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IMPLICATIONS FOR TOXICOLOGY, MEDICINE AND RISK ASSESSMENT

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ABSTRACT BOOK

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A PERSPECTIVE ON THE SCIENTIFIC, PHILOSOPHICAL, AND POLICY DIMENSIONS OF HORMESIS

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The hormesis concept has broad implications for biology and the biomedical sciences. This perspective on hormesis concentrates on toxicology and toxicological risk assessment and secondarily explores implications for other fields. It considers the varied manifestations of hormesis in the context of a broad family of biological stress responses. Evidence for hormesis is reviewed, and the hormesis model for dose-response relationships is contrasted with more widely accepted dose-response models in toxicology: a linear nonthreshold (LNT) model for mutagenesis and carcinogenesis, and a threshold model for most other toxicologic effects. Scientific, philosophical, and political objections to the hormesis concept are explored, and complications in the hormesis concept are analyzed. The perspective concludes with an opinion on the current state of hormesis, challenges that the hormesis model poses for risk assessment, and problems requiring further analysis.

SUPEROXIDE DISMUTASE AND BELL-SHAPED DOSE RESPONSE CURVES

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For forty years we have known that cellular metabolism generates the superoxide free radical, $O_2^{\cdot -}$, that the radical is cytotoxic, and that a family of enzymes called superoxide dismutases (SOD) protects us from the radical by catalyzing its conversion to oxygen and hydrogen peroxide. Furthermore, radical production has been shown to increase in a wide variety of pathological states, especially involving inflammation or ischemic injury. Most of the literature has described systems wherein added or over expressed SOD produced beneficial effects, yet certain applications were evident where SOD was detrimental, exacerbating cell injury or death. When broad dose-response studies were finally possible in a model of reperfusion injury in an isolated heart model, hormesis became clear. We propose that the mechanisms underlying the hormesis are related to the paradoxical abilities of the superoxide radical to serve as both an initiator and a terminator of the free radical-mediated chain reaction that results in lipid peroxidation. Lipid peroxidation is a universal feature of oxidative stress, causing loss of cellular structure and function. Under any given conditions, the optimal concentration of SOD is that which decreases chain *initiation* without elimination of the chain *termination* properties of the radical, resulting in a minimum of net lipid peroxidation. Mathematical modeling of this hypothesis yields predictions fully consistent with observed laboratory data.

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INDUCTION OF THE REDOX PROTEIN THIOREDOXIN MEDIATING THE HORMETIC RESPONSE OF PRECONDITIONING-INDUCED NEUROPROTECTION

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The dose-response effects of experimental hormesis can be evaluated both in vivo and in vitro. A mild and brief ischemic preconditioning stress induces hormetic responses leading to cytoprotection of myocardial cells and brain neurons against subsequent prolonged lethal ischemic stress in vivo. It has been difficult to investigate molecular mechanisms underlying hormesis in animals; ischemic preconditioning is known to induce heat shock proteins in vivo. For studying the hormesis-induced neuroprotection a preconditioning human SH-SY5Y cell model was developed in our laboratory for searching key molecules which may mediate adaptive responses and cross resistance in hormesis. A brief preconditioning stress produced by a non-lethal 2-hr serum deprivation protects SH-SY5Y cells against cell death caused by a 24-hr serum deprivation. This preconditioning-induced neuroprotective response is mediated by the NO/cGMP/PKG signaling pathway leading to the delay expression of the redox protein thioredoxin (Trx). Serum deprivation- induced preconditioning hormesis also suppresses mitochondria-dependent apoptosis caused by not only 24-hr serum deprivation but also the administration of 1-methyl-4-phenyl-pyridinium ion (MPP+) neurotoxin. Ischemia-preconditioned cells are less vulnerable to MPP+-induced oxidative stress and neurotoxicity. Preconditioning- induced Trx may regulate hormetic response in neuroprotection since it can be blocked by the inhibition of Trx redox cycling and also by the antisense that blocks Trx expression. In agreement with the neuroprotective effects of preconditioning-induced Trx the externally administered nanomolar Trx concentration-dependently inhibits the generation of reactive oxygen species, the release of cytochrome c and the proapoptotic caspases 9 and 3 all of which contribute to cell survival. Moreover, Trx also increases the expression of mitochondrial proteins such as antiapoptotic Bcl-2 and antioxidative MnSOD. In conclusion, preconditioning-induced NO/cGMP/PKG-dependent Trx expression may mediate the hormetic responses such as the survival of brain neurons and perhaps cross resistance against MPP+ neurotoxin.

