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ABSTRACT BOOK

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NON-IONIZING, ELECTROMAGNETIC STIMULATION OF HORMETIC RESPONSES: FACT OR FANCY?

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Hormesis is the nonlinear dose-response of biological systems to physical and chemical stimuli. Characterized by low-dose stimulation and high-dose inhibition, hormetic responses (HR) prove most interesting (and controversial) at low-doses. It is here where cells are temporarily hardened and activated to be more metabolically resilient or responsive to various stimuli, including most—if not nearly all—chemicals and certainly all forms of ionizing radiation. However, non-ionizing electromagnetic radiation (such as visible and infrared light, microwaves, and radio frequency (RF) radiation) has only recently been examined for its potential to elicit HR. The little research conducted thus far provides some evidence to suggest a possible link between non-ionizing radiation and hormesis. In the past decade or so, non-ionizing, low level light therapy has been shown to enhance the rate of wound healing in various tissues, including skin and brain. Other studies have demonstrated that low-level electric and magnetic fields can stimulate specific areas of the brain to enhance human cognition, thereby linking electromagnetic fields to possible HR. In the past few years, several intriguing studies have even shown that RF radiation can elicit not only HR but also adaptive responses (AR) in both human and animal tissue. Because AR are protective cellular responses activated by and dependent upon hormetic, low-dose stimulation, it seems perfectly reasonable to consider AR as just “latent” HR (i.e., AR = HR). As such, AR represent true markers of HR and provide evidence of hormesis. Together, these studies and others suggest that non-ionizing radiation may induce both HR and AR. However, much more research is needed to (a) prove and identify—with certainty—the forms, modes and doses of non-ionizing radiation that can/cannot stimulate HR, (b) understand both the mechanisms and the photo-electro-magnetic interactions with cells that trigger and control HR, and (c) exploit the practical implications that may benefit society and lead to future technologies.

EPIGENETIC RESPONSES TO LOW DOSE RADIATION

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Human epidemiological and animal experimental data indicate that the risk of developing adult onset diseases and neurological disorders is influenced by persistent adaptations to prenatal and early postnatal environmental exposures. One group of epigenetically regulated genes that links environmental exposures early in development to adult diseases are those with metastable epialleles. These genes have highly variable expression because of stochastic allelic changes in the epigenome rather than mutations in the genome. The viable yellow agouti (A^{vy}) mouse harbors a metastable *Agouti* gene because of an upstream insertion of a transposable element. We have previously used this animal model to show that nutritional and chemical toxicant exposures during early development induce persistent epigenetic changes at the A^{vy} locus that result in alterations in adult disease susceptibility. We now demonstrate that low doses of ionizing radiation ($\leq$7.6 cGy) also significantly alter DNA methylation, and induce a positive adaptive phenotype in A^{vy} offspring in a sex and dose dependent manner; an effect that is block by maternal antioxidant exposure. The importance of these studies, with regards to human health and disease, will be discussed.

MOLECULAR SIGNATURES OF ADAPTIVE STRESS RESPONSES

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Adaptive responses of biological systems to environmental stress factors characteristically display pronounced dose dependency. In many models low doses of these stressors can cause beneficial effects on the vitality of the affected organism in contrast to damaging action of high doses. Whereas a multitude of such “hormetic” stress responses has been observed in diverse biological systems the underlying molecular mechanisms are mainly unknown. To explore specific molecular reactions controlling the ambivalent responses of cells and organisms to noxious effects a Collaborative Research Center ”Molecular Signatures of Adaptive Stress responses” has been founded in 2012 at Jena University. I will report about these ongoing research activities. I also intend to specify first results of our consortium.

RADIATION / ENVIRONMENTAL SESSION

PRECIOUS KNOWLEDGE: THE LINEAR CONCENTRATION-RESPONSE NON-THRESHOLD MODEL AS THE GOLDEN RATIO

***Aalt Bast**, Maastricht University, The Netherlands, **Jaap C. Hanekamp**, Roosevelt Academy, Middelburg, The Netherlands*

NONLINEAR EFFECTS OF NANOPARTICLES: BIOLOGICAL VARIABILITY FROM HORMETIC DOSES, SMALL SIZES, AND DYNAMIC ADAPTIVE INTERACTIONS.

