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ABSTRACT BOOK

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HORMESIS IN PRECAUTIONARY REGULATORY CULTURE: MODELS PREFERENCES AND THE ADVANCEMENT OF SCIENCE

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From a number of different angles, the contemporary policies on environmental chemicals exposures will be commented on. It demonstrates the excessive use of arguments based on adverse effects and underlines the necessity to take adaptive effects seriously. It is argued that the archetypal inclination of the 'When in doubt, keep it out.' precautionary approach, results in counter-productive and costly regulations. It is essential to include hormesis in regulation, in the form of a TIE (toxicological insignificant exposure level) related to the concentration, as an initial regulatory translation of adaptive effects. This regulatory transition will not be an easy one, as shall be argued from a sociological and historical perspective.

STOCHASTIC THRESHOLDS: A NOVEL EXPLANATION FOR NONLINEAR DOSE RESPONSE

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There is growing evidence that low doses of certain agents (e.g., radiation and genotoxic chemicals) can induced a protective process that leads to a reduction in mutations, neoplastic transformation, and cancer below the spontaneous frequency. High doses, however, appear to inhibit the protection, leading to elevated risks for the indicated biological effects. In this presentation, a dose-response model will be summarized which attributes the low-dose protection to stimulation of apoptosis among genomically compromised cells. The indicated apoptosis selectively removes genomically compromised cells and has been named the **p**rotective **a**poptosis **m**ediated (PAM) process. The PAM process is mediated via reactive oxygen and nitrogen species, and in the case of fibroblast, by extracellular transforming growth factor β1 (TGF-β1). Apoptosis is presumed to occur via the mitochondrial pathway. Activation of PAM appears to require a stochastic threshold, D_{PAM}, which varies for different individuals (*in vivo*), and also *in vitro* for different replicate samples. In addition, above a stochastic threshold, D_{off}, PAM appears to be inhibited. These threshold assertions are supported by microarray data from radiation studies by others. Because of the indicated stochastic thresholds, dose-response relationships for mutagenesis, neoplastic transformation, and cancer induction are predicted to be of the nonlinear hormetic type, involving a hormetic zone where risk is suppressed below the spontaneous level for genotoxic agents that activate PAM. The level of suppression (called the hormetic intensity) as well as the width of the hormetic zone over which risk is suppressed is predicted to increase with decreasing dose rate. Cancer cells appear to attain resistance to undergoing PAM, but new research results suggest that the resistance could possibly be reversed via use of apoptosis sensitizing agents. Implications of the PAM process for low-dose cancer therapy, cancer prevention, extension of expected lifespan, and low-dose risk assessment will be discussed.

RISK COMMUNICATION CHALLENGES AND OPPORTUNITIES FOR ESTABLISHING THE HORMETIC MODEL

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The biology and psychology of risk perception suggest that human judgment about risk is a matter of intuition, values, and emotions as much as or more than it is a matter of strictly fact-based reasoning. Further, neuroscience and psychology have found that the perception of any given risk, once learned, is difficult to extinguish or "unlearn". Given that hormesis deals with potentially or actually hazardous substances, which have affective attributes that evoke relatively higher levels of concern and fear, and given that many people already assume most of these substances to be harmful regardless of dose, the evidence suggests the potential for widespread resistance to the hormetic model. On the other hand, by identifying affective attributes that make a risk more or less worrisome, the risk perception literature can inform more effective risk communication by giving the initiator of the communication a better understanding of and respect for why the audience feels the way it does. It is argued that that, in turn, gives the communication more trustworthiness, clarity, and relevance, and therefore more influence on the choices and judgments of the audience. The findings of the biology and psychology of risk perception, and judgment making under uncertainty, are discussed, and their applicability to more effective risk communication about hormesis is explored.

THE EMERGENCE OF HORMESIS IN BIOLOGY, TOXICOLOGY, AND MEDICINE

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Considerable evidence indicates that reliable examples of hormetic dose responses in the toxicological/pharmacological literature are common and highly generalizable across biological model, endpoint measured and chemical class. Further assessment revealed that the hormetic dose response model is far more common than the threshold dose response model in objective, head-to-head comparisons. However, the field of toxicology introduced a profound error by rejecting the hormetic dose response model in its teaching, research, risk assessment and regulatory activities. The hormetic dose response model was rejected primarily because of its close historical association with the medical practice of homeopathy as a result of the prolonged and bitter feud between traditional medicine and homeopathy. Opponents of the concept of hormesis were successful in their misrepresentation of the scientific foundations of hormesis and in their unfair association of it with segments of the homeopathic movement with readily discreditable views. Such misrepresentations became firmly established and integrated within the pharmacology and toxicology communities because of their origins in and continuities with traditional medicine and subsequently profoundly affected governmental risk assessment activities further consolidating the rejection of hormesis. This error of judgment was reinforced by toxicological hazard assessment methods employing only high and few doses that were unable to assess hormetic responses, statistical modeling processes that were constrained to prevent the possibility of hormetic dose response relationships and by the modest nature of the hormetic stimulatory response itself, which demanded more rigorous study designs to evaluate possible hormetic responses. The future of low dose research in toxicology and pharmacology needs to be cognizant of and influenced by the possibility of hormetic dose responses with respect to animal model selection, acceptable background disease, endpoint selection, study design features, and biostatistical modeling activities.

RADIATION

TRANSITIONS IN BIOLOGY AND THE SHAPE OF RADIATION DOSE-RESPONSE RELATIONSHIPS

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COMPLEX MUTAGENESIS DOSE-RESPONSE RELATIONSHIPS AFTER EXPOSURE OF pKZ1 MICE TO DNA DAMAGING AGENTS

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MECHANISMS UNDERLYING IONIZING RADIATION-INDUCED ADAPTIVE AND BYSTANDER RESPONSES

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ADAPTIVE RESPONSE AND BYSTANDER EFFECTS IN HUMAN AND NON-HUMAN BIOTA

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MULTICELLULAR RESPONSES TO IONIZING RADIATION

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RADIATION RISK PREDICTION AND GENETICS: THE INFLUENCE OF THE P53 GENE IN VIVO

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SUPPRESSION OF DISEASE DEVELOPMENT AND TUMORIGENESIS BY LOW LEVEL RADIATION

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TRANSITIONS IN BIOLOGY AND THE SHAPE OF RADIATION DOSE-RESPONSE RELATIONSHIPS

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The dose related mechanistic transitions at the molecular, cellular, tissue and organism level of biological organization will be discussed. The best example of a transition at the molecular level is the change in the number and types of genes changing their expression as a function of radiation dose. At doses below about 0.10 Gy, a rather large set of genes change their expression. As the dose increases, these genes down-regulate and another set of genes are altered. This reflects the transition from mechanisms involved in the response to high damaging doses to the adaptive response elicited by low doses of radiation. Similar responses have been observed for the induction of mutations, with a low dose protective response and a high dose increase in mutation frequency. At the cellular level, the observation of low dose hypersensitivity suggests that radiation-induced changes in gene expression may provide a level of protection over a narrow dose range. Following low doses of radiation (less than about 0.10 Gy) cells that are damaged are eliminated from the population. As the dose increases (to the intermediate doses 0.10-0.30 Gy), genes involved in cell survival and repair may be activated and survival increases. As the dose continues to increase, cell killing increases as single exponential function of dose. Cell transformation also shows a low dose transition. At doses below about 0.10 Gy, the transformation frequency is less than observed in the controls. As the dose continues to increases the frequency of transformed cells also increases. Observations from human studies, such as the induction of leukemia in A-Bomb survivors and bone cancer in radium dial painters, suggest that linear extrapolation across these transition points is not possible. Understanding the mechanisms of action at transition points will help define the shape of dose-response relationships. Research supported by the Office of Biological and Environmental Research, U.S. Department of Energy through a grant No. DE-FG03-99ER62787 to Washington State University.

COMPLEX MUTAGENESIS DOSE-RESPONSE RELATIONSHIPS AFTER EXPOSURE OF pKZ1 MICE TO DNA DAMAGING AGENTS

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We have observed complex dose response relationships using a chromosomal inversion end-point *in vivo* and *in vitro* for a number of known DNA damaging agents in the pKZ1 recombination mutagenesis assay. This assay is sensitive and enables the study of very low doses of DNA damaging agents. The pKZ1 assay also has a high endogenous chromosomal inversion frequency which enables detection of a reduction below endogenous frequency without the need to study a prohibitive number of cells. At high doses of DNA damaging agents an induction above endogenous inversion frequency has been observed and at lower doses a reduction below endogenous inversion frequency has been observed. In the case of low LET X-radiation exposure an induction above endogenous frequency has again been observed at ultra-low doses. These results clearly do not conform to a linear no-threshold paradigm. The ability of a DNA damaging agent to cause an inversion in a cell will be dependent on chromatin structure and DNA repair protein activity. Adaptive response and bystander effects are likely to also be playing a role in the dose responses observed. The potential implications of these results on risk assessment will be discussed. Research funded by the Low Dose Radiation Research Program, Biological and Environmental Research (BER), U.S. Department of Energy, grant # DE-FG02-01ER63227.