HEME OXYGENASE-1 AND CARBON MONOXIDE AS NOVEL ANTI-INFLAMMATORY MOLECULES

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Carbon monoxide (CO) at low concentrations confers beneficial effects in several inflammatory disorders including ischemia-reperfusion injury (IRI) and pulmonary hypertension. The most salient feature of CO-mediated cytoprotection is the lack of both an archetypical inflammatory response and cell death. Little progress has been made in understanding how CO mediates these important functions. One of the important cellular targets of CO is the macrophage (mϕ), a key innate modulator of the inflammatory response. We demonstrate that CO acts very rapidly on the mϕ and induces stabilization of the transcription factor hypoxia inducible factor 1α (HIF1α), a potent oxidant stress response gene responsible for regulating gene expression involved in angiogenesis, metabolism, and survival. We demonstrate in vitro and in vivo in the lung that exposure to a low concentration of CO [250 ppm or 0.025%] resulted in a rapid and marked induction in HIF1α stabilization. The ability of CO to increase HIF1α activity is mediated in part by a highly significant and transient burst of reactive oxygen species (ROS) arising from the mitochondria. CO-mediated HIF1α activity then leads to the induction and secretion of TGFβ, a potent anti-inflammatory cytokine. The induction of both HIF1α and TGFβ by CO were necessary to rescue mϕ from anoxia/reoxygenation-induced apoptosis and IRI in the lung. Blockade of HIF1a or TGFb abrogated CO-induced protection. Taken together, these data provide the first insight into the earliest events elicited by CO that ensure cellular homeostasis. CO, acting at the site of injury targets the mϕ to maintain a protective phenotype and as such, CO offers a powerful clinical alternative to prevent IRI as it enters clinical trials for solid organ transplantation.

MILD HEAT STRESS SLOWS DOWN AGING, INCREASES WOUND HEALING, AND ENHANCES ANGIOGENESIS IN HUMAN CELLS

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Progressive accumulation of molecular damage and increased molecular heterogeneity are the main hallmarks of cellular aging. Inefficiency of the maintenance and repair systems is the main reason for the accumulation of molecular damage. Mild stress-induced hormesis appears to be an effective way for reducing the accumulation of molecular damage. Our studies have shown that repeated mild heat stress has anti-aging effects on growth and various other cellular and biochemical characteristics of normal human skin fibroblasts and keratinocytes undergoing aging *in vitro.* Human cells exposed to 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, improved cellular resistance to ethanol, hydrogen-peroxide and UV-B rays, enhanced the levels of various antioxidant enzymes, enhanced the activity and amounts of sodium-potassium pump, and increased the phosphorylation-mediated activities of various stress kinases. Furthermore, novel hormetic effects of mild heat stress on the wound healing capacity of skin fibroblasts and on the angiogenic ability of endothelial cells have been observed. A pre-exposure of cells to mild heat stress increased their wound healing capacity by more than 25% in an *in vitro* "scratch assay", as measured by the extent of cell migration and cell elongation. Similarly, mild heat stress at 41°C for 1 hr, but not severe heat stress at 42°C, increased the total tube length and total number of junctions by 30-60% and 10-14%, respectively, in human vascular endothelial cells. These data add to the ever growing body of evidence in support of the view that repeated mild stress-induced hormesis can be applied for the modulation, intervention and prevention of aging and age-related impairments and diseases.

A SUMMARY OF DOSE-RESPONSE MODELS AND ESTIMATION IN DEVELOPMENTAL TOXICITY STUDIES

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Developmental toxicity studies are an important area in the field of toxicology. In a developmental toxicity study, fetal litters are indirectly exposed to various levels of non-carcinogenic toxic substances through direct exposure to the host animals. Endpoints that are recorded in these studies include fetal weight and length, as well as indicators of abnormality and death. Endpoints are then measured to determine litter responses, which include average weight, malformation and death rate. The dose-response pattern in these studies typically appears to exhibit at least the existence of a threshold effect. The threshold dose-response model is the default model for non-carcinogenic risk assessment, according to the USEPA, and is encouraged by the agency for the use in the risk assessment process. Several statistical models are proposed to estimate the threshold dose and to account for other important aspects of the developmental toxicity study. Use of these models to different applications will be summarized. The advantages and disadvantages of these models, and the comparison to other alternative models are discussed. We, also, summarize potentials for future research in this field.

