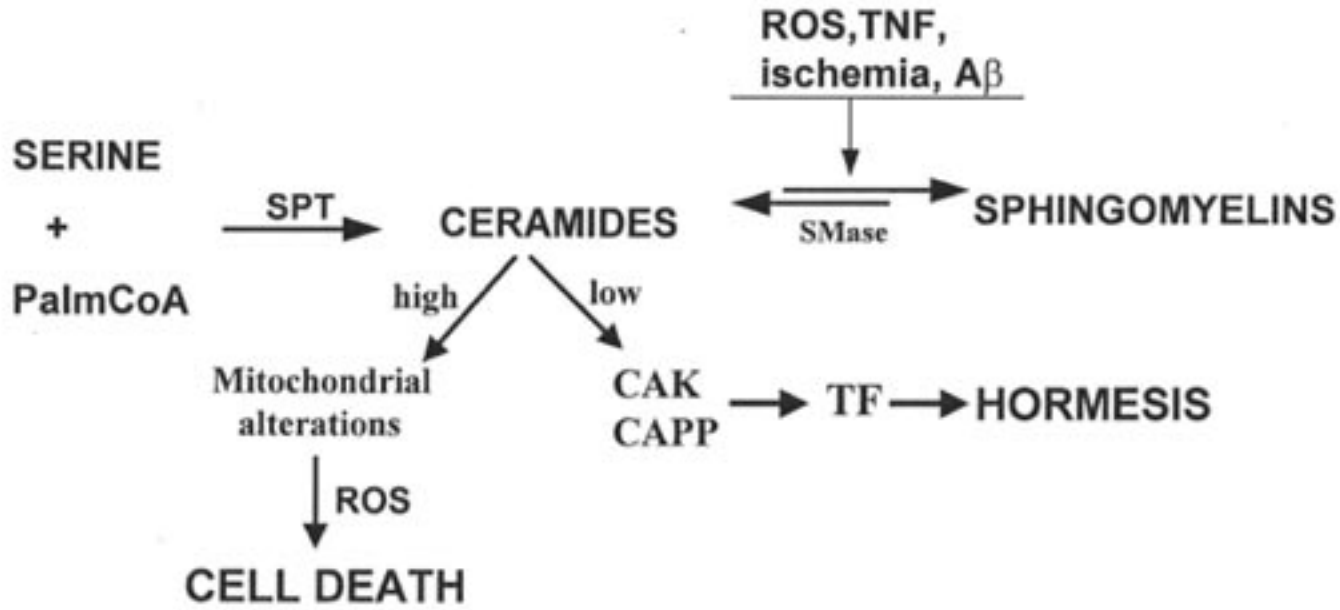


Figure 3. Ceramide is an endogenous stress-induced lipid that can induce hormesis or cell death. Ceramide is produced by the activity of serine palmitoyl transferase (SPT) and then incorporated into sphingolipids in cell membranes. When cells are exposed to stressful stimuli such as reactive oxygen species (ROS), tumor necrosis factor (TNF), ischemia and amyloid beta-peptide (A), ceramide is released from sphingomyelin by the activity of the enzyme sphingomyelinase (SMase). High levels of ceramide can be toxic to cells, while lower concentrations may have beneficial affects such as stimulation of cell growth and protection against severe stress.



LOW-DOSE RADIO-IMMUNO-THERAPY OF CANCER

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ABSTRACT

Recent research and clinical experience indicate the importance of the immune system in the prevention and treatment of cancer. The present review summarizes numerous experiments with both acute and protracted exposures of the immune system of mammals to low-doses of low-LET radiation. These investigations confirm the up-regulation of immune responses. Indeed, several studies with tumor bearing animals and also on patients with non-Hodgkin's lymphoma in various stages of malignancy used low-dose exposures at various low dose rates over several weeks. The data showed low-dosed induced prevention of metastases, as well as prolongation of disease remission with minimal side effects. A large set of other low-dose effect studies particularly over the past decade resulted in dose dependent delayed and temporary stimulation of physiological antimutagenic DNA damage-control systems involving damage prevention, repair, and removal at the molecular, cellular and tissue level, and also showed a low-dose induced reduction of malignant cell transformation in vitro or late appearing malignancies in vivo. In view of these new low-dose related radiobiological and oncological observations in contrast to data obtained at high doses, clinical trials of radioimmunotherapy appear warranted.

INTRODUCTION

Four decades of genomic, cellular, animal, and human data have shown that low-dose ionizing radiation stimulates positive genomic and cellular responses associated with effective cancer prevention and therapy and increased life span of mammals and humans.[1-8] Nevertheless, this data is questioned because it seems to contradict the well demonstrated linear relation between ionizing radiation dose and damage to DNA without providing a clear mechanistic explanation how low-dose radiation could produce such beneficial effects. This apparent contradiction is dispelled by current radiobiology that now includes DNA damage both from ionizing radiation and from endogenous metabolic free radicals, and coupled with the biological response to low-dose radiation. Acceptance of current radiobiology would invalidate long established recommendations and regulations of world-wide radiation safety organizations and so destroy the basis of the very expensive existing system of regulation and remediation.

A quantitative understanding of the antimutagenic DNA damage-control system essential for survival was recently developed (Figure 1).[9] This complex system evolved in aerobic organisms over aeons of tme in order to control an enormous burden of relentless DNA alterations produced by reactive oxygen species (ROS) generated principally by free radicals leaked from intracellular oxygen metabolism. This antimutagenic system is also operative against the DNA damage generated by ionizing radiation and chemicals. The enhanced response of the antimutagenic system to low-dose radiation provides a

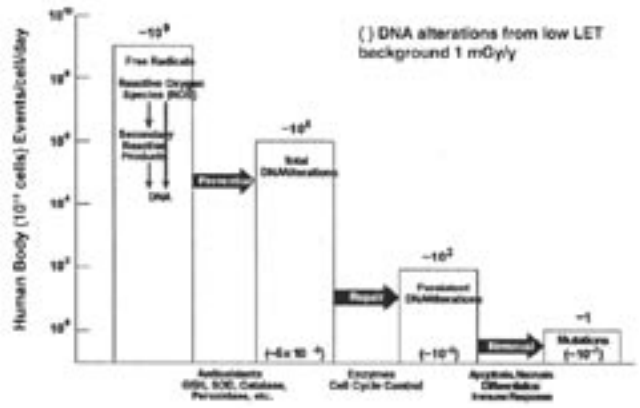

Figure 1. The antimutagenic DNA damage-control biosystem. Estimates are based on data in the literature. [9-12]

clear mechanistic explanation of the beneficial effects seen in cells, mammals, and humans.

THE ANTIMUTAGENIC DNA DAMAGE-CONTROL SYSTEM

The immune system is an essential component of antimutagenic control of cumulative DNA damage.[9,13] In addition to **removal** of persistent DNA alterations by the immune system and apoptosis, the antimutagenic system includes antioxidant **prevention** and enzymatic **repair** of DNA damage. This complex system of prevention, repair, and removal sequentially reduces DNA damage from about one million DNA alterations/cell/d to about one "mutation"/cell/d (Figure 1).[9] In contrast, low LET

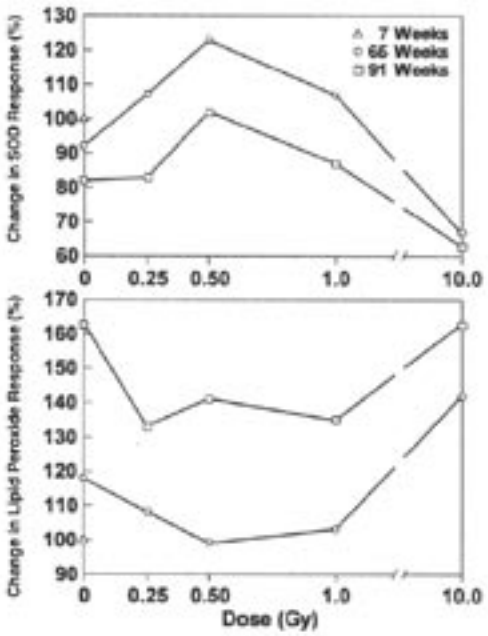

Figure 2. Antioxidant SOD and lipid peroxide response to age and radiation of rat brain cortex.[17]