MECHANISMS UNDERLYING IONIZING RADIATION-INDUCED ADAPTIVE AND BYSTANDER RESPONSES

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To understand low-dose effects and their potential impact on risk from ionizing radiation, we have investigated cellular, molecular and biochemical effects in quiescent human and rodent cell cultures exposed to low doses of γ-rays or low fluences of α-particles. Effects were also examined in cultures consisting of ^{3}H-thymidine labeled and unlabeled cells and in their progeny. The short range of the β-particles emitted by tritium confined the radiation dose to the labeled cells only.

Normal human cells were grown in a three-dimensional architecture. Pre-exposure to low dose/low dose rate (1-10 cGy, 0.2 cGy/h) γ-rays protected them from DNA damage induced by a subsequent acute challenge exposure to γ-rays. DNA repair, oxidative metabolism and induced delays in the transition from G_1 to S phase of the cell cycle regulate these protective effects. With relevance to risk assessment, a single chronic low dose exposure to γ-rays decreased the frequency of micronucleus formation (a surrogate form of DNA damage) and neoplastic transformation to a level below the spontaneous rate in human and rodent cells, respectively.

In contrast to the above studies, when cell cultures were exposed to very low fluences of α-particles, by which a small fraction of the nuclei were traversed by a particle track, stressful effects were transmitted from irradiated to adjoining non-irradiated bystander cells. Gap-junction intercellular communication, oxidative metabolism, oxygen tension, DNA repair and the p53 signaling pathway mediate the expression of these bystander effects.

When ^{3}H-thymidine labeled cells were co-cultured with unlabeled cells, the propagation of stressful or protective effects from irradiated to non-irradiated cells was dependent on the dose received by the irradiated cells and on the type of functional gap-junctions expressed in irradiated or bystander cells. The persistence of stressful effects in the progeny of isolated bystander cells and in their respective controls will be discussed.

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ADAPTIVE RESPONSE AND BYSTANDER EFFECTS IN HUMAN AND NON-HUMAN BIOTA

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Recent advances in our understanding of effects of radiation on living cells suggests that fundamentally different mechanisms are operating at low doses compared with high doses. Also, acute low doses appear to involve different response mechanisms compared with chronic low doses. Both genomic instability and so called "bystander effects" show many similarities with well known cellular responses to oxidative stress. These predominate following low dose exposures and are maximally expressed at doses as low as 5mGy. At the biological level this is not surprising. Chemical toxicity has been known for many years to show these patterns of dose response. Cell signaling and coordinated stress mechanisms appear to dominate acute low dose exposure to chemicals. Adaptation to chemical exposures is also well documented although mechanisms of adaptive responses are less clear. In the radiation field adaptive responses also become important when low doses are protracted or fractionated. Recent data from our group concerning bystander effects following multiple low dose exposures suggest that adaptive responses can be induced in cells which only receive signals from irradiated neighbours. We have determined using genetically distinct mice, with different radiosensitivities, that bystander effects occur in vivo and vary according to genetic background. We also have data showing delayed and bystander effects in fish and in prawns following in vitro irradiation of haematopoietic tissue. These data have implications for environmental radiation protection of human and non human species alike. Simple extrapolations from high to low dose exposure may need to be re evaluated. This presentation will discusses our knowledge about these low dose radiobiological effects in both human and non-human biota.

MULTICELLULAR RESPONSES TO IONIZING RADIATION

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Our global hypothesis is that multicellular responses directed via extracellular signaling affect the probability of adverse outcomes following radiation exposure. At low doses, in normal tissues, a major role of extracellular signaling is to inhibit carcinogenesis by eliminating abnormal cells and suppressing neoplastic behavior. In premalignant tissues, radiation can induce signaling that promotes neoplastic progression. At high doses, the tissue response to damaged cells may drive organ failure. We have shown that radiation exposure not only affects the composition of the microenvironment, but can also persistently alter cell phenotype, genome stability, and interactions between cells and with the microenvironment. These multicellular responses are often non-linear.

Understanding radiation effects in terms of coordinated multicellular responses may necessitate reevaluation of radiation dose and risk concepts. Paradigms that place DNA damage as the central event initiating cellular radiation responses, which in turn dictate tissue effects, lead to models that are hierachical and linear. Rather than considering an organism an assembly of cells, multicellularity should be considered as an asset of a system and be modeled as a combinatorial interactions and networks. Systems biology may be applied to understand how the behaviors of multiple cell types are coordinated in response to an external stimulus like radiation. By regarding the tissue, organ or organism as the primary responder, then extracellular signaling through the microenvironment can impose directives, act as a register for damage, record response, and coordinate the response of muliple cell types.

Funded by the Low Dose Radiation Program of the Office of Biological and Environmental Research, United States Department of Energy DE AC03 76SF00098 .

RADIATION RISK PREDICTION AND GENETICS: THE INFLUENCE OF THE P53 GENE *IN VIVO*

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Dose limits for human radiation exposure are based on the assumption that risk is proportional to total dose. However, there is concern about the appropriateness of those limits for people who may be genetically cancer prone. The p53 gene product functions in regulatory pathways for DNA repair, cell cycle checkpoints and apoptosis, processes considered critical in determining ionizing radiation risk. Mice heterozygous for p53 are highly prone to spontaneous cancers and p53 null mice are extremely cancer prone. In fetal mice exposed to high doses, reduced p53 gene function reduced the risk of teratogenesis, with the maximum protection in p53 null fetuses. The linear risk assumption predicts that higher total doses would increase risk, but that p53 deficiencies would also reduce teratogenesis in mice exposed to higher total doses. On the contrary, low doses prior to the high dose had a maximally protective effect in the p53 normal fetuses, a reduced protective effect in the p53 heterozygous fetuses and were detrimental in the p53 null fetuses. Both p53 normal and heterozygous adult mice exposed to high doses showed a dose proportional loss of life from both cancer and non-cancer endpoints, and p53 heterozygosity had no effect on the dose response for either process. Extrapolation of this high dose effect to low doses would predict less detrimental effect, and still no influence of p53 heterozygosity as total dose increased. However, a low dose alone reduced risk, as did a low dose prior to a high dose, but the protection was reduced in p53 heterozygotes. The results show that a p53 functional deficiency that results in spontaneous cancer proneness can influence radiation risk, but that, as for p53 normal mice, the dose response is not linear and the high dose response does not predict the low dose response.

SUPPRESSION OF DISEASE DEVELOPMENT AND TUMORIGENESIS BY LOW LEVEL RADIATION

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To elucidate the biological effects of low level radiation we have examined the effects of low dose rate gamma irradiation on disease model mice and mice treated with tumorigenic agents. In Type II diabetic C57BL/KsJ-*db/db* mice, the urine glucose level was improved in some of the mice irradiated at 0.70 mGy/hr but not in the non-irradiated control mice. Mortality was delayed and a healthy appearance was prolonged in the irradiated mice by about 20-30 weeks compared with the control mice; the last mouse in the non-irradiated control group died at the 121st week, and the one in the irradiated group, at the 147th week. In severe autoimmune MRL-*lpr/lpr* mice, the immunological status was kept better and the incidence of a number of symptoms, including lymphadenopathy, splenomegaly and proteinuria, was suppressed in the mice irradiated at 0.35 or 1.2 mGy/hr. Furthermore, prolonged life span was observed in the irradiated mice. The incidence of skin tumors in ICR mice injected with methylcholanthrene was suppressed from 94 % in control mice to 70 % in those continuously exposed to gamma rays at 1.2 mGy/hr. High frequency (90 %) induction of thymic lymphomas in C57BL/6 mice by 4 weekly repeats of whole-body X irradiation (1.8 Gy) was suppressed to 43 % by continuous whole-body irradiation at a dose rate of 1.2 mGy/hr. These results implied that the low dose rate irradiation enhanced some bio-protective functions.

HORMESIS IN AGING RESEARCH AND INTERVENTIONS

HORMETIC MODULATION OF AGING AND LONGEVITY BY MILD HEAT STRESS

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THE HORMETIC EFFECTS OF HYPERGRAVITY ON LONGEVITY AND AGING IN THE FRUIT FLY *DROSOPHILA MELANOGASTER*

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THE UNEXPECTED ANABOLIC PHENOTYPE AND EXTENDED LONGEVITY OF SKIN FIBROBLASTS AFTER CHRONIC GLUCOCORTICOID EXCESS

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EXERCISE ACTIVATION OF CELLULAR ANTIOXIDANT SIGNALING PATHWAY

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HORMESIS AND REGULAR EXERCISE IN AGING: POSSIBLE BENEFICIAL EFFECTS OF OXIDATIVE STRESS

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HORMETIC MODULATION OF AGING AND LONGEVITY BY MILD HEAT STRESS

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Heat shock (HS) response is a primordial intracellular defence mechanism against stressful conditions. A wide variety of stresses can induce the cellular HS response in terms of preferential transcription and translation of heat shock genes and protein chaperones, respectively. Experiments performed with nematodes and fruitflies have demonstrated that those animals who could tolerate and survive high temperatures generally had longer lifespans and were more resistant to other stresses. Single or multiple exposures to mild heat stress, which is sufficient to induce about 30% of the maximum HS response in terms of heat shock proteins synthesis, results in the slowing down of aging and prolongation of lifespan of nematodes and fruitflies. Our studies have shown that repeated mild heat stress (RMHS) has anti-aging effects on growth and various other cellular and biochemical characteristics of normal human skin fibroblasts undergoing aging in vitro. RMHS at 41°C for 1 hr twice a week increased the basal levels of various chaperones, reduced the accumulation of oxidatively and glycoxidatively damaged proteins, stimulated proteasomal activities for the degradation of abnormal proteins, improved cellular resistance to ethanol, hydrogenperoxide and UVB rays, enhanced the levels of various antioxidant enzymes, and increased the phosphorylation-mediated activities of various stress kinases. Mild HS-exposed human skin fibroblasts are also better protected against glucose, fructose and glyoxal-induced growth inhibition and apoptosis. Further studies are in progress to determine if a pre-exposure of telomerase-immortalised human bone marrow stem cells to mild HS can improve their differentiation ability into specific cell types. We are also testing the effects of mild HS in combination with other hormetic molecules such as curcumin on aging and longevity of human keratinocytes in culture.