SEPARATING STIMULANT AND IMPAIRING FUNCTIONS IN HORMETIC PROFILES WITH INDEPENDENT COMPONENT ANALYSIS (ICA)

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Mean response profiles can obscure lawful underlying processes that may differ substantially from the average functions. The purpose of this research is to test specific hypotheses concerning the mathematical properties of underlying processes that may account for hormetic dose-response functions and temporal hormesis. We hypothesize that hormesis is produced by the combination of two separate functions: (1) a stimulant/activation function that is nonlinear—it increases nonlinearly with dose and time; and (2) a sedative/impairing function that is roughly linear with dose and time. We predict further that the sedative/impairing function is recruited and decays more slowly than does the stimulant function. The stimulant and sedative functions may combine nonadditively. We will analyze two large, existing datasets using Independent Component Analysis (ICA), a powerful statistical technique that can isolate sources of variation into separate, independent functions. ICA has been applied successfully in many domains containing mixtures of components that must be separated, but without recourse to a reference model. ICA appeals instead to statistical independence conditions to achieve separation, and as such is suitable for noisy signals when the noise is independent of the useful signals.

We will test the hypotheses above as well as assess the degree to which the combination of underlying functions is additive, synergistic, or inhibitory. Importantly, ICA may help explain why some dose-response experiments exhibit hormesis, while others do not. In addition, the ability to dissociate source functions that generate hormetic dose-response and temporal effects may be an important first step in determining their physiological mechanisms. The physiology of the stimulant function may be very different from that of the sedative/impairing function. In turn, statistical noise is likely to be different altogether from either source function, which would confirm the suitability of ICA as an analysis tool.

HORMETIC AND NON-HORMETIC DOSE-RESPONSES IN EMOTIONAL NEUROSCIENCE

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Stress exerts a profound, yet complex, influence on learning and memory. Over a century of behavioral research has shown that stress can exert both positive and negative effects on memory. This presentation will address how the complex effects of stress on learning and memory can be characterized by hormetic- and non-hormetic dose-response functions. Thus, stress may either stimulate or impair brain memory mechanisms, depending, in part, on the timing and duration of the stress experience. We suggest that brief stress exerts a rapid enhancement of memory-related functions of the hippocampus, a temporal lobe structure which is necessary for memory formation. The stress-induced enhancement of memory is generated, in part, by excitatory actions of neuromodulators, including glucocorticoids, norepinephrine, corticotropin-releasing hormone, acetylcholine and dopamine. The rapid stress-induced activation of the hippocampal brain memory system results in a linear (non-hormetic) dose-response relation between emotional strength and memory formation. In response to more prolonged stress a delayed inhibition of hippocampal function develops, which can be attributed to compensatory cellular responses which protect hippocampal neurons from excitotoxicity. In addition, stress exerts a global inhibitory influence on the functioning of the prefrontal cortex, a brain region which is necessary for higher order cognitive processing, such as decision-making and multi-tasking. The inhibition of hippocampal and prefrontal cortex functioning in response to stress is potentially relevant to the well-described curvilinear (hormetic) dose-response relationship between arousal and memory. In summary, our emphasis on the temporal features of stress-brain interactions addresses how stress can activate, as well as impair, hippocampal and prefrontal functioning to produce different shaped (non-hormetic/hormetic) stress-memory dose response functions.