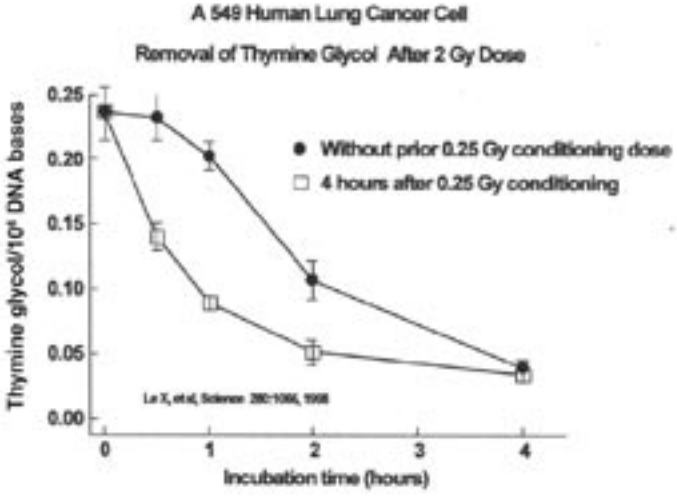

Figure 3. Low dose induced DNA repair.[18]

	1 r/y HBRA ± SE	0.1 r/y NBRA ± SE	P-value
MCAPC (0 Gy)	0.013 ± 0.003	0.016 ± 0.004	Not Significant
MCAPC (1.5 Gy)	0.098 ± 0.012	0.176 ± 0.017	P < 0.001

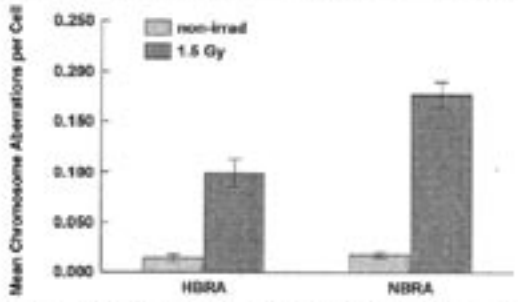

Figure 4. Mean chromosomal aberrations per cell in lymphocytes before and after exposure to 1.5 Gy. Lymphocytes were obtained from Ramsar residents in a high background radiation area of about 10 mGy/y and residents in a normal background radiation area of about 1 mGy/y.[13]

background radiation of 1mGy/year produces only one DNA alteration/500 cells/d and about 10^{-7} "mutations"/cell/d.[9] Double strand breaks/cell/d generated by oxygen metabolism is 1000 times greater than the double strand breaks produced by low LET background radiation.[9] UNSCEAR 1994 Report[11] and recent studies[15,16] furnish extensive documentation of low-dose stimuation of many cellular functions including antioxidant prevention (Figure 2),[17] enzymatic repair (Figures 3,4),[18,19] and immunologic and apoptotic removal (Figure 5)[20] of DNA damage. Stimulation of each of these antimutagenic responses by low-dose radiation, in contrast to their suppression by high-dose radiation, predictably precludes a linear dose–response relation of radiation and health effects.[21] Enhanced prevention of gene mutations by increased low-dose radiation (Figure 6)[9] is associated with decreased mortality and decreased cancer mortality observed in human populations exposed to low-dose radiation.[5, 21-24]

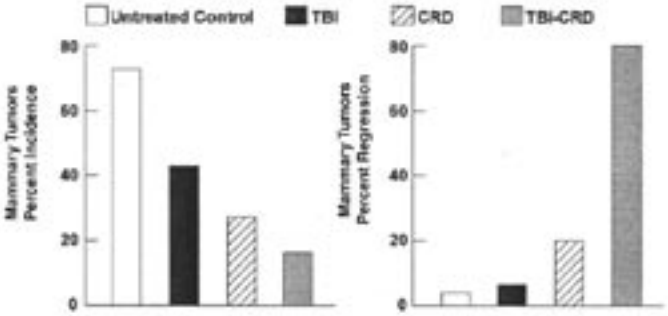

Figure 5. Eight month old, mammary tumor-susceptible, female C3H/He mice were first adjusted in a stepwise manner to chronically restricted diet (calorically 70% of ad libitum diet) (CRD) over a period of 3 weeks. The mice were maintained on CRD until completion of the study. After their diet was adjusted, the mice were exposed to TBI (0.04 Gy, 3 alternating days/week, 4 weeks) and were observed for 35 weeks. Tumor regression of the CRD + TBI group was very rapid and large numbers of CD8+ T cells were found infiltrating the regressing tumors, which were not seen in mice of the untreated control, LDR and CRD groups.[20]

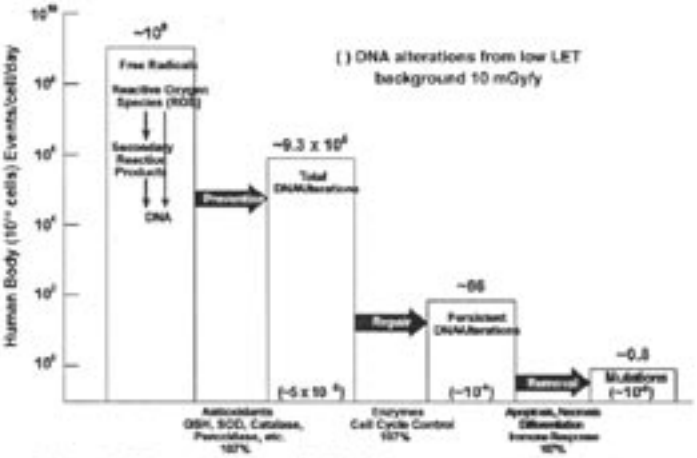

Figure 6. The antimutagenic DNA damage-control biosystem response to high background radiation = 120%. Estimates based on data in literature.[9-12]

IMMUNE SYSTEM RESPONSE TO RADIATION

Low-dose total body irradiation (TBI) and chronic TBI

(LDR) stimulate immune system prevention and removal of cancer metastases. This has been observed in mice for about 40 years[25,26] and more recently in rats[27] and humans.[3-6,8, 28-32]

The maximal immune response of mouse spleen T lymphocytes to sheep red blood cells, both *in vitro* and *in vivo*, occurs after a single dose occurs at 0.25 Gy (25 r) (Figure 7).[26] Maximal *in vitro* response is 180% with suppression to 50% of control after 100 r. The maximal *in vivo* response 145%, but more than 260 r is required for suppression to 50% of control.

TBI given with subimmunogenic tumor antigen induces tumor immunization, Subcutaneous inoculation of sham irradiated controls with 100 non-viable tumor cells does not suppress growth of 10,000 viable tumor cells inoculated subcutaneously 21 days later. Strikingly, TBI 15 r given simultaneously with inoculation of 100 non-viable tumor cells does induce marked suppression of tumor cell growth exceeding that induced by 100,000 non-viable tumor cells without TBI (Figure 8).[22]

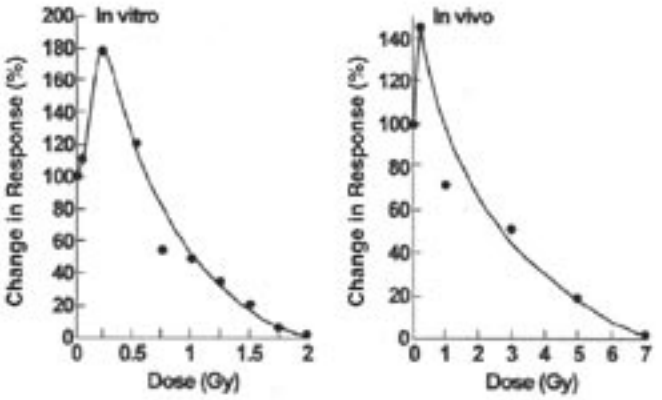

Figure 7. Immune system response to radiation. Mouse splenic cells primed with antigenic sheep red blood cells.[26]

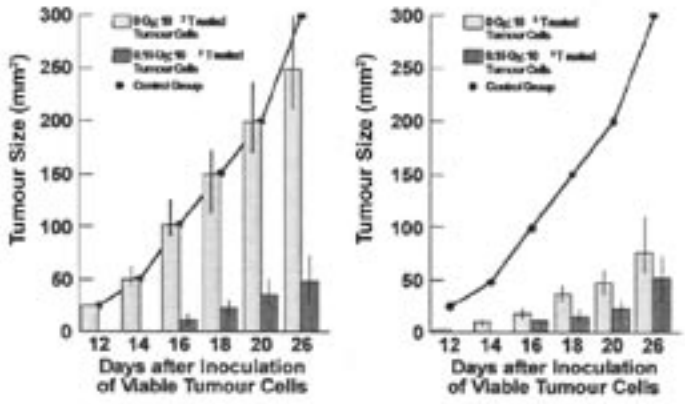

Figure 8. Effect of 0.15 Gy upon response of A/J mice to subimmunogenic and immunogenic numbers of non-viable mitomycin-treated fibrosarcoma (Sal) tumor cells. Groups of 60 mice were exposed to whole-body irradiation or sham-irradiated and inoculated subcutaneously with the indicated numbers of mitomycin-treated tumor cells. Twenty-one days later, all animals received 10^4 untreated Sal cells and were followed for tumor size. A control group did not receive mitomycin-treated cells.[25]

TBI stimulates immune suppression of tumor metastases to lung (Figure 9).[8] Lung colonies counted 20 days after TBI given 12 days after tumor cell transplantation into axilla of mice, were decreased by TBI doses less than 50 r; 15 r induced the maximaldecrease of 60%. High doses 50-100 r suppressed the immune system with increased metastases to lung.