THE HORMETIC EFFECTS OF HYPERGRAVITY ON LONGEVITY AND AGING IN THE FRUIT FLY *DROSOPHILA MELANOGASTER*

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Gravity is one of the few constant environmental parameters. As an effect, the impacts of changes in gravity levels have not been studied before the development of inhabited space missions, when it was observed that organisms subjected to lack of gravity exhibited muscle mass reduction and decalcification. Thereafter, the first studies on hypergravity (levels of gravity higher than terrestrial gravity level, 1g) showed that hypergravity increased metabolism. It could also lead to a decreased longevity. These findings made hypergravity an interesting tool to study mechanisms of aging. The team I have been working with on this topic had shown that a chronic exposure to hypergravity of adult *Drosophila melanogaster* decreased longevity (at levels higher than 4g), speeded up the rate of decline with age of locomotor activity, and affected slightly fertility and viability. I have shown that in contrast, on a learning task, young animals subjected to hypergravity were impaired but not older ones. I thus develop the hypothesis that hypergravity was a "normal" stress in physiological terms, to which organisms adapt. If this is true, an exposure to hypergravity should increase resistance to other stress: I showed that flies having lived in hypergravity were more resistant to heat. No effect of hypergravity was observed on resistance to starvation, desiccation and cold. The second implication of hypergravity being a stress is that a short exposure to hypergravity should have beneficial effects. I have shown that such an exposure increased longevity in male flies, long term resistance to heat in both sexes and more importantly, it is able to slow down the speed of aging measured with behavioral variables. The last part of the talk will focus on possible mechanisms that could explain the effects of hypergravity, such as the involvement of heat shock proteins and antioxidant defense mechanisms.

THE UNEXPECTED ANABOLIC PHENOTYPE AND EXTENDED LONGEVITY OF SKIN FIBROBLASTS AFTER CHRONIC GLUCOCORTICOID EXCESS

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Chronic exposure to elevated glucocorticoid (GC) concentrations, one of the primary hormonal responses to stress, induces detrimental effects in several tissues, while it has been suggested that it accelerates the ageing process. In the skin, glucocorticoids provoke intense alterations on various parameters of fibroblasts' physiology, cumulatively leading to skin atrophy and impaired wound healing. Accordingly, we have investigated whether chronic *in vivo* exposure to GC excess results in persisting defects in skin fibroblasts. To this end, we have studied primary skin fibroblast cultures obtained from patients suffering from endogenous Cushing's syndrome, characterized by increased, although not pharmacological, GC levels. Interestingly, Cushing's fibroblasts (CF), grown under standard culture conditions in the absence of a hypercortisolemic milieu, exhibit an anabolic phenotype, that is in contrast to the well known catabolic effect of GC. In particular, CF exhibited an increased proliferative capacity and a higher final cell culture density compared to normal fibroblasts (NF). Furthermore, no reduction of collagen synthesis was observed in CF cultures in comparison to NF ones. In addition, CF secreted comparatively lower levels of matrix metalloproteinases (MMP-1, MMP-2). They also exhibited an increased ability to contract gels of polymerized collagen. Finally, we have observed that CF exhibit a significant increase in their proliferative lifespan when cultured in vitro. In parallel, these cells secrete lower levels of transforming growth factor-β (TGF-β), which is implicated in stress-induced premature senescence. Furthermore, they also exhibit an intense stress reaction (near two-fold, compared to normal cells) in terms of heat-shock protein-70 (HSP70) induction by sodium arsenite. Collectively, these results support the hypothesis that stress response may have long-term beneficial consequences in cellular longevity, and tissue homeostasis in general.

EXERCISE ACTIVATION OF CELLULAR ANTIOXIDANT SIGNALING PATHWAY

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Contraction-induced production of reactive oxygen species (ROS) has been shown to cause oxidative stress to skeletal muscle. As an adaptive response, muscle antioxidant defense systems may be upregulated after heavy exercise. Nuclear factor (NF) κB is one of the major oxidative stress-sensitive signal transduction pathways in the mammalian tissues. Activation of NFκB signaling cascade has been shown to enhance the gene expression of several enzymes containing NFκB binding sites on their DNA, including the mitochondrial superoxide dismutase (MnSOD), inducible nitric oxide synthase (iNOS) and γ-glutamylcysteine synthatase. We investigated the effect of an acute bout of treadmill running on NFκB signaling and the time course of activation in rat skeletal muscle. NFκB binding intensity in muscle nuclear extracts reached maximal at 1-2 h post exercise, accompanied by an elevated P65 content, and returned to the resting level at 48 h. Cytosolic content of IκB, the inhibitory subunit of NFκB complex, was decreased, whereas phosphor-IκB content and IkB kinase (IKK) activity were increased immediately after exercise. The exercise-induced activation of NFκB signaling cascade was mimicked by treatment of lipopolysaccharride, but not *t*-butylhydroperoxide, and could be partially abolished by NFκB inhibitor pyrrolidine dithiocarbamate (PDTC) in the rat. These data suggest that exercise-induced ROS generation may serve as messenger molecules to activate adaptive responses using the NFκB signaling pathway. This cascade may explain the transcriptional activation of MnSOD by exercise reported in our previous studies, as well as the training-induced protection in muscle mitochondria against oxidative insult.

HORMESIS AND REGULAR EXERCISE IN AGING: POSSIBLE BENEFICIAL EFFECTS OF OXIDATIVE STRESS

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Reactive oxygen species (ROS) is generally thought to be harmful except under specific situations such as in killing bacteria by leukocytes and in cellular signal transduction. Aging and age-related diseases are associated with damage of proteins, lipids and nucleic acids due to ROS that are not replaced or repaired efficiently and therefore potentially detrimental to an organism. Adaptive response can, however, possibly attenuate accumulation of such damage due to increase in gene expression of proteins responsible for replacement and repair of the damaged molecules as well as reduction of ROS. We have studied possible attenuation of oxidative stress in aging rats by regular exercise. Regular swimming for 9 weeks augmented cognitive functions with concomitant decrease in protein oxidation as measured by carbonyl content in the brain of young and middle-aged rats. Proteasome activities were increased in the brain, heart and skeletal muscle. Regular treadmill running for 8 weeks of adult (18 months) and aged (28 months) rats resulted in reduced nuclear factor (NF)-kB binding to responsive element on DNA in the liver. This was accompanied by reduction of total ROS and increase in reduced glutathione. Age-associated increase in the oxidative modification (8-hyroxy-2'-deoxyguanosine) of nuclear DNA was attenuated in the skeletal muscle by the exercise. The repair activity for the lesion was increased by the regimen. These findings suggest that regular exercise can reduce oxidative stress in protein and DNA and/or upregulate the repair and turnover capacities of the modified macromolecules even at old ages in rats. While over training is obviously harmful due to excessive generation of ROS, moderate regular exercise may be protective against usual and accidental oxidative stress such as those in ischemia/reperfusion episode. Regular exercise is thus a form of hormesis that may retard aging and reduce the risk of age-related diseases.

TOXICOLOGY

EFFECT OF LOW-DOSE RADIATION ON MALE GERM CELL DEATH

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EXAMINATION OF THE HORMESIS RESPONSE IN THE MICROTOX BIOASSAY AS INDUCED BY BINARY MIXTURES OF CHEMICALS

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HORMESIS MECHANISMS IN HEPATOCARCINOGENESIS

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HORMETIC INDUCTION OF ANTIOXIDATIVE AND ANTIAPOPTOTIC PROTEINS FOR cGMP-MEDIATED ADAPTIVE TOLERANCE

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MULTIPLE EFFECTS OF LOW LEAD LEVELS IN SWISS MICE

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IS HORMESIS IN CELLULAR REPAIR MECHANISMS INDUCED BY WATER DISINFECTANT BYPRODUCTS HARMFUL OR BENEFICIAL?