IS POSSIBLE FOR LDR ABLE TO INDUCE HORMETIC EFFECT ON NORMAL CELL GROWTH, BUT NOT HUMAN TUMOR CELL GROWTH? CLINICAL IMPLICATION

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The biological effect induced by low-dose radiation (LDR) is distinguishable from that induced by high-dose radiation (HDR). Stimulating effect, i.e. hormesis, is one of such distinguishable effects between LDR and HDR. We have demonstrated the hormetic effects of LDR (75 mGy) on bone marrow cell proliferation and peripheral mobilization. Whether such hormetic effects induced by LDR also occur in tumor cells remains to be determined. Recently we found that significantly stimulating effect was found in the normal cell lines, but not in the two leukemic and five solid tumor cells, in response to LDR exposure in vitro. Examination of cell cycling changes and cell death for these cells by flow cytometry at different post-LDR times did not find any change attributable to the distinct effects of LDR on cell proliferation between tumor and normal cells. To further provide evidence for the absence of LDR-induced hormetic effect in tumor cells in vivo, tumor-bearing models were established by implanting two solid tumor cell lines to nude mice. When tumor cells before implanted to nude mice or tumor-bearing mice on day 0, 10 and 15 after tumor cells were implanted were exposed to LDR (75 mGy X-rays), no stimulating effect on the tumor growth rate was observed as compared to the mice without LDR exposure of either animals or tumor cells, confirming the absence of LDR-induced hormetic effect in these two solid tumor cells under an in vivo condition. These results suggest that LDR induces a hormetic effect on cell proliferation in normal cell, but not in leukemia and solid-tumor cells in vitro, and solid tumor cells in vivo.

Furthermore, three tumor-bearing animal models were also used to further define whether LDR induces adaptive response in tumor cells in vivo. Adaptive response was observed only in normal cell line, but not in four tumor cell lines, in response to LDR, showing a resistance to subsequent D2-induced cell growth inhibition. Three tumor-bearing mouse models with U251, NCI-H446 or S180 tumor cells were used to confirm that pre-exposure of tumor-bearing mice to D1 did not induce the resistance of tumor cells in vivo to D2-induced tumor growth inhibition. Furthermore, a higher apoptotic effect, along with higher expression of apoptosis-related genes P53 and Bax and lower expression of anti-apoptosis gene Bcl-2, was found in tumor cells of the tumor-bearing mice exposed to D1 + D2 than those in the tumor cells of the tumor-bearing mice exposed to D2 alone. These results suggest that LDR does not induce adaptive response in the tumor cells under both in vitro and in vivo conditions, which is a very important, clinic-relevant phenomenon.

Therefore, the presentation will discuss the current status regarding the possibly distinct responses between human normal cells and tumor cells in response to LDR.

GENETIC DISSECTION OF HORMESIS: PONCE D'ELEGANS

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We have shown that increased longevity and stress resistance in *Caenorhabditis elegans* can be induced by sub-lethal exposure to any one of several stressors (hormesis). We have studied hormesis both by subsequent exposure to the same stressor (or one in the same class) but also find that many such treatments have a general effect on life prolongation as well. Several genes specifying regulation of dauer formation are also involved in a signal transduction pathway that is known to modulate life span in *C elegans* and several of these genes are also required for hormesis. We find that loss-of-function mutations in any of three genes (daf-16, daf-18, or daf-12) not only reduce or abolish the ability to form dauers but also block the hormetic response increasing life span following sub-lethal heat stress. Indeed, the life expectancy of these dauer-defective mutants is decreased by the same pretreatments that increase the life expectancy of wild-type animals. Surprisingly, daf-16 and daf-12 are not required for the induction of thermotolerance, but daf-18 is required for its full induction.

We have also examined in detail the U-shaped dose-response relationships (hormesis) in response to a mild heat shock of 35^{o}C with regards to longevity. By applying the concept of heterogeneity, we found that the inverted U-shaped dose-response in longevity is driven by the U-shaped dose-response in initial mortality. When worms are subjected to mild heat shock, the initial mortality decreases as compared to the control. This benefit in the initial mortality increased with moderate increases in the length of heat shock, peaking at a point that coincided with the induction of damage to the worms. We have also been able to use response to heat, when coupled with a visible biomarker, (HSP-16::GFP) as a fruitful way to predict subsequent differential longevity of individual worms.