LDR stimulates immune response of spleen T lymphocytes proliferation in mice (Figure 10).[26] Mice irradiated 5 days/week for 4 weeks with LDR courses of 10 r (0.5 r/d), 20 r (1.0 r/d) and 80 r (4.0 r/d) showed lymphocyte responses of 115%, 140%, and 160%, respectively, relative to 100% proliferation in the unirradiated control group.

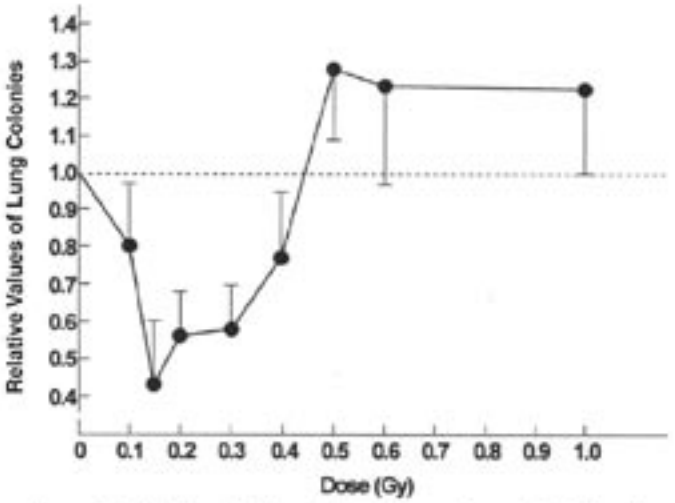

Figure 9. TBI given 12 days after tumor cell transplantation in to axilla. Lung colonies counted 20 days after TBI. Low dose TBI ineffective with spleen blocked. Low dose splenic irradiation, half-body irradiation (HBI) and TBI equally effective.[8]

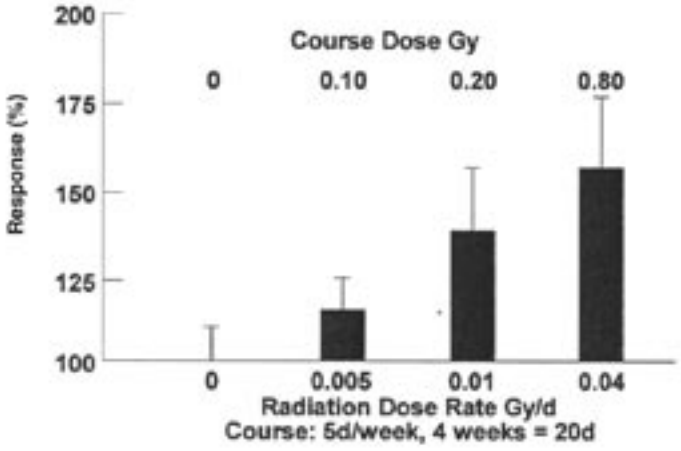

Figure 10. Dose-response analysis of splenic T cell proliferative response 3-5 days after the last radiation exposure of immunologically normal, long-lived C57B1/6J+/+ mice. Results are expressed as the mean percent increase in ^{3}H-thymidine uptake relative to 0 Gy control group as 100%. The vertical bars = 1 SEM.[26]

LDR with a calorically restricted diet (CRD) of 70% ad libitum diet calories, prevents and removes spontaneous breast cancer tumors in mice (Figure 5).[20] Eight month-old breast tumor susceptible female mice, after 3-week adjustment to CRD were exposed to a 48 r 4-week course of LDR (4 r 3d/week) and then observed for 35 weeks. While 70% of ad libitum diet mice and 27% of CRD mice developed breast cancer, only 16% of CRD + LDR mice developed breast cancer. Most impressive was the very rapid 80% tumor regression of CRD = LDR mice compared to the 20% and 4% regression in CRD and control mice, respectively. Large numbers of "killer" cytotoxic CD8+ T cells were observed infiltrating the regressing tumors of CRD=LDR mice, but not in control and CRD mice.[20] Half body LDR of women given 15-30 r by 75-150 fluoroscopic lung examinations similarly decreased breast cancer mortality. Breast cancer mortality of those receiving doses between 10-20 r was reduced to 66% of controls (Figure 11).[21, 27, 28]

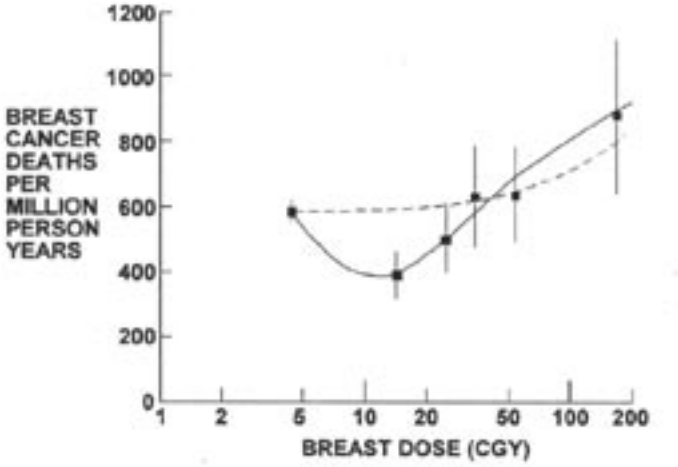

Figure 11. Reduced breast cancer mortality of tuberculosis patients who received LDI during fluoroscopy.[27] Breast dose is on log scale.[28] Dashed line is the linear best fit by authors in original publication that omits any figure of plotted data.

Metastasis is also suppressed by TBI of tumor-bearing rats (Figure 12).[29] TBI, or irradiation localized to tumor implanted into the leg, or control sham irradiation were given 14 days after tumor implantation. The number of visible metastases in the lung and the incidence of metastases in mediastinal and axillary lymph nodes were obtained 50 days after implantation. The number of tumor-infiltrating lymphocytes/microscopic field was observed 21 days after implantation. Metastases to lung, mediastinum, and axillary lymph nodes in TBI rats were reduced by more than 70% of that in control and locally irradiated rats. Tumor infiltration by lymphocytes in TBI rats was more than 900% of that in control and locally irradiated rats. Cytotoxic CD8+ T cells in the spleen of TBI rats were increased to 176% of those in control and locally irradiated rats.

HUMAN LOW-DOSE RADIATION CANCER IMMUNOTHERAPY

Two Harvard University clinical trials of LDR therapy in patients with non-Hodgkin's lymphoma were published in 1976[30] and 1979 (Figure 13).[31] The protocols were very similar. The Chaffey, et al. 1976 trial used a 150 r LDR course with TBI doses of 15 r 2x/week for 5 weeks. The Choi, et al. 1979 trial also used a 150 r LDR course with TBI doses of either 15 r 2x/week or 10 r 3x/week for 5 weeks. In both studies transient low platelets and interruption of scheduled therapy occurred in 35-40% of patients, irrespective of 10 r or 15 r dose schedule. Both chemotherapy and LDR patients had previously received chemotherapy and localized tumor high-dose radiation. Histologic tumor grades of LDR and chemotherapy patients were similar. COP chemotherapy used in the

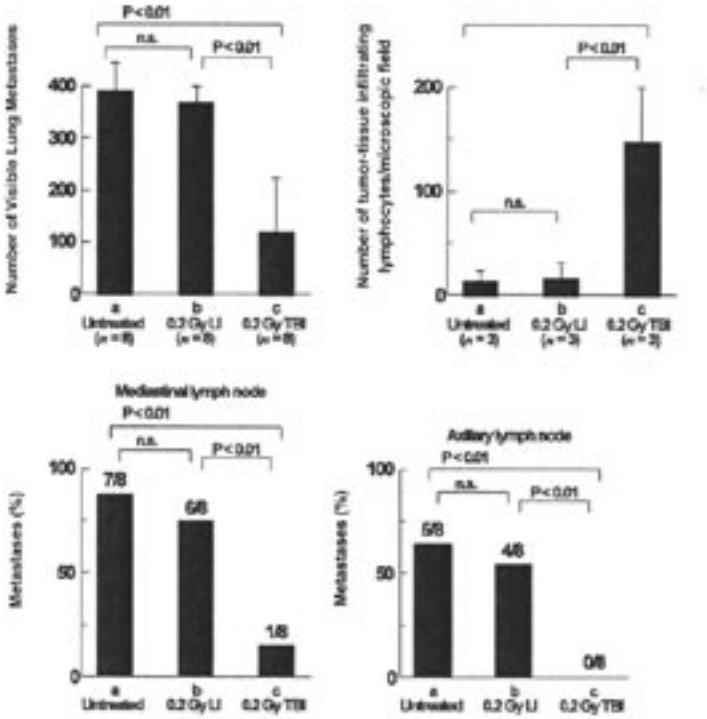

Figure 12. The number and incidence of metastases in lung and lymph nodes of mediastinum and axilla 50 days after intramuscular (leg) tumor implantation in rats, and the number of tumor infiltrating lymphocytes 21 days after implantation. Total body or localized tumor irradiation, with 0.2 Gy was given 14 days after implantation 5 x 10^5 allogenic hepatoma cells.[29]