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EFFECT OF LOW-DOSE RADIATION ON MALE GERM CELL DEATH

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Cytotoxic effect, including apoptotic and necrotic cell death, is a major cellular event of cells in vitro or in vivo in response to ionizing radiation. Hormetic and adaptive response of low-dose radiation (LDR) on hematopoietic and immune systems were observed, showing a stimulatory effects of cell growth and increased resistance of these cells to subsequent radiation-induced cytotoxicity. However, in term of apoptotic cell death, the effects of LDR were controversy: some studies found LDR-induced decrease in apoptosis, and some studies found LDR-induced an increase in apoptosis. These controversy of these results may be related with dose levels of LDR, dose rates of LDR and also, more important, with types of cells. Testes is one the most radiosensitive organs. The loss of male germ cells by radiation has recently been attributed to apoptosis, especially in low-dose range of radiation In the present study, therefore, the effects of LDR at dose up to 200 mGy on mouse spermatogenesis in term of apoptotic cell death and apoptosis-related proteins were examined different times after mice were exposed in whole body to LDR. In addition, effect of LDR pretreatment on subsequent high dose radiation (HDR)-induced cell death was also examined to explore the possibility of LDR-induced adaptive response. Results showed that within the dose range of 25 – 200 mGy LDR, 50 and 75 mGy significantly induced increase in apoptosis in both spermatogonia and spermocytes, which may be associated with the increased expression of p53 expression. Furthermore, 50 or 75 mGy LDR-pre-irradiation leaded to an adaptive response of seminiferous cells to subsequent at 4 – 12 hr HDR-induced apoptotic cell death. However, if the challenging dose was up to 3 Gy, no adaptive response was noted. These results showed that LDR induces an increased apoptotic cell death in male germ cells, but significantly induced an adaptive response to subsequent LDR-induced cell death.

EXAMINATION OF THE HORMESIS RESPONSE IN THE MICROTOX BIOASSAY AS INDUCED BY BINARY MIXTURES OF CHEMICALS

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The Microtox assay using the bioluminescent bacteria *Vibrio fischeri* was used to assess the presence of a nonmonotonic response to binary mixtures of chemicals. The assays were conducted in M500 Microtox Analyzers operated at 15^{o} and 23^{o} C. A hormetic response was characterized by increased light output indicating stimulation of the bioluminescent bacteria. Binary mixtures were produced for seven common environmental contaminants: chromium, lead, nickel, phenol, sodium lauryl sulfate, diethylene glycol, and ethylene glycol. Pairs of these chemicals were tested with one another in binary combinations, resulting in a total of twenty-one binary mixtures. For each binary mixture, twenty-four concentrations were analyzed. A nonmonotonic, hormetic, response was observed for all twenty-one binary mixtures at both 15^{o} and 23^{o} C. The ethylene glycol-diethylene glycol mixture induced the highest level of light simulation (75%). In general metal-metal mixtures induced the highest hormetic response, followed by metal-organic mixtures, then organic-organic mixtures. Hormesis levels of mixtures were more-than-additive for the majority of the combinations tested in this study. Increasing temperature was found to significantly depress hormesis levels for binary mixtures. Dose response curves containing hormesis regions were successfully generated for chromium, lead, ethylene glycol, and diethylene glycol.

HORMESIS MECHANISMS IN HEPATOCARCINOGENESIS

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Liver tumors are the most common neoplasia detected in chronic rodent carcinogenicity studies. Chemically induced hepatocarcinogenesis involves a multi-step process that incorporates both mutation and cell proliferation events. The cellular and molecular mechanisms involved in this multistage process have been extensively studied in rodents. The initial stage involves genomic DNA damage that if not repaired may result in the formation of a mutated, intitiated cell (initiation stage). This initiated cell requires subsequent clonal expansive growth to form a preneoplastic lesion (tumor promotion stage). For preneoplastic cells to progress to neoplasia, additional DNA damaging events need to occur (progression stage). Multiple investigations have clearly defined many of the mechanisms involved in liver neoplasm induction by chemicals. Initiated cells may be produced through either mis-repaired DNA damage following chemical carcinogen adduct formation or through spontaneous DNA mutation (i.e. following oxidative insult). The initiation process is dose dependent with demonstrable threshold mechanisms. The promotion stage involves the selective increase in preneoplastic cell population through either increased cell proliferation and/or decreased cell apoptosis, both of which involve modulation of gene expression by the chemical agent. Multiple biological processes including, changes in methylation status, receptor mediated events, blockage of cell to cell communication, and increased oxidative stress have been associated with the selective preneoplastic cell growth. As with the initiation stage, tumor promotion involves a dose dependant process. Thus it is clear that the cellular and molecular mechanisms involved in multi-step hepatocarcinogenesis are dose dependent and exhibit thresholds. The cellular and molecular processes involved in hepatic carcinogenesis are frequently dependent on the overwhelming of normal cellular functions (i.e. DNA repair enzymes, antioxidant defense system). These processes are inducible thus it is not surprising that a protective effect on hepatocarcinogenesis (Hormesis) is observed in studies employing low dose exposure. The presence of dose response, threshold, and low dose protective effects of chemicals on liver neoplasia development is supported in several chronic carcinogen bioassays and hepatic carcinogenesis models.

HORMETIC INDUCTION OF ANTI-OXIDATIVE AND ANTI-APOPTOTIC PROTEINS FOR MEDIATING cGMP-MEDIATED ADAPTIVE TOLERANCE

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Preconditioned organs are more resistant than normal ones against subsequent severe oxidative stress in both basic and clinical studies *in vivo*. We recently developed a human cell model for investigating molecular mechanism underlying adaptive tolerance or hormesis produced by preconditioning evoked by a brief non-lethal serum deprivation (Andoh, Lee and Chiueh, 2000). This brief preconditioning procedure activates the $^{\bullet}$NO/cGMP/PKG signaling pathway in human SH-SY5Y cells increases thioredoxin-1 (Trx1) a redox protein with anti-oxidation, anti-apoptosis, and anti-inflammation while retards $p66^{shc}$ a longevity impeding adaptor protein. Based on the fact that Trx anti-sense pretreatment blocked this preconditioning-induced survival we thus hypothesize that induction of survival genes and related proteins such as Trx1 may mediate cyto-protection evoked by adaptive tolerance or hormesis (Andoh, Chock, and Chiueh, 2002). Early studies indicate that preconditioning increases the expression of mitochondrial K(ATPase) channel for maintaining mitochondria functioning. Moreover, our recent studies infer that a cGMP-dependent up-regulation of Trx1 phospho-activates CREB in the nucleus resulting in a delay increase of the transcription of anti-oxidative protein MnSOD and anti-apoptotic Bcl-2 in the mitochondria lasting for more than 24 hours. Furthermore, preconditioned cells develop drug resistance against oxidative stress and apoptosis caused by 1-methy-4-phenylpyridinium ion (MPP^{+}) the toxic metabolite of a parkinsonism producing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Andoh, Chiueh, and Chock, 2003). These results are consistent with early *in vivo* findings that preconditioned mice are less vulnerable to MPTP-induced neurotoxicity and ischemic brain injury. It is concluded that in addition to K(ATPase) expression the $^{\bullet}$NO/cGMP/PKG-dependent induction of survival proteins such as cytoplasmic redox protein Trx1 and mitochondrial cytoprotective proteins MnSOD and Bcl-2 may play a pivotal role in adaptive cardio- and neuroprotection during early phases of hormesis.

MULTIPLE EFFECTS OF LOW LEAD LEVELS IN SWISS MICE

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This paper will present the results of various experiments that were designed to assess the effects of dietary lead on male and female Swiss mice at concentrations that would result in blood levels that are above and below that normally present (~2 µg/dL blood) in humans. The blood lead levels assessed ranged from 0.6 to equal to or greater than 15 µg/dL depending on the specific experiments. Separate experimental studies assessed the effects on such levels of lead on red blood cell parameters (i.e. hemoglobin, hematocrit, and red blood cell count) and multiple developmental measures indicative of time to puberty in female. In general, the findings revealed striking differences in response in mice depending on the concentration of lead in the blood in comparison with normal background lead concentrations. For example, in female mice, at concentrations greater than normal background time to puberty of 33-35 days in controls was notably delayed while at lower blood lead concentrations the time to puberty was accelerated such that time to puberty was reduced to 21 days. Similar enhancements in red blood cell parameters were observed at blood lead level below the 2.0 µg/dL concentration while in female and male mice they were inhibited at the higher concentrations. These findings are notable for their capacity for replication and the observations that lead has biologically significant effects below current background concentrations. Whether such findings can be generalized to other biological models remains to be investigated.

IS HORMESIS IN CELLULAR REPAIR MECHANISMS INDUCED BY WATER DISINFECTANT BYPRODUCTS HARMFUL OR BENEFICIAL?

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Disinfection byproducts (DBPs) are produced during chlorination of drinking water and are detected at concentrations up to 200 μg/L (ppb). Experimentally, several DBPs are mutagenic or carcinogenic and the mechanisms of these effects are rarely defined. Previous mechanistic studies using sub-lethal doses of DBPs indicated elevation in oxidative stress (OS) markers; DNA (8-OHdG) and protein oxidation (carbonylated proteins). Currently, we evaluated the effect of low range of doses of the DBP dibromoacetonitrile (DBAN) on cellular repair mechanisms. Base excision repair (BER) pathway is involved in the repair of oxidative DNA damage. Oxidized proteins are predominantly degraded by the ATP independent proteasomal proteins. In a dose response and time course study we measured markers of OS and BER activity in mouse embryonic fibroblasts exposed to DBAN. The results indicated time dependent up-regulation of BER activities at 1.0-5.0 μM DBAN with 30-60 min exposure times. At 10-20 μM, however, DNA repair activities were down-regulated and were inhibited at 2 hr following treatment. We also investigated the effect of 0-400 ppb DBAN on the proteolysis processes in neuroglial cells. The results indicated that DBAN induced a concentration dependent significant increase (125%) followed by a decrease (65-58% of control) in proteasomal activities. 2- DGE indicated distinctive differences in the cytosolic protein profiles of control and treated cells. MALDI-TOF MS and subsequent database search revealed a concentration dependant up-regulation of several proteins including the C5 component of proteasomal proteins. In conclusion, Up-regulation of BER activities and proteasomal protein levels and activities indicate that the cellular repair mechanisms are provoked to overcome the accumulation of DBAN-damaged macromolecules. Hormetic effects of DBPs on biochemical repair processes appear to be quite evident and may be involved in biological amplification of adaptive responses leading to improved cellular functions. However, theses adaptive processes are highly demanding on the cellular biochemical environment and that demand could burden the cell leading to the initiation of detrimental signals.