TOXICOLOGY SESSION

ANTIBIOTICS HAVE DUAL MODES OF ACTION

Julian Davies, University of British Columbia, Vancouver BC

THE DIVERSE EFFECTS OF ARSENIC ON HUMAN DNA REPAIR: IMPLICATIONS FOR RISK ASSESSMENT

Peter Sykora, Deakin University, Melbourne, Australia
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THE POLYACETYLENES FALCARINOL AND FALCARINDIOL AFFECT STRESS RESPONSES IN MYOTUBE CULTURES IN A BIPHASIC MANNER

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U-SHAPED DOSE-RESPONSES AT LOW DOSES: EXPLANATION WITH A NEW MODEL FOR IN VITRO NEOPLASTIC TRANSFORMATION

Helmut Schöllnberger, PhD., University of Salzburg

HORMESIS WITHOUT CELL KILLING IN A COMPETING-RISKS MODEL OF CARCINOGENESIS

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COMPLEX MIXTURE-ASSOCIATED HORMESIS AND TOXICITY: THE CASE OF LEATHER TANNING INDUSTRY

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ANTIBIOTICS HAVE DUAL MODES OF ACTION

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Antibiotics are low molecular weight products of bacteria and fungi that have been used as treatments for infectious and other diseases for more than 50 years. Evidence is accumulating that these compounds have quite different roles in the environment compared to therapeutic applications. The difference lies in the available concentrations that determine inhibitory activity compared to transcription modulation. As will be discussed, low concentrations may be responsible for cell-cell signaling activity in microbial communities.

THE DIVERSE EFFECTS OF ARSENIC ON HUMAN DNA REPAIR: IMPLICATIONS FOR RISK ASSESSMENT

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Arsenic is an established non genotoxic carcinogen however its mechanism of action is not well understood. The DNA damage produced by arsenite induced reactive oxygen species is repair primarily by base excision repair (BER). We investigated the affect of low, physiologically relevant concentrations of arsenite on BER in human cells. Despite previous research showing that DNA ligase I is not directly inhibited by low doses of As(III) in vitro, our results showed that in vivo ligase I mRNA, protein and activity levels were significantly reduced after 24 hours exposure to concentrations as low as 5 μM As(III). In contrast, sub micromolar arsenite concentrations comparable to levels of total As in the blood of arsenicosis patients caused a significant increase in both ligase I protein and activity levels. The research also investigated arsenite regulation of core BER enzymes; Polymerase β (Pol β) and AP endonuclease (APE1) with both polβ and ape1 mRNA being down regulation at concentrations above 1 μM. In contrast, levels of arsenite below 1 μM, Pol β mRNA, protein levels and subsequently BER activity were significantly increased. These hormetic changes in BER activity correlated with an overall protection against sunlight UVR-induced toxicity although produced synergistic toxicity after exposure to higher concentrations of As(III). The increase in Pol β protein levels reported after 24 hours exposure dissipate with longer exposure though became significantly increased again after chronic exposure. Importantly, our results showed that even very low concentrations of arsenite coupled with chronic exposure can affect the rate of damage repair. The increased rate of repair correlated with a greater rate of survival after exposure to sunlight UVR. These results demonstrate that low biologically relevant doses of arsenite can cause hormetic regulation of DNA repair genes which can serve to protect rather than sensitize cells exposed to additional oxidative damage.

THE POLYACETYLENES FALCARINOL AND FALCARINDIOL AFFECT STRESS RESPONSES IN MYOTUBE CULTURES IN A BIPHASIC MANNER

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Epidemiological studies indicate some correlation between high intake of fruit and vegetables and certain beneficial health effects. This has lead to a great interest in studying effects of single compounds originating from fruit and vegetables, e.g. abundantly occurring bioactive compounds as caroteonoids and flavonoids. Other highly bioactive, but less abundant compounds co-occur in fruit and vegetables and may contribute to overall effects. Some of these less abundant compounds include the aliphatic C_{17}-polyacetylenes falcarinol and falcarindiol, which are mainly present in carrots, celery, celeriac and other umbelliferous vegetables. We investigated the effects of these compounds on the stress responses in primary myotube cultures isolated from porcine *semimembranosus* muscle. The myotube cultures were exposed to various concentrations of falcarinol and falcarindiol for 24 h before testing effects of 100 μM H_2O_2 on the intracellular formation of reactive oxygen species (ROS) and mRNA abundance of the heat shock proteins HSP70 and HO1. Prior exposure to both polyacetylenes at concentrations from 1.6 to 25 μM caused a slightly accelerated intracellular ROS formation when exposed to H_2O_2 compared to controls (without pre-incubation). However, the ROS formation following H_2O_2 exposure was substantially decreased after pre-incubation with falcarinol and falcarindiol at concentrations of 50 and 100 μM. The heat shock proteins HSP70 and HO1 were regulated in a similar way as their mRNA abundance were lower when exposed to H_2O_2 after pre-incubation with 6.25-25 μM of either falcarinol or falcarindiol as compared to the controls. In contrast, the mRNA abundance after pre-incubation with 50 μM polyacetylene approached the levels of the respective controls. Both of the tested stress responses; ROS formation and HSP regulation were affected in a biphasic manner by increasing doses of the polyacetylenes, but the relation and mechanisms needs further investigation.