1976 trial was superceded by more effective CHOP chemotherapy, still considered most effective. Both trials furnish 4-year survival data. Four–year survival in the 1976 trial of 25 LDR patients is 70% compared with 40% survival of 24 matched patients treated with COP.[30] The 1979 trial shows a similar 74% survival of 39 LDR patients compared with improved 52% survival of 225 patients treated with CHOP (Figure 13).[31]

Sakamoto, et al., Tohoku University, Sendai, Japan, published a 1997 review of their experimental studies in mice and a clinical trial of LDR. In mice, 15 r TBI induced

maximal suppression of tumor metastasis (Figure 9).[8] TBI given 6-12 hours before localized high-dose tumor therapy increased the effectiveness of tumor therapy. TBI, upper half body irradiation (HBI), and localized spleen irradiation were equally effective in stimulating the immune system.[8]

The protocol used by Sakamoto, et al. in their clinical trial of LDR therapy of patients with non-Hodgkin's lymphoma is similar to that used by Choi, et al. Both used a 150 r LDR course with equally effective TBI doses of either 15 r 2x/week or 10 r 3x/week for 5 weeks in patients relapsing from previous CHOP chemotherapy and localized high-dose tumor irradiation. Choi, et al. used TBI, while Sakamoto, et al. used TBI or HBI (Figure 14) with equal effectiveness without interruption of scheduled therapy by low platelets.

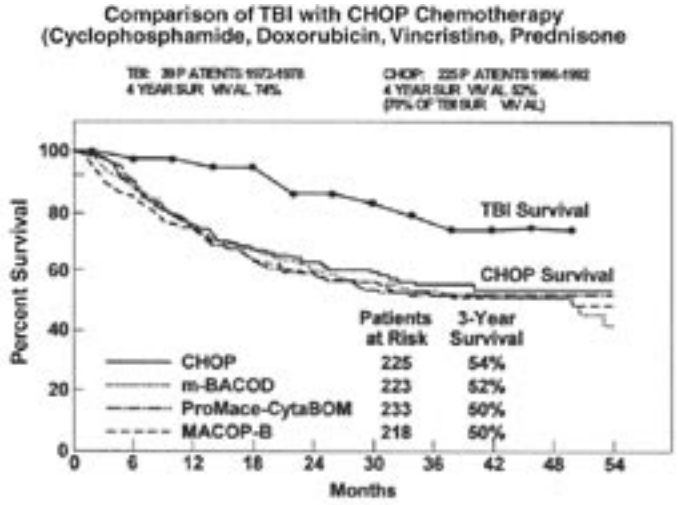

Figure 13. Comparison of TBI with CHOP chemotherapy.[31] CHOP remains the best available chemotherapy treatment for patients with advanced-stage intermediate grade or high-grade non-Hodgkin's lymphoma. *N Engl J Med 1993;328:1002-6.*

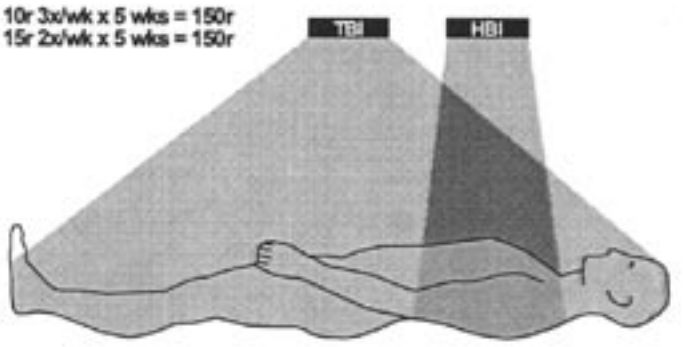

Figure 14. Treatment of patients with Non-Hodgkins Lymphoma with half (HBI) or total (TBI) body irradiation.[8]

Sakamoto, et al. report 9-year survival of 23 LDR patients and 94 CHOP chemotherapy patients with similar histologic tumor grades; approximately 75% of each group having intermediate or high grade lymphoma (Figure 15).[8] Tumors outside the HBI field regressed completely in response to LDR (Figure 16).[32] Nine-year survival of patients treated with LDR is 84%, unchanged from their 3.5-year survival and 12-year survival continues to be 84% (personal communication with author). In comparison, the 9-year survival of CHOP chemotherapy patients is 50 %.

Comparison of 4-year survival in the Harvard and Tohoku LDR *vs* CHOP trials are consistent in that both show about a 20% better survival of LDR patients compared with CHOP patients. In the Japanese trial, however, the 4-year survival of both LDR and CHOP patients is increased about 10% above those of the United States trial. This may be related to the well established benefits to the immune system of lower caloric intake and more exercise in the Japanese population. Though racial differences may be a factor, this has not been demonstrated in Japanese living in the United States. As shown by Makinodan (Figure 5),[20] LDR therapy is more effective when administered to mice with optimal caloric intake and better initial immune system activity. In marked contrast to chemotherapy, LDR stimulates, rather than depresses, all components of the antimutagenic system and is asymptomatic without significant side effects.

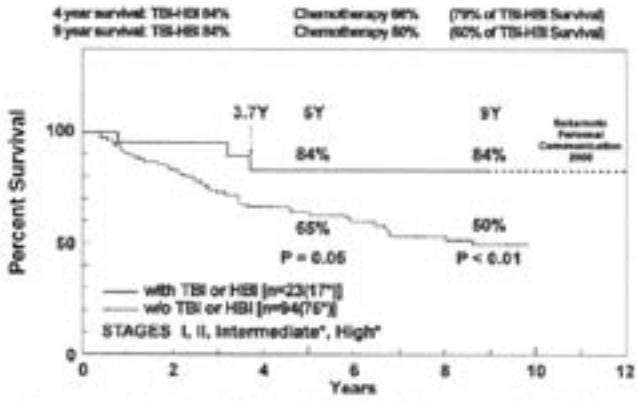

Figure 15. Equally effective TBI or HBI low-dose irradiation of patients with non-Hodgkin's lymphoma. Patients in both groups received chemotherapy and localized tumor high-dose radiation.[8]

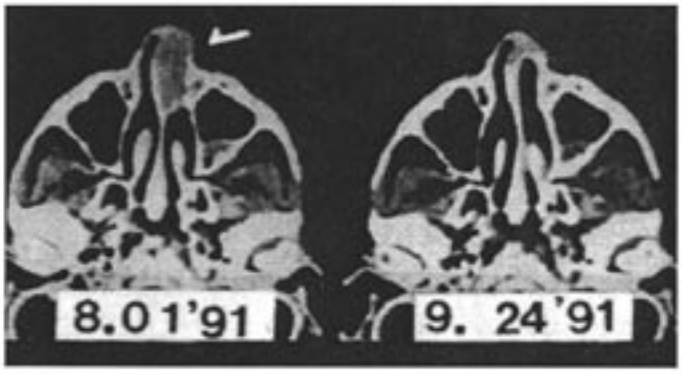

Figure 16. CT scans of upper nasal cavity before and after HBI therapy. Though entirely outside the HBI field, the nasal tumor completely disappeared.[32]

NEED FOR CLINICAL TRIALS OF LDR IMMUNOTHERAPY

Despite thousands of clinical trials of chemotherapy during the past 50 years, breast cancer death rate has not decreased significantly, prostate cancer death rate has risen steadily until recently, and colon and rectum cancer death rates also remain high,[33] Chemotherapy is not winning the war against cancer. In contrast, during this same time period, research in mice, rats and humans has revealed with high statistical confidence that LDR is very effective in preventing and treating cancer. Human clinical trials in the United States and confirmed in Japan have shown LDR immunotherapy to be much more effective than chemotherapy in treating intermediate and high grade stages of non-Hodgkin's lymphoma. Further research is needed during clinical trials to optimize LDR protocols and intervals between courses. Published results of LDR immunotherapy justify current initiation of clinical trials in patients with lymphomas, breast, prostate, colorectal, and ovarian cancer.