***Iris R. Bell**, University of Arizona College of Medicine, Tucson, AZ*
John A. Ives, Samueli Institute, Alexandria, VA
Wayne B. Jonas, Samueli Institute, Alexandria, VA

TWENTY-FOUR MONTHS EATING RADIUM

***Carmel Mothersill**, McMaster University, Hamilton, ON, Canada, RW Smith, McMaster University, Hamilton, ON,Canada, D Lariviere, Laval University, Quebec City, Canada, CB Seymour, McMaster University, Hamilton, ON,Canada*

THE P53/NFKB-MEDIATED METABOLIC RESPONSE TO IRRADIATION

*Rajuli Lall, Suthakar Ganapathy and **Zhi-Min Yua,** Harvard University School of Public Health, Boston, MA*

~400-FOLD NATURAL BACKGROUND RADIATION DOES NOT INDUCE DETECTABLE DNA DAMAGE *IN VIVO*

*Werner Olipitz, Dominika Wiktor-Brown, Joe Shuga, Bo Pang, Jose McFaline, Pallavi Lonka, Aline Thomas, James T. Mutamba, Massachusetts Institute of Technology, Cambridge, MA, Joel S. Greenberger, University of Pittsburgh, Pittsburgh, PA, Leona D. Samson, Peter C. Dedon, Jacquelyn C. Yanch, **Bevin P. Engelward**, Massachusetts Institute of Technology, Cambridge, MA*

THE IMPORTANCE OF RADIATION DOSE-RATE

***Jacqueline Yanch**, MIT, Cambridge, MA*

HORMESIS, GUILT AND MARKET SCIENCE

***Colin Seymour**, McMasters University, Hamilton, ON, Canada*

PRECIOUS KNOWLEDGE: THE LINEAR CONCENTRATION-RESPONSE NON-THRESHOLD MODEL AS THE GOLDEN RATIO

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For many centuries, man pursued the logic of aesthetics in architecture, art, music, and etceteras. Pyramids, temples, churches and objects of art, for instance, have been constructed following the golden ratio. Two quantities, a and b, (where a is bigger than b) are in golden ratio if the ratio of the sum of the quantities (a+b) to the larger quantity (a) is equal to the ratio of the larger quantity (a) to smaller one (b). Mathematically: $[(a+b)/a] = (a/b) = \varphi$ (phi). Numerous examples can be provided in which not only human artifacts but also nature seems to follow this golden ratio. Whether or not phi is a natural mathematical denominator in physiology is beyond the context of our paper, obviously.
Yet, toxicology seems to be under the spell of analogous logical aesthetics, especially considering the linear concentration-response non-threshold model. Comparable considerations exist: aesthetically pleasing, constant, orderly, universally applicable, and the like.

However, although the golden ratio is not underscrutiny here, the logic of the LNT is not unwavering, as there seem no physiological reasons to argument its occurrence. Rather non-linear threshold models should be assumed. The arguments are manifold and include: mild toxic responses lead to health benefit, induction of protection systems, combination of mechanisms leading to effects, and life long exposure to natural carcinogenic compounds.

Many books have been devoted to the golden ratio; many scientific papers to the linear concentration-response non-threshold model. The beauty and simplicity of the model is attractive to outline nature and to model reality. The artistic beauty of Da Vinci's Mona Lisa is arguably painted via the golden ratio. As with the Mona Lisa, we have to smile about so much ignorant perseverance to sculpture toxicology through a linear concentration-response non-threshold model. Therefore we will here critique this perseverant model with artistic history in mind.

TWENTY-FOUR MONTHS EATING RADIUM

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Purpose: Radium is a long lived actinide in the uranium decay chain. In living organisms it completes with calcium and thus can accumulate in organisms exposed to radium as a result of uranium mining and milling activities associated with the nuclear industry. Currently actinides are assigned an RBE (relative biological effectiveness) factor of between 20 and 40 but this value is highly uncertain as there are very few studies which measure biological effects following chronic exposure to low doses of radium. The aim of this study was to assess the impact of lifetime ingestion of environmentally relevant levels ^{226}Ra on a common freshwater fish species.

Methods: Fathead minnow (Pimephales promelas, Rafinesque) were obtained at the first feeding stage and established on a commercial fish food diet containing ^{226}Ra in the activity range 10mBq/g-10Bq/g. They remained on this diet for 18 months and were sampled invasively at 1,6 and 18 months to assess biochemical indices and accumulated dose and non-invasively also at 12 and 15 months to assess growth.