RISK ASSESSMENT

LOW-DOSE DOSE-RESPONSE NONLINEARITIES AS ADAPTIVE INVENTORY CONTROL RISK MANAGEMENT STRATEGIES FOR TISSUES

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THE USE OF AN EVIDENCE-BASED APPROACH FOR EVALUATING THE CONCEPT OF HORMESIS

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THE COLLISION OF HORMESIS WITH ENVIRONMENTAL CHEMICAL AND NATURAL CHEMICAL EXPOSURES: OPPORTUNITIES AND CHALLENGES FOR INTEGRATING HORMESIS INTO AN IMPROVED UNDERSTANDING OF CHEMICAL HEALTH RISKS

James S. Bus, Dow Chemical Company, Midland, MI

THE HORMETIC HYPOTHESIS AND ITS RISKS ASSESSMENT IMPLICATIONS

Michael Dourson, TERA, Cincinnati, OH

IMPLICATIONS OF HOMEOSTASIS FOR DOSE-RESPONSE AT DIFFERENT LEVELS OF BIOLOGICAL ORGANIZATION

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LOW-DOSE DOSE-RESPONSE NONLINEARITIES AS ADAPTIVE INVENTORY CONTROL RISK MANAGEMENT STRATEGIES FOR TISSUES

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Why do low-level exposures to environmental toxins often elicit over-compensating responses that reduce risk to the organism? Conversely, if these responses improve health, why wait for an environmental challenge to trigger them? This paper presents a mathematical model that answers both questions using the principle that evolution favors tissues that optimally hedge their bets against uncertain environmental challenges.

We consider a tissue composed of differentiated cells performing essential functions (e.g., lung tissue, bone marrow, etc.). The tissue seeks to maintain adequate supplies of these cells, but many of them may occasionally be killed relatively quickly by cytotoxic challenges. The tissue can "order replacements" (e.g., via cytokine network signaling) from a deeper compartment of proliferative stem cells, but there is a delivery lag because these cells must undergo maturation, amplification via successive divisions, and terminal differentiation before they can replace the killed functional cells. Therefore, a "rational" tissue maintains an *inventory* of relatively mature cells (e.g., the bone marrow reserve for blood cells) for quick release when needed. The reserve is ultimately replenished by stimulating proliferation in the stem cell compartment. Normally, stem cells have a very low risk of unrepaired carcinogenic (or other) damage, due to extensive checking and repair. But when production is rushed to meet extreme demands, error rates increase. We use a mathematical inventory model to show that "rational" decision rules for managing the inventory of mature cells to maintain tissue function across a wide range of unpredictable cytotoxic challenges imply that small increases in average levels of fluctuating cytotoxic challenges can increase average inventory levels and reduce the average error rate in stem cell production. Thus, hormesis emerges as a result of rational cell-inventory risk management by tissues.

THE ROLE OF EVIDENCE BASED TOXICOLOGY IN RISK EVALUATION

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Hormesis challenges the typical assumption in toxicology that the dose-response relationship exhibits a threshold. Hormesis advances the idea that at least for some chemicals low doses are not simply inactive (or adaptive) but actually cause inverse reactions. Incorporating hormesis into a "risk assessment" structure raises the more general question of deficiencies in some current practices of causation and risk evaluation. Some relations between agents and events are identified risks, meaning unwanted events known to occur at some frequency. However, other relations that are only possibilities – not known to occur (and may never be realized) – also are sometimes called risks and are even expressed quantitatively. For clarity in "risk" communication, we label as 'nomological possibilities' (not as risks) all predictions of harm that are known *not* to be physically or logically impossible. Some of these nomological possibilities are known to be causal. We term them 'epistemic.' Epistemic possibilities are risks. The remaining nomological possibilities are called 'uncertainties.' Distinguishing risks (epistemic relationships) from among all nomological possibilities requires knowledge of causation. Causality becomes knowable when scientific experiments demonstrate, in a strong, consistent (repeatable), specific, dose-dependent, coherent, temporal, and predictive manner, that a change in a stimulus determines an asymmetric, directional change in the effect. In contrast, most risk assessment methodologies assume the truth of causality and then, proceed to calculate probabilities for occurrence of the hazard. For claims of hormesis (or any dose-response relationship) to stand as Evidence-based conclusions rather than Authority-based opinions (Guzelian and Guzelian. 2004. Authority-based explanation. Science. 303:1468-1469). such relationships must be held to the same standards of scientific evidence. Evidence-based toxicology derived from Evidence-based medicine (EBM) makes a conscientious, explicit, and judicious use of current best evidence assembled and appraised by a structured, 'transparent' protocol in a deliberate, objective, unbiased, and systematic manner to decided about causal relations of harm. When, as may be anticipated for study of all low dose relationships, there are shortcomings in the evidence reliance upon Authority-based opinions, rather then Evidence-based conclusions should be acknowledged explicitly. Evidence-based toxicology can be used to solidify the importance of hormesis to understanding the risks of exposure to environmental chemicals.

THE COLLISION OF HORMESIS WITH ENVIRONMENTAL CHEMICAL AND NATURAL CHEMICAL EXPOSURES: OPPORTUNITIES AND CHALLENGES FOR INTEGRATING HORMESIS INTO AN IMPROVED UNDERSTANDING OF CHEMICAL HEALTH RISKS

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In the absence of appropriate human data, the human health risks of both new and existing synthetic chemicals are commonly extrapolated from responses in animal toxicity studies using several risk evaluation models. For non-cancer endpoints, acceptable human exposure levels are estimated by multiplying a series of potential Uncertainty Factors (UFs) of either 3- or 10X, generally resulting in application of cumulative UFs of 100-3000X to the No-Observed-Effect-Level (NOEL) doses in animals. For genotoxic carcinogens or carcinogens of unknown modes of action, human risk is estimated with linear, no-threshold models of animal tumor responses. However, when these risk estimation approaches, and particularly the no-threshold cancer models, are applied to the many natural chemicals found in everyday diets (e.g., acrylamide in potatoes/breads), they result in estimations of risk suggesting basic high quality fruits, vegetables and other foods represent the greatest health risks to the public. Since such estimates are intuitively and scientifically inconsistent with the intrinsic health benefits of food, it suggests that existing models for estimating potential human health risks from animal toxicity dose-responses are likely extremely conservative, and particularly so for human exposures that are significantly below the operative range of the toxicity dose response curves. These observations, and the growing body of hormetic responses noted near conventional chemical NOEL values, strongly suggest that gaining a better understanding of the biology underpinning responses at the low end of the dose response curve will inevitably lead to development of toxicology and risk assessment methods capable of distinguishing toxins of true human health impact from the many thousands of exposures to natural chemicals experienced every day. These future methods will be facilitated not only by improved toxicology study designs emphasizing more environmentally relevant exposures, but also by new research tools such as toxicogenomics that can reveal the biology of low-dose responses.

THE HORMETIC HYPOTHESIS AND ITS RISK ASSESSMENT IMPLICATIONS

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Whether or not hormesis is better characterized as a hypothesis or theory, a study of its potential implications for risk assessment is important. Not only is the practice of risk assessment moving in the direction of better understanding modes of action, compensatory and adaptive effects, toxicokinetics and toxicodynamics---concepts clearly related to hormesis---but regulatory scientists are now more willing to contemplate the use of data over defaults positions. Examples are discussed for both threshold and non-threshold toxicity where hormesis might help or hinder the risk assessment process.

IMPLICATIONS OF HOMEOSTASIS FOR DOSE-RESPONSE AT DIFFERENT LEVELS OF BIOLOGICAL ORGANIZATION

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The body can be thought of as a machine with sensors and feedback loops operating across multiple levels of organization to maintain homeostasis. For example, the intracellular signal transduction pathways MAPK and NFκB-IκB respond to external stimuli by generating a suite of dynamic behaviors including oscillations, bistability, history-dependence, and switch-like dose-response. These dynamic capabilities allow the cell to adjust its biochemical state in response to stress.

This view of the cell as, among other things, an assembly of regulatory networks maintaining the internal environment suggests a paradigm for characterizing the dose-response effects of xenobiotics. Any xenobiotic that enters a cell will interact either specifically or non-specifically with the molecules that comprise regulatory networks. Initial interactions will perturb but not overwhelm the network. Progressively higher concentrations of xenobiotic will eventually overwhelm network regulatory modules, leading to toxicity.

This paradigm also challenges the argument about expected linearity of dose-response curves when an exogenous stressor adds to an ongoing endogenous process. If the endogenous process is part of normal biology, then it is a component of the normal homeostatic environment. The low-dose-linear argument would only be expected to apply when homeostasis is not operative, as has been suggested for some phases of development and perhaps old age.