SUMMARY

Recent research has led to recognition of the importance of the immune system in the prevention and treatment of cancer as well as infectious disease.[34] LDR cancer immunotherapy is highly effective in rodents and humans. Clinical trials of LDR immunotherapy are needed to maximize the effectiveness of immune response to LDR Optimal protocols would be established by determining the magnitude and duration of immune response coupled with minimal marrow radiation achieved by reduced irradiated body volume while maintaining maximal immune stimulation. Clinical trials are also indicated to determine the effectiveness of LDR immune stimulation in patients with early HIV and other infectious diseases. LDR of patients is asymptomatic with minimal side effects, a rational and promising way of using our antimutagenic system to control cancer and infection. Current rapid increase of published genetic, molecular biology, and animal data that confirm the stimulatory effects of low-dose ionizing radiation will overcome the resistance of the regulatory and medical status quo. Clinical trials of effective cancer immunotherapy will resume in the United States after a lapse of more than 30 years.

REFERENCES

1. Liu SZ. Nonlinear dose-response relationships in the immune system following exposure to ionizing radiation: Mechanisms and implications. *Nonlinearity in Biol, Toxicol,& Med* 2003;**1**(1):71-92.
2. Iyer, Lehnert BE. Low dose, low LET ionizing radiation-induced radioadaptation and associated early responses in unirradiated cells. *Mutat Res* 2002;**503**:1-9.
3. Calabrese EJ, Baldwin LA. Radiation hormesis and cancer. *Human Ecol Risk Assessment* 2002 **8**:227-353.
4. Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/reduction and cellular responses to ionizing radiation: A unifying concept in stress response biology. *Cancer and Metastasis Reviews* 2004;**23**:311-322.
5. Burkart W, Sohrabi M, and Bauer A. High Levels of Radiation and Radon Areas: Radiation Dose and Health Effects. Elsevier, Amsterdam 2002.
6. Safwat A, Bayoumi Y, El-Sharkawy N, et al. The potential palliative role and possible immune modulatory effects of low-dose total body irradiation in relapsed or chemoresistant non-Hodgkin's lymphoma. *Radiother Oncol* 2003;**69**(1):33-36.
7. Richaud PM, Soubeyran P, Eghbali J, et al. Place of low-dose total body irradiation in the treatment of localized follicular non-Hodgkin's lymphoma: results of a pilot study. *Int J Radiat Oncol Biol Phys* 1998;**40**(2):387-390.
8. Sakamoto K, Myogin M, Hosoi Y, et al. Fundamental and clinical studies on cancer control with total or upper half body irradiation. *J Jpn Soc Ther Radiol Oncol* 1997;**9**:161-175**.**
9. Pollycove M, Feinendegen LE. Radiation-induced versus endogenous DNA damage: possible effects of inducible protective responses in mitigating endogenous damage. *Human & Exp Toxicol* 2003;**22**:290-306.
10. Rothkamm K, Löbrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci US 2003; 100: 5057-5062
11. Vilenchick MM, Knudson AG. Endogenous DNA double-strand breaks: Production, fidelity of repair and induction of cancer. Proc Natl Acad Sci. US 2003; 100: 12871 - 12876
12. Sedelnikova OA, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, Barrett JC. Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nature Cell Biol 2004; 6(2):168-70
13. Mille RA. The aging immune system: primer and prospectus. *Science* 1996;**273**:70-74.
14. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and Effects of Ionizing Radiation; UNSCEAR 1994 Report to the General Assembly, with Scientific Annexes. New York; Annex B. Adaptive Responses to Radiation in Cells and Organisms: 185-272.
15. Feinendegen LE, Loken MK, Booz J, et al. Cellular mechanisms of protection and repair induced by radiation exposure and their consequences for cell system responses. *Stem Cells* 1995;**13**(Suppl 1):7-20
16. Feinendegen LE, Sondhaus CA, Bond VP, Muhlensiepen H. Radiation effects induced by low doses in complex tissues and their relation to cellular

adaptive responses. *Mutat Res* 1996;**358**:199-205.

17. Yamaoka K Increased SOD activities and decreased lipid peroxide in rat organs induced by low X-radiation. *Free Radical Biol Med* 1991;**11**:3-7.
18. LE XC, Xing JZ, Lee J, et al. Inducible repair of thymine glycol detected by an ultra sensitive assay for DNA damage. *Science* 1998;**280**:1066-1069.
19. Ghiassi-nejad M, Mortazavi SMJ, Cameron JR, et al. Very high background radiation area in Ramsar, Iran: preliminary biological studies. *Health Phys* 2002;**22**:87-93.
20. Makinodan T. Cellular and subcellular alteration in immune cells induced by chronic, intermittent exposure in vivo to very low dose of ionizing radiation (ldr) and its ameliorating effects on the progression of autoimmune disease and mammary tumor growth. In: *Low Dose Irradiation and Biological Defense Mechanisms.* Ed: Sugahara T, Sagan LA, Aoyama T. Exerpta Medica, Amsterdam 1992:233-237.
21. Pollycove M, Feinendegen LE. Biological responses to low doses of ionizing radiation: detriment versus hormesis. Part 2: Dose response of organisms. *J Nucl Med* 2001;**42**(9):26N-37N.
22. Pollycove M, Feinendegen LE.Molecular biology, epidemiology and the demise of the linear no-threshold (LNT) hypothesis. In: CR Acad Sci, Paris, *Life Sciences* 1999;**322**:197-204.
23. Pollycove M. Nonlinearity of radiation health effects. *Env Health Perspec* 1998;**106**:363-368.
24. Pollycove M. Positive health effects of low level radiation in human populations. In: *Biological Effects of Low Level Exposures: Dose-Response Relationships.* Ed: Calabrese EJ. Chelsea,MI; Lewis Publishers 1994:171-187..
25. Anderson RE. Effects of low-dose radiation on the immune response. Chap 5 In: *Biological Effects of Low Level Exposures: Dose-Response Relationships.* Ed: Calabrese EJ. Chelsea,MI, Lewis Publishers 1992:95-112.
26. Makinodan T, James SJ. T cell potentiation by low dose ionizing radiation: possible mechanisms. *Health Phys* 1990;**59**(1):29-34.
27. Miller AB, Howe GR, Sherman GJ, et al. Mortality from breast cancer after irradiation during fluoroscopic examination in patients being treated for tuberculosis. *N Engl J Med* 1989;**321**:1285-1289.
28. Cuttler JM, Pollycove,M. Can cancer be treated with low doses of radiation? *J Am Phy Surg* 2003;**8**(4):108-11.
29. Hashimoto S, Shirato H, Hosokawa M, et al. The suppression of metastases and the change in host immune response after low-dose total-body irradiation in tumor bearing rats. *Radiat Res* 1999;**151**:717-724.
30. Chaffey JT, Rosenthal DS, Moloney WD, Hellman S. Total body irradiation as treatment for lymphosarcoma. *Int J Radiat Oncol Biol Phys* 1976;**1**:399-405.
31. Choi NC, Timothy AR, Kaufman SD, et al. Low dose fractionated whole body irriation in the treatment of advanced non-Hodgkin's lymphoma . *Cancer* 1979;**43**:1636-1642.
32. Takai Y, Yamada S, Nemoto K, et al. Anti-tumor effect of low-dose total or half-body irradiation and changes in the functional subset of peripheral blood lymphocytes in non-Hodgkin's lymphoma patients after TBI (HBI). *Low dose Irradiation and Biological Defense Mechanisms.* Eds: Sugahara T, Sagan LA, Aoyama T. Amsterdam: Elsevier Science BV 1992:113-116.
33. Amereican Cancer Society. Cancer Statistics 2004. *CA* 2004;**54**(1):13-16.
34. Blattman JN, Greenberg PD. Cancer immunotherapy: A treatment for the masses. *Science* 2004;**305**:200-205.

LOW-DOSE RADIATION RISK EXTRAPOLATION FALLACY ASSOCIATED WITH THE LINEAR-NO-THRESHOLD MODEL

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ABSTRACT

Managing radiation risks typically involves establishing regulations that limit radiation exposure. The linear-no-threshold (LNT) dose-response model has been the traditional regulatory default assumption. According to the LNT model, for low-linear-energy-transfer (LET) radiation-induced stochastic effects (e.g., neoplastic transformation and cancer) the risk increases linearly without a threshold. Any radiation exposure is predicted to increase the number of cancer cases among a large population of people. Cancer risk extrapolation from high to low doses based on this model is widespread. Here, indirect evidence is provided that the excess cancer risk calculated at very low doses of low-LET radiation (e.g., around 1 mGy), based on extrapolating from high dose data for an irradiated human population using the LNT model, is likely a phantom excess risk. Indirect evidence is provided, suggesting that for brief exposures to low-LET radiation doses on the order of 1 mGy, that a decrease below the spontaneous level is many orders of magnitude more probable than for any increase in risk as would be predicted by extrapolating from high to low doses using the LNT model. Such a decrease is, however, not expected after exposure to high-LET alpha radiation. The risk reduction has been largely attributed to the induction of a protective apoptosis mediated (PAM) process that selectively eliminates cells that contain genomic instability (e.g., mutant and neoplastically transformed cells). The PAM process appears to require a dose-rate-dependent stochastic threshold for activation whose minimum is estimated to possibly be as low as 0.01 mGy for X- and gamma rays. However, if the dose is too high (e.g., above 250 mGy for brief exposure at a high rate to X- or gamma rays), the PAM process is not expected to be activated. For protracted exposure to X- or gamma rays, doses as high as 400 mGy (and possibly higher) may activate the PAM process.