U-SHAPED DOSE-RESPONSES AT LOW DOSES: EXPLANATION WITH A NEW MODEL FOR IN VITRO NEOPLASTIC TRANSFORMATION

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In vitro studies have shown that low doses of low-LET radiation can lead to neoplastic transformation frequencies significantly below the spontaneous level. One possible explanation for this phenomenon is bystander-induced apoptosis (Portess et al. 2007). To simulate this biological endpoint, a new compartment model has been developed. It consists of states for normal, mutated and transformed cells that are linked through rate constants that describe the formation of simple and complex damage clusters formed by endogenous processes and ionizing radiation. The rate of lesions removal by correct or incorrect repair is considered together with the misrepair probabilities. The latter are derived the MCER (Monte Carlo Excision Repair) software (Semenenko et al. 2005). Coupled differential equations are applied for the calculation of the expected number of simple or complex damage clusters caused by radiation or endogenously per cell at time t. The model is also equipped with a pathway for bystander-induced apoptosis of transformed cells. The related rate constant is treated as a free parameter. The model is tested on an important and representative data set by Redpath et al. (2001) which shows U-shaped dose-response curves for in vitro neoplastic transformation of CGL1 cells. The current study also attempted to estimate the onset and cessation of the protective apoptosis-mediated effect. The new model approach will be compared to an earlier one with the State-Vector Model (SVM). Advantages of the new model compared to the SVM approach will be discussed and uncertainties for model predictions and parameter estimates will be presented. The SVM has also been applied to the data of Redpath et al. (2001) and to other low dose data that show supralinearities at low doses (Schöllnberger et al. 2007a, 2007b).

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HORMESIS WITHOUT CELL KILLING IN A COMPETING-RISKS MODEL OF CARCINOGENESIS

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Stochastic two-stage clonal expansion (TSCE) models of carcinogenesis offer the following clear theoretical explanation for U-shaped cancer dose-response relations. Low doses that kill initiated (pre-malignant) cells thereby create a protective effect. At higher doses, this effect is overwhelmed by an increase in the net number of initiated cells. Ths sum of these two effects, from cell killing and cell proliferation, gives a U-shaped or J-shaped dose-response relation.

This paper shows that exposures that do not kill or decrease cell populations, but that only hasten transitions that lead to cancer, can also generate U-shaped and J-shaped dose-response relations in a competing-risk (modified TSCE) framework where exposures disproportionately hasten transitions into carcinogenic pathways with relatively long times-to-tumor. Quantitative modeling of the competing effects of more transitions toward carcinogenesis (risk-increasing) and a higher proportion of transitions into the slower pathway (comparatively risk-reducing) shows that J-shaped dose-response relation can occur even if exposure increases the number of initiated cells at every positive dose level.

This suggests a possible new explanation for hormetic dose-response relations in response to carcinogenic exposures that do not have any protective (cell-killing) effects. In addition, the examples presented emphasize the role of time in hormesis: exposures that monotonically increase risks at younger ages may nonetheless produce a U-shaped or J-shaped dose-response relation for lifetime risk of cancer.