A limited amount of information is available about how regulatory modules in individual cells influence dose-responses at higher levels of biological organization. Some of the data suggest coordinated switch-like changes across large numbers of cells, but generalizations are difficult at this time. However this question may be answered, I think it is likely that the phenomenon of hormesis will come to be seen as an aspect of the homeostatic capabilities that insulate us from environmental stress.

POSTER SESSION

COMPLEX LOW-DOSE DOSE-RESPONSE NONLINEARITIES AND ABRUPT TRANSITIONS: INSIGHTS FROM CADMIUM LUNG CARCINOGENESIS

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DYNAMICS OF HORMESIS AND OF INVERSE HORMESIS FOR CYTOTOXICITY-MEDIATED CARCINOGENESIS

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CIPROFLOXACIN-INDUCED CYTOTOXICITY IN PRIMARY CULTURES OF RAT ASTROCYTES AND PROTECTION BY VITAMIN E

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MONITORING STORMWATER TOXICITY: HORMESIS IN THE MICROTOX BIOASSAY AS AN INDICATOR OF LOW DOSE EFFECT

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EFFECTS OF LOW-LEVEL EXPOSURES TO X-RAYS ON THE ANTI-TUMOR FUNCTIONS OF MURINE NK CELLS

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THE HEALTH EFFECTS OF DIFFERENT RADIATIONS

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EFFECTS OF DILUTED LOW MOLECULAR MEDIATORS ON ACTIVITY AND PATTERNS OF FUNGAL PHENOL-DEGRADING ENZYMES
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UNDERSTANDING THE HORMETIC EFFECT OF AZADIRACHTIN IN AN INSECT SYSTEM, IMPLICATIONS IN PEST MANAGEMENT
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POSSIBLE MOLECULAR UNDERPINNINGS OF HORMESIS-INDUCED LONGEVITY
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RADON TREATMENT CONTROVERSY
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ADAPTIVE RESPONSE IN pKZ1 MOUSE PROSTATE AFTER EXPOSURE TO VERY LOW DOSES OF X-RADIATION
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THE pKZ1 INVERSION ASSAY IS A SENSITIVE ASSAY FOR LOW-DOSE X-RADIATION STUDIES
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COMPLEX LOW-DOSE DOSE-RESPONSE NONLINEARITIES AND ABRUPT TRANSITIONS: INSIGHTS FROM CADMIUM LUNG CARCINOGENESIS

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Cadmium is an extraordinary carcinogen in several respects. Relatively slight (e.g., 2-fold to 10-fold) increases in dose concentration actually *reverse the direction* of several of its key biological effects (e.g., from tumor-inducer to tumor-suppressor) *in vivo* and *in vitro*, while other effects (e.g., on DNA methylation) have opposite directions for short-run and longer-run exposures. Cd exerts important effects (e.g., inhibiting repair of oxidative DNA damage in lung cells) even at very low (non-cytotoxic) levels. To explain and describe the complex set of nonlinear dose-response relations observed for Cd in recent experiments, we expand the conventional MVK two-stage clonal expansion model of carcinogenesis in two ways: (1) Add an explicit model of DNA repair dynamics (allowing normal stem cells to make a transition to a transient "pre-initiated" state from which they may be repaired until mitosis locks in any unrepaired damage, completing the transition to the "initiated", i.e., pre-malignant, state); and (2) Model the effects of exposure on tumor progression (e.g., on selection and apoptosis rates of more *vs.* less tumorigenic cells, which requires modeling the malignant cell population in more detail than in the usual MVK model.) Mathematical analysis of this expanded model of carcinogenesis shows that U-shaped dose-response relations can arise not only by dose-dependent killing of initiated cells, as is now well understood and experimentally verified, but also by dose-dependent interference with the DNA repair and mitotic rates of normal stem cells (not yet initiated) and/or with the rates of selection and apoptosis of malignant cells. In addition, the expanded model breaks new ground by showing how the more complicated dose-response relations (e.g., inverse u-shaped, or "n-shaped" relations and direction-reversals with increasing dose) recently observed in experiments with Cd carcinogenesis can arise naturally from the interaction of simple effects on the repair and progression processes preceding and following the traditional MVK process.

DYNAMICS OF HORMESIS AND OF INVERSE HORMESIS FOR CYTOTOXICITY-MEDIATED CARCINOGENESIS

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We present an MVK-type two-stage stochastic model of carcinogenesis in which proliferation of normal stem cells is realistically regulated by feedback from more mature and differentiated cell populations to help maintain or restore homeostasis following cytotoxic challenges. This model describes (by a relatively simple system of equations) key features of biologically important feedback loops revealed by detailed experimental investigations of normal and perturbed hematopoietic stem cell proliferation. Its main components are: (a) A population of fully differentiated cells that gradually die and must be replaced; they can also be killed more quickly by cytotoxic exposures. (b) A "reservoir" of relatively mature cells that are released to help maintain or restore equilibrium cell population levels in compartment (a) following cytotoxic exposures. In the absence of cytotoxic damage, relatively mature cells pass through the reservoir to replace dying cells. (c) A proliferative stem cell compartment where proliferation and differentiation (leading to entry into the reservoir) take place. Feedback signals from the mature and differentiated cell populations, as well as from the stem cell population itself, affect the rates at which the stem cells proliferate and differentiate to produce replacements for dead cells, but the rates remain between maximum and minimum allowed rates (reflecting the constraints on physiologically realistic production rates observed in experiments). We show that dynamic systems with these features can reproduce/explain previously published experimentally observed patterns of compensating proliferation following cytotoxic stress, including over-compensation. Hormetic (U-shaped) cancer dose-response functions can arise due solely to normal stem cell kinetics following cytotoxic exposures that kill the fully differentiated cells (but not the stem cells directly involved in carcinogenesis). The kinetics of normal stem cells in response to cytotoxic exposures can also explain the *inverse-U shaped* ("n-shaped") relation between exposure concentrations and cancer risk observed in experimental data for some carcinogens, such as isoprene.

CIPROFLOXACIN-INDUCED CYTOTOXICITY IN PRIMARY CULTURES OF RAT ASTROCYTES AND PROTECTION BY VITAMIN E

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The aim of this study was to investigate the possible cytotoxic and oxidative stress inducing effects of ciprofloxacin (CPFX) on primary cultures of rat astrocytes. The cultured cells were incubated with various concentrations of CPFX (0.5-3000 mg/l), and cytotoxicity was determined by neutral red (NR) and MTT assays. Survival profile of cells was biphasic in NR assay: While CPFX did not caused any alteration at any concentration for 7 h, ≤50 mg/l concentrations induced significant cell proliferation in incubation periods of 24, 48, 72, and 96 h. However, cell proliferation gradually decreased at higher concentrations, and 200 and 300 mg/l of CPFX exposure was found to be significantly ($p < 0.05$) cytotoxic in all time periods. With MTT assay, no alteration was noted for incubation period of 7 h, as observed with NR assay. But, cell survival decreased with ≥50 mg/l CPFX exposure in all other time periods. Cell proliferation was only seen in 24 h of incubation with 0.5 and 5 mg/l CPFX. Vitamin E pretreatment of cell cultures were found to be providing complete protection against cytotoxicity of 300 mg/l CPFX when measured with both NR and MTT assays. The SOD pretreatment was partially protective with NR assay, but no protection was noted when measured with MTT. A significant enhancement of lipid peroxidation was observed with the cytotoxic concentration of the drug, but total glutathione, oxidised glutathione, and catalase content of cells did not change. The data obtained in this study suggest that, in accordance with our previous results with fibroblast cells, CPFX-induced cytotoxicity is related to oxidative stress. And the biphasic effect of CPFX possibly resulted from the complex dose-dependent relationships between reactive oxygen species, cell proliferation, and cell viability.

MONITORING STORMWATER TOXICITY: HORMESIS IN THE MICROTOX BIOASSAY AS AN INDICATOR OF LOW DOSE EFFECT

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Monitoring stormwater presents a major challenge to environmental management. The primary challenge is that during a single event concentrations of potentially toxic contaminants may vary widely over very short time scales. Further, the effects of high concentrations of exposure for short time periods are not well documented. As part of a comprehensive research effort to identify test systems appropriate to stormwater toxicity assessment and develop protocols for sampling and analysis of stormwater events for toxicity a battery of toxicity tests were used to assess stormwater toxicity at multiple sites. The test battery included the Microtox bioassay and test systems incorporating *Ceriodaphnia dubia*, *Hyalella azteca*, and the fathead minnow. A consistent result of testing was the observation of stimulation in the Microtox bioassay when no response was noted in other test systems. Quality assurance and quality control reviews and laboratory testing determined that the observed stimulation was due to organism response, not an artifact of test procedures. Laboratory testing revealed a hormetic response in the Microtox bioassay for common environmental contaminants. A dose response curve was fit to this response indicating that the Microtox bioassay can be an effective tool to determine toxic response at low concentrations of environmental contaminants. The Microtox bioassay was also shown to be an effective test system for stormwater because toxicity response can be quickly measured and tests are responsive to short exposure times.