INTRODUCTION

The risk of harm to humans (e.g., induced mutations, neoplastic transformations, or cancers) after low doses of ionizing radiation of any type is generally assessed based on the linear-no-threshold (LNT) model whereby risk of harm increases linearly without a threshold as shown for neoplastic transformation in Figure 1A. Neoplastic transformation is considered a critical early step in the cancer induction process. Little consideration has been given to other forms of dose response such as the hormetic-type curve (Figure 1B) whereby low doses suppress (i.e., provide a protective effect) rather than increase risk. The LNT model is used in establishing radiation protection guidelines for nuclear workers and the general public, and is often applied in epidemiological studies of radiation induced cancer. Don Higson in his recent article, "The Bell Tolls for LNT," wrote the following (Higson 2004):

"The linear no-threshold (LNT) model has been a convenient tool in the practice of radiation protection, but it is not supported by scientific data at doses less than about 100 millisievert or at chronic dose rates up to at least 200 millisievert per year. Radiation protection practices based on the LNT model yield no demonstrable benefits to health when applied at lower annual doses. The assumption that such exposures are harmful may not even be conservative and has helped to foster an unwarranted fear of low-level radiation."

Here I discuss evidence for what I call the "low-dose radiation risk extrapolation fallacy associated with the LNT-model." The indicated fallacy is reflected by the general statements in the following paragraph:

After very low doses of ionizing radiation in humans, cancer risk is presumed by many experts to increase according to the LNT model. However, cancer cases that are presumed to be induced by very low radiation doses (e.g., 1 mGy) are considered too small in number to be shown to be statistically significant in epidemiological studies. Radiation-induced cancers are presumed to exist — and we humans, therefore, need to be protected from such radiation exposures, irrespective of a lack of evidence of harm. Experts insist that it's because of the relatively large fluctuations in the spontaneous cancer incident (background incidence) that we are unable to identify the excess cancers due to low doses or ionizing radiation. A very large irradiated population of humans (not presently feasible) is needed to show such a small increase after low radiation doses.
However, for those who believe in the above notions, no consideration is usually given to the possibility that

cancer risk might decrease after low doses (e.g., 0.01 – 100 mGy) of low-LET radiation. Further, unlike the difficulty of demonstrating an increase in cancer cases after low doses, it might be much easier to demonstrate a significant decrease in cancer cases if the focus is on low radiation doses rather than on extrapolating from high to low doses. This article presents evidence supporting the view that a decrease in risk for stochastic effects, such as neoplastic transformation and cancer after low doses of low-linear-energy-transfer (LET) radiation, may be orders of magnitude more likely than an increase. Thus, it may be much easier to demonstrate a reduction of these effects after exposure to very low doses of low-LET radiation than to demonstrate an increase.

LET is the average radiation energy loss when radiation traverses a small thickness of matter divided by the thickness size. Typical units for LET are kiloelectron volts per micrometer. X-rays, gamma rays, and beta particles are examples of low-LET radiation. Neutrons, alpha particles, and heavy ions (which are encountered in space travel) are examples of high-LET radiation. High-LET radiation, such as alpha particles, does not penetrate very far in matter. For example, alpha particles can be stopped by a piece of paper. However, low-LET gamma rays can penetrate the entire body of a human. Low-LET beta particles have an intermediate penetration between that for alpha particles and gamma rays. Low-LET X-rays and gamma rays have similar characteristics. Low-LET radiation is therefore more penetrating than high-LET radiation.

After moderate and high radiation doses, it is widely recognized that cancer risk for humans increases as the dose increases. Results are presented here which indicate that after low doses of low-LET radiation (around 1 mGy), the probability of a decrease in the risk of stochastic effects, such as neoplastic transformation and cancer, could be **orders of magnitude greater** than for the probability for an increase in risk (as predicted based on extrapolating from high dose data using the LNT model) for a range of doses. Thus, the notion that cancer risk at low doses could decrease and then increase at moderate and high radiation doses implicates a **hormetic type** dose-response relationship (Calabrese and Baldwin 2003).

RADIATION ABSORPTION IN TISSUE OR CELL CULTURES

The absorption of ionizing radiation in biological tissue or cell cultures involves stochastic interactions with constituent atoms and molecules, and generates energy deposition (track) events accompanied by bursts of reactive oxygen species (ROS) (Feinendegen 2005). Induced radiogenic DNA damage increases as radiation dose increases. The ROS are similar to those that arise constantly by normal oxidative metabolism. Endogenous ROS alone induce about a million DNA oxyadducts per cell per day compared to 5×10^{-3} total DNA-damaging events per average cell per day from background radiation exposure at 1 mGy over a year (Feinendegen 2005).

In the case of penetrating low-LET radiation, particle tracks arise stochastically throughout the exposed tissue with relatively low density at low doses. For low doses of high-LET radiation, the distribution of ionized molecules and of ROS bursts is more heterogeneous (Feinendegen, 2005). Intercellular communication after radiation damage probably depends on the spatial distribution of the hit cells as well as the cellular environment. Biological response to irradiation of humans is known to depend on the spatial distribution of the radiation hits in the body, the total radiation dose, and how rapidly the dose is delivered (i.e., dose-rate history).

STOCHASTIC RADIOBIOLOGICAL EFFECTS

For ionizing radiation doses in the range 0 – 100 mGy, biological effects of interest include induced genomic instability, mutations, neoplastic transformation, and cancer. These effects, along with genetic effects, are called stochastic, since their occurrence is governed by probabilistic considerations.

PROTECTIVE AND DELETERIOUS BYSTANDER EFFECTS

Radiation hits in cells may cause non-hit neighboring cells to become affected by signaling substrates from hit cells (bystander effect) (Azzam *et al.* 2004). The bystander effect can either be deleterious (enhancing the net biological damage) or protective (suppressing the net biological damage). Protective effects appear to predominate over the dose range of 0.1 – 100 mGy after brief exposure to low-LET photon radiation and appear to be mediated via ROS, reactive nitrogen species, and specific cytokines, including transforming growth factor β (Scott 2004, 2005a, 2005b). Deleterious bystander effects may predominate over the ultra low dose range of 0 – 0.01 mGy so far as inducing inversion mutation in mice (Hooker *et al.* 2004). The indicated inversion mutation assay is more a measure of DNA damage than of actual mutations. The indicated inversion mutations results implicate a threshold for induction of the protective bystander effect. At moderate (e.g., 250 – 500 mGy) and high (> 500 mGy) radiation doses, there is no evidence for a protective bystander effect after brief exposure at a high rate to low-LET photon radiation (Scott 2004). Thus, moderate and high doses appear to inhibit the protective bystander effect. However, when the dose rate is low, the protective bystander effect may occur over a much wider dose range, possibly to doses exceeding several hundred mGy (Scott 2004, 2005b).

ADAPTIVE RESPONSE

The classical two-dose, adaptive-response study involves exposing cell cultures or animals to a very small low-LET

radiation dose and after a few hours or longer irradiating them with a much larger dose that is expected to produce easily measurable enhanced biological effects. For the comparison group, only the higher dose is given. The hypothesis is that the adapting low dose will protect the cells from damage from the subsequent high dose (e.g., by inducing enhanced repair capacity). Thus, if the frequency of biological effect (e.g., mutations, neoplastic transformations) is lower in the group that received both the adapting and larger test dose than for the test dose alone, then one concludes that the small dose caused the cells to adapt so that they were less affected by the subsequent high dose.

Redpath *et al.* (2001) and Azzam *et al.* (1996) introduced a novel experimental single-dose protocol whereby only the small adapting dose was administered. The yield of biological effect (neoplastic transformation *in vitro*) was then compared to the spontaneous frequency for unirradiated cells. To the surprise of many, the adapting dose protected against spontaneous neoplastic transformation, yielding a decrease (rather than an increase) in the transformation frequency. Similar results were reported by Hooker *et al.* (2004) for mutation induction in mice exposed to very low doses of X-rays. However, their mutation data also showed elevated risk at ultra low doses (< 0.01 mGy), suggestive of reduced DNA repair activation after such low doses. I have characterized the neoplastic transformation data of Redpath *et al.* (2001) and the mutation data of Hooker *et al.* (2004) using my recently published NEOTRANS$_3$ model. A poster (http://www.radiation-scott.org/Scott_4C_for_web.pdf) presented at a recent workshop provides the resultant dose-response relationships (Scott 2005a). The NEOTRANS$_3$ model and how it can be used to clarify issues related to the shape of the dose-response curves for specific stochastic effects at very low doses is discussed in the section that follows.