COMPLEX MIXTURE-ASSOCIATED HORMESIS AND TOXICITY: THE CASE OF LEATHER TANNING INDUSTRY

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Leather tanning was investigated in a series of studies focused on the toxicities of tannery-derived complex mixtures, i.e. wastewater from tannin-based vs. chromium-based tanneries, and vegetable tannin (VT, from *Acacia* sp.) or phenolic synthetic tannin (ST). Toxicity was evaluated by means of multiple bioassays including developmental defects and loss of fertilization success in sea urchin embryos and sperm (*Paracentrotus lividus* and *Sphaerechinus granularis*), algal growth inhibition (*Selenastrum capricornutum* and *Dunaliella tertiolecta*), and *Daphnia magna* immobilization. When comparing tannin-based effluent (TBE) vs. chromium-based tannery effluent (CBE), a concentration-related shift from hormesis to toxicity was observed for TBE in terms of: (i.) developmental toxicity and spermiotoxicity in sea urchins, and (ii.) algal growth inhibition. Hormetic effects were observed at effluent concentrations of 0.1 to 0.2%, whereas effluent levels ≥1% resulted in concentration-related increases in the respective adverse effects in sea urchin embryos or sperm, or in algal cultures. On the other hand, CBE invariably showed a steady concentration-related toxicity from the lowest tested level of 0.1% and the toxicities observed at higher CBE levels were significantly more severe than induced by TBE. No shift from hormesis to toxicity could be detected in *D. magna* bioassays, due to the assigned zero value of daphnid immobilization in controls, preventing any observation of hormetic effects. The overall results suggested that the TBE-associated shift from hormesis to toxicity could be related to tannins as a major component of tannin-based tanning operations. This was the case, as both *Acacia* VT and phenolic ST exerted the same concentration-related trends displaying hormesis a environmentally realistic low tannin levels (0.1 to 0.3 mg/L) and toxicity at higher tannin levels (≥1 mg/L). Altogether, the data support the view that leather production utilizing tannins might be regarded as a relatively more environmentally friendly procedure than chromium-based tanning process.

BIOTERRORISM, LOW DOSE EFFECTS AND HORMESIS SESSION

LOCAL, REGIONAL AND NATIONAL RESPONSES FOR MEDICAL MANAGEMENT OF A RADIOLOGICAL/NUCLEAR INCIDENT

Nicholas Dainiak, MD, Bridgeport Hospital, Bridgeport, Connecticut

LOW-DOSE-RADIATION-ACTIVATED NATURAL PROTECTION AGAINST CANCER AND OTHER DISEASES

Bobby R. Scott, Lovelace Respiratory Research Institute, Albuquerque, NM

MECHANISMS OF BYSTANDER EFFECTS WHICH COULD UNDERLIE HORMETIC EFFECTS FOLLOWING LOW DOSE EXPOSURE

Prof. Carmel Mothersill, McMaster University, Hamilton, ON, Canada

THE CUTANEOUS RADIATION REACTION AT THE MOLECULAR LEVEL

V. Meineke, Bundeswehr Institute of Radiobiology, Munich, Germany

LOCAL, REGIONAL AND NATIONAL RESPONSES FOR MEDICAL MANAGEMENT OF A RADIOLOGICAL/NUCLEAR INCIDENT

Nicholas Dainiak, MD, Clinical Professor of Medicine, Yale University School of Medicine, Chairman of Medicine, Bridgeport Hospital, Bridgeport, Connecticut

Radiological and nuclear devices may be used by terrorists or may be the source of accidental exposure. A tiered approach has been recommended for response to a terrorist event wherein local, regional, state and federal assets become involved sequentially, as the magnitude in severity of the incident increases. A variety of US national response assets may be called upon for use in radiological incidents. These include agencies and programs that have been developed by the Department of Homeland Security, the Department of Energy and the Environmental Protection Agency. Recently, the National Marrow Donor Program and the American Society for Blood and Marrow Transplantation have established the Radiation Injury Treatment Network which has partnered with the Office of the Assistant Secretary for Preparedness and Response, US Department of Health and Human Services, to provide care to radiation injury victims requiring intensive or highly specialized care. Regional response programs such as the New England Regional Health Compact (consisting of 6 member states, including Connecticut, Rhode Island, Massachusetts, New Hampshire, Vermont and Maine) have been developed to manage consequences of radiation injury. Recently, a state-wide hospital plan for radiologic emergencies has been developed for Connecticut which addresses delineation of responsibilities of various categories of health professionals, protection of healthcare providers, identification and classification of individuals who might have been exposed to and/or contaminated by radiation, and early management of individuals who have had or might have had a radiologic exposure. Hospital admission pathways are described, and general guidelines for referral to transplant centers are suggested Other states are in the process of developing a similar plan based upon the Connecticut model. Together, these resources may be used to augment the response for which local authorities have the primary responsibility in a domestic disaster. Coordination of federal, regional and state efforts is necessary in order to provide an efficient response to a radiologic/nuclear incident.