EFFECTS OF LOW-LEVEL EXPOSURES TO X-RAYS ON THE ANTI-TUMOR FUNCTIONS OF MURINE NK CELLS

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As we have been led to believe the most important late effect of ionizing radiation is an increased incidence of cancer in the exposed population. On the other hand, experimental evidence from the recent years indicates that low-level irradiations with X or gamma rays may inhibit the development of both primary and secondary tumors. In fact, the results of our previous studies demonstrated that whole body irradiation of mice with 0.1 or 0.2 Gy of X-rays led to a significant inhibition of the development of artificial tumor colonies in the lungs. In the present investigation, the NK cells were prepared from spleens of BALB/c mice exposed to 0.1, 0.2, or 1.0 Gy X-rays. The anti-mouse Pan-NK and anti-Fas ligand (FasL) antibodies were used to label NK cells. The anti-asialo GM_1 antibody was injected i.p. to block the NK cell-mediated activity *in vivo*. Cytotoxic activity of NK cells was estimated *in vitro* using the classical ^{51}Cr-release assay. Production of IFN-γ was estimated with use of the ELISA test. Apoptosis and necrosis of NK cells were examined using annexinV and PI, respectively. We showed that the cytotoxic activity of NK cells collected from the irradiated mice was significantly stimulated compared to the cells obtained from the sham-exposed mice. This effect was totally abrogated by injection of the anti-asialo GM_1 antibody. Moreover, NK cells obtained from the irradiated mice exhibited reduced surface expression of FasL. Exposure of mice to 0.1 or 0.2 Gy of X-rays did not affect the rate of apoptosis and necrosis in the collected NK cells, whereas irradiation with 1.0 Gy increased the number of apoptotic and necrotic NK lymphocytes. Collectively, the obtained results suggest that the inhibitory effect of the low-level irradiations with X-rays on the development of pulmonary tumor nodules may be directly associated with stimulation by such exposures of anti-neoplastic functions of NK cells.

EFFECTS OF LOW-LEVEL EXPOSURES TO X-RAYS ON ANTI-TUMOR FUNCTIONS OF MURINE PERITONEAL MACROPHAGES

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A number of epidemiological and experimental data indicate that exposures to low doses of ionizing radiation may inhibit tumor growth by triggering the activity of natural anti-tumor immune mechanisms. Previously, we showed that whole-body exposure (WBE) of mice to a single irradiation with 0.1 or 0.2 Gy but not 1.0 Gy X-rays resulted in a significant reduction of the development of pulmonary tumor metastases induced by i.p. injection of syngeneic L1 sarcoma cells. In the present experiments, peritoneal macrophages were collected from BALB/c mice exposed to 0.1, 0.2, or 1.0 Gy X-rays. Cytotoxic activity of these cells was estimated in the *in vitro* assays using the [^{3}H]thymidine-labeled L1 and P815 neoplastic cells as targets. Colorimetric assay with the Griess reagent and the NBT-reduction assay were used for the detection of nitric oxide (NO) and superoxide anions' synthesis in the collected macrophages, respectively. Finally, production of TNF-α by these cells was examined using the ELISA assay. The results indicate that all the tested parameters were significantly up-regulated in macrophages obtained from mice exposed to 0.1 or 0.2 Gy X-rays compared with the cells obtained from non-irradiated and 1.0 Gy-exposed mice. Pretreatment of mice with carrageenan inhibited the above functions of the macrophages (cytotoxicity and NO production) and led to a significant rise in the number of pulmonary tumor colonies in all the tested groups of the animals. As indicated by the results of the annexinV-based assay WBE of mice to 0.1, 0.2, or 1.0 Gy X-rays did not affect the rate of apoptosis in the collected macrophages. The obtained results suggest that the inhibitory effect of low doses of X-rays on the induction of pulmonary tumor nodules may be causatively related to stimulation by such exposures of the macrophage-mediated natural anti-tumor defense reactions.

THE HEALTH EFFECTS OF DIFFERENT RADIATIONS

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The radiation from different sources might have dramatic different biological effects to humanity. There were two clinical studies of radiation health effects with human beings ever done: one was with the radiation from the atomic bomb explosions in Hiroshima and Nagasaki, Japan, the other one was the radiation from the Co-60 contaminated apartments in Taipei City and many counties in Taiwan. The radiation suddenly exposed to the Japanese in one minute is nomenclature here as acute radiation, the radiation exposed in low-dose-rate to the residents living in the contaminated apartments in Taiwan for 21 years nomenclature as chronic radiation. The dose rate over 1 mSv/hr were ever detected in many key positions in the contaminated apartments, the chronic radiation could thus tentatively defined as radiation in dose rate < 1 mSv/hr. When the two atomic bombs exploded in the high air in August 1945, one half of the Japanese of about eighty thousands were killed by the blast and heat close to ground zero in the 2.5 kilometer radius of the explosion, the other half far from the ground zero suffering the harmful health effects of acute radiation, with dose no matter how small received. Many radiation scientists in the world asserted that the doses < 200 mSv exposed to the Japanese survivors might not be harmful, even beneficial to them. But the residents living in the Co-60 Contaminated apartments in Taiwan received the average doses of chronic radiation much higher than the Japanese Survivors, had been not harmed, but on the contrary greatly benefited. The average dose received by all residents in 1983 (All the Co-60 contaminated apartments were constructed in 1982-84, and the half-life of Co-60 is 5.3 year) was roughly estimated to be 50 mSv, and accumulated dose in 21 years was about 0.4 Sv, high up to 6 Sv; yet they did not have excess cancer deaths according to LNT model, and their spontaneous or natural cancer deaths were reduced to only 2-3% of the general population due to the beneficial health effects of chronic radiation, and the hereditary defects of their offspring were also reduced only 5-7 % of the population. Therefore the health effects of radiation from the atomic bomb and the Co-60 contamination incident in Taiwan were completely different to each other. Both the atomic bomb explosion and the Co-60 contamination incident would seldom occur, but they had distinctly revealed the different biological effects of radiation to humanity, and the chronic radiation from the Co-60 contaminated houses is quite similar to the radiation received by the workers and public in the peaceful of the nuclear energy and medical application of radiation. The radiation from a nuclear accident could harm limited number of people, but might benefit tremendous amount of general public. So that the public should not fear the radiation and the traditional radiation protection policy and practices used in past 50 years should be earnestly revised based on the experience of chronic radiation observed in Taiwan that has no harm but only benefit to humanity.

Key works: Co-60 contaminated apartments, Chronic Radiation, Low-dose -rate, Beneficial Health Effects

EFFECTS OF DILUTED LOW MOLECULAR MEDIATORS ON ACTIVITY AND PATTERNS OF FUNGAL PHENOL-DEGRADING ENZYMES

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Many substances with small particles, so call mediators, are effective in degradation of lignin-cellulose complex during the natural decay of phenol-rich wood wastes. Some of them, as formaldehyde or oxalic acid, can be produce as the endogenous products by the bacteria or fungi and are important for acceleration of wood utilization for many microbes as a source of carbon. Other accelerated substances have the exogenic, chemical nature and their presence in the fungal environment can be regulated by man. They create the group of mediators, important for industrial wood degradation. In presented paper the effect of high diluted exogenous mediators on activity and isoenzymatic electrophoretic patterns of laccase or peroxidase (HRP) during the cultivation of some species of white-rot fungi (Basidiomycetes) with phenolic substances was studied. Dilutions of formaldehyde, a very important effector of many demethylated-dependent reactions, were tested in cultures of *Trametes versicolor* and *Pleurotus sajor-caju* cultivated on ferulic acid (4-hydroxy-3-methoxycynnamic acid). Very low doses of lignin-degradation mediators, ABTS and HBT, were useful for modifications of laccase and peroxidase activities in the culture of *Trametes versicolor*. and *Cerrena unicolor* growing on guajacol-rich background. All experiments showed that high diluted effectors have the bimodal effect on extracellular laccase and peroxidase in all tested white rot fungal cultures. In each cultures the enzyme activities changed in sinusoidal manner according to step of effector's dilution. The optimal dilutions of mediators activate the broad spectrum of laccase or peroxidase isoenzymes.

UNDERSTANDING THE HORMETIC EFFECT OF AZADIRACHTIN IN AN INSECT SYSTEM, IMPLICATIONS IN PEST MANAGEMENT

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Azadirachtin incorporated in wheat flour, favored survival, growth and development of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae) at doses of less than 1ppm. The present results conform to the β-curve, which has a single stimulatory peak at concentrations immediately below those that are inhibitory. To further understand the process at the molecular level, the involvement of esterases was studied in the hormetic action of azadirachtin on *T. castaneum*. Electrophoretic patterns of esterases from larvae of *T. castaneum* fed on diet, which included hormetic concentrations of azadirachtin (viz. 0.001, 0.01 and 0.1 ppm) for 10 days were studied. The results showed a dose-dependant variation in the multiple molecular forms of the esterases. The variations, however, were not limited to the synthesis of new isoforms or the deletion of the existing ones; there was also variation in their relative abundance. Hence, it is suggested that hormetic concentrations of azadirachtin affect the synthesis or repression of esterases in *T. castaneum* larvae in a manner that will help them to grow and survive more efficiently. Since esterases have been implicated in hormone metabolism and digestion, a model, which may possibly explain such stimulation, is that the inhibitor may increase the activity of the polymorphic allosteric enzymes, and possibly of other protein systems. It could equally well be the case in the present study that selective synthesis and/or deletion of esterase isoforms, in response to different levels of azadirachtin, contributed towards detoxification of the inhibitor molecule(s) or modulated the endogenous larval hormonal levels in a manner which conducive to larval growth and survival. Thus the use of azadirachtin based natural insecticides assume serious dimensions when viewed in context of critical dose thresholds and inadequate or inefficient application strategies as well as formulation types.