NONLINEAR DOSE-RESPONSE CURVES

The NEOTRANS$_3$ model can be used to characterize nonlinear (or linear) dose-response relationships for mutation or neoplastic transformation induction by low doses of ionizing radiation (Scott 2004). With the model, mutations arise due to misrepair of DNA damage induced by radiation. Viable mutants can divide, producing progeny that can undergo spontaneous neoplastic transformation. However, after low doses of low-LET radiation above a stochastic threshold (D_{PAM}), a protective apoptosis mediated (PAM) process can be activated that selectively removes some of the already present mutant and neoplastically transformed cells. PAM is a protective bystander effect as well as an adaptive response. For low-LET photon sources (e.g., X-rays and gamma rays) the minimum threshold for activating PAM may be as low as 0.01 mGy (Scott 2005a). However, above a stochastic low-LET dose D_{off} (150 – 250 mGy for brief exposure to gamma rays at a high rate), the PAM process is not activated (Scott 2004, 2005a). Both the activation and inactivation thresholds are expected to vary for different individuals and, possibly, between different organs. They are also expected to vary for replicate cell cultures. The inactivation threshold D_{off} also appears to decrease as LET increases, possibly approaching zero for alpha particles (Scott 2005a). Stated differently, alpha particles do not appear to activate PAM after low doses. For X-rays and gamma rays, doses above 250 mGy delivered briefly at a high rate would not be expected to activate the PAM process (Scott 2005a).

When activated, the PAM process is predicted to cause the dose-response curve to have a hormetic shape. If not activated, the NEOTRANS$_3$ model essentially yields an LNT dose response (e.g., for alpha irradiation [Scott 2004, 2005b]). The model includes pathways for DNA damage induction, error-free repair, misrepair (leading to mutations), normal apoptosis, and PAM. The model allows evaluating both the probability for an increase ($P_{increase}$) in frequency of the biological effect of interest (mutant induction, neoplastic transformation) under the LNT model when extrapolating from high to low doses and also for the probability for a decrease ($P_{decrease}$), due to PAM at a given dose. The ratio $P_{decrease}/P_{increase}$, therefore, can be quantified for a specific low dose of radiation. Note that this ratio is just the odds of a decrease in risk relative to the odds for an increase under the LNT model (extrapolated from high dose data).

Published research results (Redpath *et al.* 2001) have demonstrated that the relative risk (*RR*) for radiation-induced neoplastic transformation in HeLa x skin fibroblast hybrid cells is quite similar to the *RR* for cancer induction in humans. Further, both the dose-response curves for the transformation frequency and for the *RR* for transformation were of the hormetic type. The indicated dose-response curves have shapes similar to that shown for the curve in Figure 1B. Based on the observations of Dr. Redpath's group, I have adapted *RR* relationships for neoplastic transformation *in vitro* for application to cancer *RR* evaluation in humans (Scott 2005b). Based on the *RR* relationships for both endpoints, the ratio $P_{decrease}/P_{increase}$ is expected to be roughly similar for neoplastic transformation of HeLa x skin fibroblast hybrid cells and for cancer induction in humans for the same type of radiation and exposure history.

One can, therefore, justifiably apply the NEOTRANS$_3$ model to *in vitro* data for transformation of HeLa x skin fibroblast cells to get an estimate of the ratio $P_{decrease}/P_{increase}$ for neoplastic transformation *in vitro* and use that estimate as a **very crude ballpark estimate** for cancer induction in humans. This has been done using Bayesian methods. Thus, results are based on posterior distributions of model parameters obtained via Bayesian inference. Evaluations were carried out using Markov chain Monte Carlo as previously described (Scott 2004, 2005b). Results (posterior distribution statistics) are presented in Table 1 for exposure to 1 mGy of gamma rays or 28-kVp X-rays or 60-kVp X-rays. The gamma-ray data used are

from Redpath *et al.* (2001). The 28-kVp data are from Ko *et al.* (2004). The 60-kVp X-ray data are from Redpath *et al.* (2003). All three studies were conducted by the same research team using the same type of cells.

Using the results in Table 1 as crude ballpark estimates for cancer induction by brief exposure of humans to 1 mGy of gamma rays, the odds for an observable reduction in cancer risk (evaluated based on the NEOTRANS$_3$ model with PAM activated) is more than 5 orders of magnitude greater than for an increase in risk (based on linear extrapolation from high to low doses). For protracted exposure, the odds are expected to be even higher in favor of a decrease (Scott 2004, 2005b). Further, the dose range over which risk is suppressed below the spontaneous level may be greatly increased (possibly to doses exceeding several hundred mGy).

Regarding protracted exposure to gamma rays, Chen *et al.* (2004) recently published a paper entitled "Is Chronic Radiation an Effective Prophylaxis Against Cancer?" They conducted a study of cancer occurrence among about 10,000 residents of 180 apartment buildings in Taiwan that were built with cobalt-60 contaminated steel. Cobalt-60 is a gamma-ray source. The building inhabitants resided there from about 9 – 20 years, during which they unknowingly received radiation doses that averaged about 400 mGy (same as 400 mSv for gamma rays) to the total body. The cancer incidence among this population was reported by Chen *et al.* to be reduced by more than 95% below the expected level for the general population. Thus, the protracted exposure at low rates seems to have expanded the protective zone (over which deleterious stochastic effects are suppressed) to at least 400 mGy. The researchers concluded the following (Chen *et al.* 2004):

"The experience of these 10,000 persons suggest that long-term exposure to radiation, at a dose rate of the order of 50 mSv (5 rem) per year, greatly reduces cancer mortality, which is a major cause of death in North America."

Thus, the title of an article by Hooker *et al.* (2004), "The Linear Nonthreshold Model Does Not Hold for Low Dose Ionizing Radiation," is a statement that applies to low-LET radiation.

The focus here has mainly been on exposure only to low-LET radiation. Currently available evidence suggests that the ratio $P_{decrease}/P_{increase}$ for alpha-radiation-induced neoplastic transformation is essentially zero (Scott 2004, 2005a). Cancer risk would, therefore, be expected to increase in accordance to the LNT model in the case of alpha irradiation. However, what about combined exposure to alpha and gamma radiation? Could the gamma rays activate the PAM process and protect against cancer induction by alpha radiation? This indeed appears to be the case for low doses of alpha radiation in combination with chronic gamma irradiation at low rates (Scott 2005b).

What about a 25-year smoker who has precancerous (neoplastically transformed) cells in their lung due to smoking? Could a little dab of low-LET X- or gamma rays to the lung protect from cancer via activating the PAM process? Possibly, if not likely! The PAM process could also be exploited related to developing novel low-dose therapy for cancer (Scott 2005b).

CONCLUSIONS

Information has been provided pointing out that after brief exposure to low doses (around 1 mGy) of low-LET radiation, stochastic effects such as neoplastic transformation and cancer induction could decrease rather than increase as would be expected under the LNT model. Further, the odds for a decrease is expected to be more than 5 orders of magnitude more likely than for an increase as would be expected under the LNT model when extrapolated from high-dose data. The results clearly point out the low-dose radiation risk extrapolation fallacy associated with the LNT model where the argument usually presented is that after low radiation doses, the increase in risk will be too small to take on statistical significance. Rather, results presented here indicate that for exposure of humans to low doses of low-LET radiation, what one should also be looking for is a decrease in risk below the spontaneous level (i.e., hormetic type dose-response curve). Thus, using the LNT model to extrapolate to very low radiation doses to assess cancer risk to humans from exposure to low-LET radiation is likely creating phantom excess risk (possibly by many order of magnitude). This may be especially true for chronic exposure at low rates.

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REFERENCES

Azzam E.I., de Toledo S.M, Raaphorst G.P., Mitchel R.E. (1996). Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. *Radiation Research* 146:369-373.

Azzam E.I., de Toledo S.M., and Little J.B. (2004). Stress signaling from irradiated to non-irradiated cells. *Current Cancer Drug Targets* 4:53-64.

Calabrese E.J. and Baldwin L.A. (2003). The hormetic dose-response model is more common than the threshold model in toxicology. *Toxicological Sciences* 71:246-250.

Chen W.L., Luan Y.C., Shieh M.C., Chen S.T., Kung H.T., Soong K.L., Yeh Y.C., Chou T.S., Mong S.H., Wu J.T., Sun C.P., Deng W.P., Wu M.F., and Shen M.L. (2004). Is chronic radiation an effective prophylaxis against cancer? *Journal of American Physicians and Surgeons* 9(1):6-10.