LOW-DOSE-RADIATION-ACTIVATED NATURAL PROTECTION AGAINST CANCER AND OTHER DISEASES

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The fact that low doses and dose rates of some sparsely ionizing forms of radiation (e.g., x rays, gamma rays, and beta radiation) activate transient natural chemical and biological protection against cancer and some other diseases is not widely known. The disease preventative properties relate to radiation adaptive response (radiation hormesis) and involve stimulated protective biological and chemical signaling (a mild stress response). The biological processes associated with the protective signaling are now better understood and include: increased availability of efficient DNA double-strand break repair (p53-related and in competition with normal apoptosis), stimulated auxiliary apoptosis of aberrant cells (presumed p53-independent), and stimulated immune functions. The stimulated immune functions include increased antibody formation, natural killer cell activity, and secretion of interferon and other cytokines. This system of low-dose-radiation activated natural protection (ANP) appears to require protection-mode-specific thresholds that vary for different individuals but when jointly exceeded, can lead to suppression of cancer and other diseases. Repeated small radiation doses and low-rate extended exposure appears to enhance the level of protection. In this presentation, data from different research groups on low-dose radiation ANP will be discussed. Implications of the data regarding the use of low-dose-radiation to counter harm to humans from deployment of biological, chemical, and radiological weapons by terrorists will also be discussed.

MECHANISMS OF BYSTANDER EFFECTS WHICH COULD UNDERLIE HORMETIC EFFECTS FOLLOWING LOW DOSE EXPOSURE

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Following a "dirty bomb" attack involving radiation, the key issues will involve prediction of outcomes from low dose exposures to humans and the environment. Traditionally such predictions are based on the ICRP's use of the LNT hypothesis and collective dose. As in the case of Chernobyl, these extrapolations result in wildly different numbers of predicted cancers than what is actually observed. Part of the problem is that the ICRP models do not allow for any beneficial or adaptive effects at the biological level. However the reality of the situation is that cells have numerous mechanisms for dealing with radiation damage and at the tissue, organism and even population level, signaling mechanisms such as bystander effects occur and "manage" responses at the level leading to the best possible outcome. In this presentation the concept of hierarchical levels of response will be discussed and we will present new data suggesting that the bystander proteome is actually protective and restorative not damaging. Other data from our laboratory suggests that several response thresholds can be identified where increasing the dose results in abrupt changes in response. In relation to hormesis, an example is the turning on of the protective bystander response at a dose of 2mGy. Other work reported that the adaptive and repair responses are also "off" until a certain low dose has been exceeded. In relation to bio-terrorism, this means that simple use of LNT/collective dose concepts will not be useful and will increase panic and psychosocial consequences of any attack while if a hormetic concept were to be used, much better management of the health consequences of any radiation attack would result.

THE CUTANEOUS RADIATION REACTION AT THE MOLECULAR LEVEL

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The skin is one of the key organs in radiation induced multi-organ involvement and -failure. Moreover radiation damage to the skin is a key diagnostic and prognostic parameter for patients who have accidentally been exposed to radiation. Although the timely course of the cutaneous radiation reaction is an accepted diagnostic parameter on the clinical level, the pathophysiological details underlying these clinical pictures are not fully understood yet and there is a strong need for further research in this specific area. This paper sums up the current understanding of molecular events underlying radiation induced skin reactions both at high and low dose exposure conditions. The role of adhesion molecules (such as ICAM-1 and ß1-integrins) but also cytokines (e.g. IL1, IL-6, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β) is discussed. Subsequently the degranulation of cutaneous mast cells and the release of its contents (such as tryptase and serotonin) is presented as one of the key steps both in early inflammatory radiation effects in skin as well as in mediation of late effects such as fibrosis.