VALID ASSESSMENT OF LDR HEALTH EFFECTS: ESSENTIAL FOR EVALUATING RADIOLOGICAL RISKS

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When Three Mile Island showed that the extensive radioactivity release and dispersion predicted by computer models vastly overestimated the hazard, this confirmed earlier test results and stimulated a massive government-industry program to measure release and dispersion of radioactivity from simulated casualties. November 18, 1980, Starr, Levenson et al. put these results together in an NRC Briefing, "Realistic Estimates of the Consequences of Nuclear Accidents." Using documented test data and reasonable analyses, they concluded that no acute fatalities and few latent deaths are likely to follow any realistic casualty. They promulgated this story and its supporting data world-wide and encountered no convincing refutation.

After 9/11, nuclear critics raised a new issue: terrorists attacking a nuclear plant or its spent fuel could release its radioactivity, killing hundreds of thousands and devastating the land for generations. Nuclear defenders have been unwilling to present the evidence that such consequences are precluded by natural law. So 19 nuclear experts, all members of the National Academy of Engineering, prepared a heavily documented paper for SCIENCE, reviewing and updating the evidence, showing that even the worst realistic casualty would result in few if any casualties, even if the fuel and containment were both seriously damaged.

The only substantive criticism of the paper was that, although no individual would get a serious dose, the "collective dose" of the population would be large. But a century of laboratory and epidemiological data on medical radiotherapy and natural radiation background show that low-dose radiation is not harmful and is usually beneficial, and collective dose cannot predict health effects.

Yet our policies, regulations and practices still require that collective dose, and unrealistic premises about radioactivity release and dispersion, be used to assess hazards and predict deaths. This discrepancy between science and policy must be faced and resolved.

POSSIBLE MOLECULAR UNDERPINNINGS OF HORMESIS-INDUCED LONGEVITY

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It has been shown that aging can be delayed by a hormetic agent such as mild stress, a phenomenon known as hormesis-induced longevity. We propose that the primary underlying mechanism of hormesis-induced longevity consists of the classic heat shock response complemented by a simultaneous acceleration of protein turnover. More specifically, a hormetic agent activates the heat shock response and, subsequently, the rate of protein turnover is accelerated to accommodate the dramatic increase in protein synthesis associated with the heat shock response.

THE HORMETIC EFFECTS OF COPPER ON THERMOTOLERANCE IN ROTIFERS

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Hormesis describes a phenomenon in which low dosages of a toxin are more beneficial for an organism than no exposure at all. In this study, rotifers were used as a model organism to demonstrate the hormetic effects of copper on thermotolerance. It was hypothesized that rotifers pretreated with low dosages of copper would develop greater thermotolerance and thus, have a lower mortality rate to heat shock than rotifers that were unexposed to copper. The results produced a hormetic dose-response curve with rotifers pretreated with copper between 50 ppb and 5 ppm showing a statistically significant ($p<0.05$) decrease in mortality to heat shock versus the unexposed control. We propose that in the experiment, the copper pretreatment may have disturbed copper homeostasis within the rotifers; in response, the rotifers overproduced MTs and HSP70. Thus, when the rotifers were exposed to the oxidative stress induced by heat shock, the MTs and HSP70 effectively prevented apoptosis by acting as antioxidants. This may explain copper hormesis because organisms will only produce the optimal level of MTs and HSP70 when they are exposed to low dosages of copper; no exposure results in a lack of MTs and HSP70 and high dosages overwhelm the organism to a point where the optimal level of MTs and HSP70 cannot prevent apoptosis and, ultimately, death.

RADON TREATMENT CONTROVERSY

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While health spas had been in use since the Roman times, treatments utilizing radon-rich air or water have not been unequivocally embraced by modern medicine. The objective of this work is to examine medical, biological, scientific, societal and political factors that contribute to this continuing controversy.

The exact mechanism of radon's effect on human body is not completely understood, however, we do have an insight into numerous biochemical processes that occur at low-level exposures to ionizing radiation and our knowledge in this area is rapidly growing. Recent advances in radiobiology, specifically the "molecular revolution", allow us to probe into functioning cells. Also growing is the body of medical evidence and patients' testimonials regarding both scientifically confirmed as well as perceived effectiveness of radon spa treatments of a variety of ailments, most notably rheumatoid arthritis. At the same time, a dismissal of any potential benefit of low-level radiation exposure continues to be promulgated by proponents of the Linear-No-Threshold (LNT) theory.

Just as observed for many chemical agents in the environment, there is an unquestionable evidence of detrimental effects of high-level exposures to ionizing radiation in general and to radon in particular. What is questionable is an arbitrary extension of these observations to low-dose region by linear extrapolation. While lacking any scientific basis, LNT theory gained wide acceptance mainly due to obvious convenience. Historically, such inference overshadowed scientific inquiries into the low-dose region and lead to a popular belief that no amount of radiation can be good. Fortunately, the LNT theory did not remain unchallenged.
As the reviewed literature suggests, a paradigm shift, reflected in the consideration of hormetic effects at low-doses, is gaining momentum in the scientific community worldwide. The impetus comes from significant evidence of adaptive and stimulatory effects of low-levels of radiation on human immune system.

ADAPTIVE RESPONSE IN pKZ1 MOUSE PROSTATE AFTER EXPOSURE TO VERY LOW DOSES OF X-RADIATION

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An adaptive response is a decrease in biological effect when a small conditioning dose of radiation is given several hours prior to a larger "challenge" dose of radiation. Adaptive responses are important modulators of biological responses to low dose radiation exposure which contradict the linear-no-threshold model. The pKZ1 mouse recombination assay enables detection of chromosomal inversions as a mutation end-point. The pKZ1 assay is the first mutation assay to detect modulation of a mutagenic endpoint after exposure to doses of X-radiation lower than 0.001 Gy, and a non-linear dose response is observed between 1 μGy and 10 mGy (Hooker *et al*, 2004. Radiat. Res. 162:447-52.).

pKZ1 mice were exposed to a priming radiation dose and then to a challenge radiation dose 4 hours later. Three days post-irradiation, prostate tissue was analysed to quantify inversion frequency. We demonstrated that very low (10 mGy, 1 mGy and 10 μGy) doses of X-radiation induced an adaptive response when mice were subsequently challenged with a high dose (1 Gy). These are the lowest doses of X-radiation reported to induce an adaptive response.

We have observed a significant increase in inversion frequency in pKZ1 prostate after exposure to 10 μGy X-radiation, and a significant decrease below endogenous inversion frequency after 1 mGy. Exposure to 1 mGy, either 4 hours before or 4 hours after a dose of 10 μGy, reduced the inversion frequency to a level below the endogenous frequency in prostate. This is the first study where an adaptive response has been observed using such low X-radiation doses, and the first demonstration of an adaptive response after challenge with a lower dose than the priming dose.

These experiments show that an adaptive response is induced even when a lower dose is delivered after a higher dose, suggesting that, at very low doses, the adaptive response is induced independent of the relative magnitude of the priming and challenge doses.

THE pKZ1 INVERSION ASSAY IS A SENSITIVE ASSAY FOR LOW-DOSE X-RADIATION STUDIES

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The pKZ1 assay is a sensitive assay for studying the effect of low-dose X-radiation on chromosomal inversion *in vivo*. The transgenic construct in the pKZ1 mice consists of the *E.coli* β-galactosidase gene (*lacZ*) in an inverse orientation with respect to a chicken β-actin enhancer-promoter. If an inversion occurs, facilitated by the immunoglobulin recombination signal sequences flanking the *lacZ* gene, then the *lacZ* gene can be expressed within that cell. The *E. coli* β-galactoside protein product can be detected in cells which have undergone an inversion in the transgene by using the chromogenic substrate X-gal which forms a blue colour.

Three to six month old pKZ1 transgenic mice were exposed to a single acute whole body dose of X-rays ranging from 0.001 up to 2000 mGy. The mice were sacrificed 3 days later and the spleen and prostate removed for inversion analysis. Ultra-low dose (0.005 - 0.01 mGy) and high dose (> 100 mGy) acute X-radiation resulted in an increase in inversion frequency. Low dose (1-10 mGy) acute X-radiation resulted in a reduction below the endogenous inversion frequency. The pattern of dose response for inversion frequency was similar in both spleen and prostate. Prostatic glands from untreated pKZ1 control mice exhibited ≤ 3 inversions/gland. At high doses and ultra-low doses the pKZ1 mice exhibited ≥ 3 inversions/gland. At low doses there were more prostatic glands with 0 inversion events and less prostatic glands with 2 or 3 inversion events than was observed in control animals.

This is the first study to identify statistically significant chromosomal changes *in vivo* at doses ≤ 1 mGy. The linear no-threshold dose response model does not hold for chromosomal inversion response in spleen and prostate in pKZ1 mice in the low and ultra-low dose range. The inversion frequency dose-response pattern is similar in both tissues suggesting that the pKZ1 assay is measuring a fundamental DNA repair response.

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