Feinendegen L. E. (2005). Low doses of ionizing radiation: relationship between biological benefit and damage induction. *World Journal of Nuclear Medicine* (in press).

Higson DJ. (2004). The bell tolls for LNT. *Operational Radiation Safety*, 87(5): S47-S50.

Hooker A.M., Bhat M, Day T.K., Lane J.M., Swinburne S.J., Morley A.A., and Sykes P.J. (2004). The linear no-threshold model does not hold for low-dose ionizing radiation. *Radiation Research* 162:447-452.

Ko S.J., Liao X-Y., Molloi S., Elmore E., and Redpath J.L. (2004). Neoplastic transformation *in vitro* after exposure to low doses of mammographic-energy x rays: quantitative and mechanistic aspects. *Radiation Research* 162:646-654 (2004).

Redpath J.L., Liang D., Taylor T.H., James C., Christie E., and Elmore E. (2001). The shape of the dose-response curve for radiation-induced neoplastic transformation *in vitro*: evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation. *Radiation Research* 156:700-707.

Redpath J.L., Lu Q., Lao X., Molloi S., and Elmore E. (2003). Low doses of diagnostic energy x-rays protect against neoplastic transformation *in vitro*. *International Journal of Radiation Biology* 79(4):235-240.

Scott B.R. (2004). A biological-based model that links genomic instability, bystander effects, and adaptive response. Fundamental and Molecular Mechanisms of Mutagenesis, *Mutation Research* 568: 129-143.

Scott B.R. (2005a). NEOTRANS$_3$ Model for low-dose-induced stochastic radiobiological effects. Poster presentation given at the Department of Energy, Low Dose Radiation Research Program Workshop V, Hyatt Regency, Bethesda, MD, April 25-27, 2005,http://www.radiation-scott.org/Scott_4C_for_web.pdf .

Scott B.R. (2005b). Low-dose radiation-induced protective process and implications for risk assessment, cancer prevention, and cancer therapy. *Nonlinearity in Biology, Toxicology, and Medicine* (in press).

Table 1. Ratios $P_{decrease}/P_{increase}$ for gamma-ray induced transformation of HeLa x skin fibroblast hybrid cells *in vitro* based on applying the NEOTRANS$_3$ model to published data.

Radiation type	Posterior mean	Posterior 5% value	Posterior 50% value	Posterior 95% value
Gamma rays	9.74×10^6	4.17×10^6	9.23×10^6	1.7×10^7
60-kVp X-rays	1.56×10^7	7.64×10^6	1.35×10^7	3.10×10^7
28-kVp X-rays	2.77×10^6	9.22×10^5	2.18×10^6	6.4×10^6

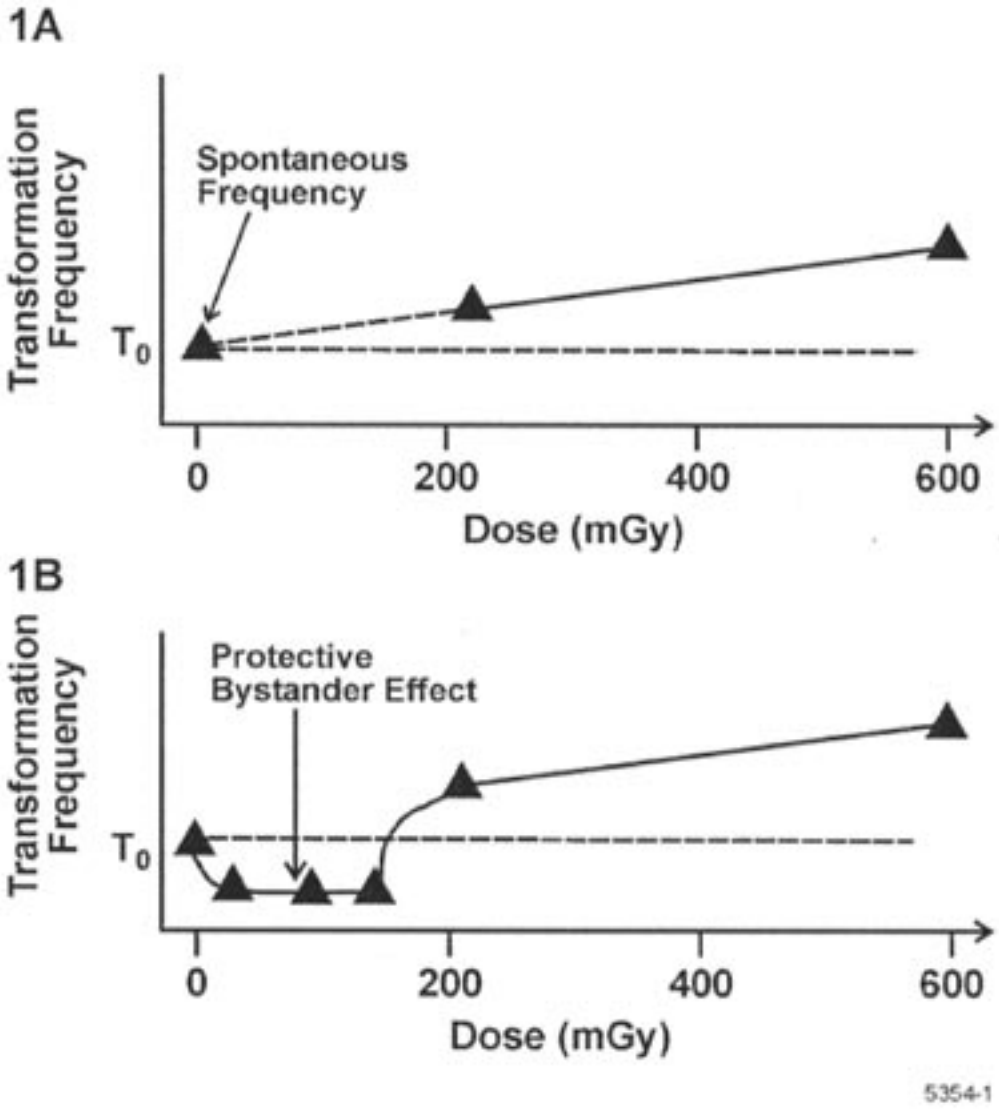

Figure 1. A comparison of general dose-response curve shapes for low-LET photon-induced neoplastic transformation in irradiated cells in culture, based on the LNT model (1A) extrapolated from high to low doses and what has been demonstrated experimentally (1B). Similar curve shapes are expected to apply to cancer induction in humans and laboratory animals by brief exposure to low-LET radiation.

INTERNATIONAL HORMESIS SOCIETY

GOAL

A growing number of scientists, including toxicologists, pharmacologists, biostatisticians, epidemiologists, occupational and environmental medical researchers and others have begun to display considerable interest in the topic of hormesis, a dose response phenomenon characterized by a low dose stimulation and a high dose inhibition. While there are many professional societies that have a general interest in dose response relationships, none explicitly is devoted to the topic of understanding the nature of the dose response in general and hormesis in particular. The diversity of professional societies that may consider dose response issues, including hormesis, is nonetheless quite broad ranging from the agricultural to the biomedical and clinical sciences. However, nearly without exception, these societies tend to be strongly organized around professional advancement and not focused on specific scientific concepts. This makes the issue of hormesis one of diffuse interest across a broad range of professions. The present situation represents a major obstacle for the integrated assessment of the dose response in general and hormesis in particular. In order to provide intellectual and research leadership on the topic of hormesis, a new professional association has been created called the International Hormesis Society (IHS).

The Society will be dedicated to the enhancement, exchange and dissemination of ongoing global research efforts in the field of hormesis. In addition, the Society will also strongly encourage the assessment of the implications of hormesis for such diverse fields as toxicology, risk assessment, risk communication, medicine, numerous areas of biomedical research, and all other biological disciplines including relevant engineering domains dealing with the dose response.

LOCATION

The International Hormesis Society is administered by BELLE, School of Public Health & Health Sciences at the University of Massachusetts at Amherst.

MEMBERSHIP

The IHS is a professional society designed to enhance understanding of the nature of the dose response and its implications for science and society. Those individuals with a professional interest in these areas will be eligible for membership. Applicants for membership must complete the attached membership application form. Corporate memberships are $5000.00 (U.S.) per year while Individual membership dues are $125.00 (U.S.) per year. Student memberships are encouraged with an annual dues set at $10.00. Applications should be mailed to the **BELLE Office, Environmental Health Sciences Program, Morrill I, Room N344, University of Massachusetts, Amherst, MA, 01003.**

As part of IHS membership, each corporate and individual member will receive a subscription to the journal **Dose-Response** (formerly called Nonlinearity in Biology, Toxicology and Medicine), which is currently a peer-reviewed quarterly journal. In addition, there will be a Society Newsletter developed for the membership. There will also be an annual conference to which all society members will receive a reduction in registration fees.

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