Results: Fish fed 10 and 100mBq/g diets showed a transitory dysregulation of growth at 6 and 12 months. Fish fed higher activities showed similar or less significant effects. Bioaccumulation at 1 month was below detection levels. At six months significant amounts of ^{226}Ra were present but at 18 months they were gone and radium levels were at background in spite of the continued ingestion of the isotope. Assessment of bystander stress signaling throughout the time period showed a constant fish to fish signaling at all times and doses measured.

Conclusions: Fathead minnow appear to bioaccumulate ^{226}Ra initially and this is associated with growth dysregulation. However after 18 months an effective purging mechanism appears to be in place. The results may be important in the assessment of long-term environmental impacts of ^{226}Ra.

THE P53/NFκB-MEDIATED METABOLIC RESPONSE TO IRRADIATION

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Because of a lack of meaningful biological outcomes for assessing the effect of low-level ionizing radiation (IR) exposure, there is great uncertainty of the potential health risk caused by low-dose irradiation. Currently, a linear, no-threshold dose model has been advocated to predict low-dose radiation-induced biological effects (Siegel and Stabin, 2012). This model assumes that the smallest dose has the potential to increase health risk and harmful effects increase as a function of exposure dose.

We investigated the effect of low-dose radiation by examining its ability to modulate the cellular response to high dose irradiation [we defined doses below 10 cGy or 0.1 Gy as low-dose radiation, according to the level set by the International Commission of Radiological Protection (ICRP.org), and 2-4 Gy as high-dose radiation that causes substantial DNA damages to cells]. Specifically, we examined whether a pretreatmentn of cells with low-dose IR (priming) could affect the subsequent high-dose (challenge) IR-induced cell killing. We focused on p53 and NFκB, two transcription factors that play an important role in determining cellular fate. We observed a functional antagonism between p53 and NFκB in mediating low dose radiation-induced protection, known as the radio-adaptive response. We expanded the crosstalk between p53 and NFκB beyond the general processes in cell survival or apoptosis to cellular metabolic programs. By inhibiting p53 activity and permitting NFκB to function, the priming dose treatment induces a metabolic shift from oxidative phosphorylation to aerobic glycolysis. We show that this shift to glycolysis is necessary for priming cells and mice to mount a defense mechanism against challenging dose IR-induced toxicity.

While both p53 and NFκB have been reported to contribute to the radio-adaptive response, there is little information on how they are involved, and even less is known regarding their interaction in regulation of cellular response to radiation. Consistent with their very distinct role in regulation of cellular fate (p53 as pro-death and NFκB as pro-survival), we observed a clear mutually exclusive interaction between these two transcription factors in that upon activation of one, the other is inhibited during the radio-adaptive response. Within the context of IR-induced apoptosis, p53 activation was dominant while NFκB was suppressed. Conversely, the protective priming dose treatment altered the interaction favoring survival as evident by a reciprocal p53 inhibition and NFκB activation. Of interest is the finding that p53 suppression seemed to be pre-requisite for NFκB activation, as shown that Nutlin-3A-induced p53 activation blocked priming dose-induced NFκB activation. As these transcription factors are critically involved in

the maintenance of homeostasis, it is not unexpected that the crosstalk between p53 and NFκB contributes to the determination of cellular fate – what is novel however, is the induction of metabolic reprogramming. Increasing evidence indicates that both p53 and NFκB participate in the regulation of cellular metabolism. p53 favors mitochondrial oxidative phosphorylation whereas NFκB stimulates aerobic glycolysis. We demonstrate that by shutting down p53 and permitting NFκB to act on the metabolic pathway, a priming dose of IR induces a metabolic shift from oxidative phosphorylation to glycolysis.

The effect of irradiation on cellular metabolic behavior is an under-explored area of radiation biology. Our study underscores the importance of cellular metabolic response to radiation (low dose irradiation in particular). Within the context of the radio-adaptive response, in addition to generating ATP more rapidly, glycolysis also provides many intermediates for *de novo* synthesis of amino acids and lipids. Moreover, increased glucose flux also stimulates the pentose pathway that produces NADPH, as an important reducing agent to counter oxidative stress, and ribose-5P as a substrate for synthesizing nucleic acids important for DNA repair. Thus, by engaging the glycolytic metabolism, cells can mount a strong protective mechanism to preserve viability in response to the damaging effects inflicted by a challenging dose of IR. Our data support a non-linear dose response to radiation, which may have important implications in assessing the health risk of radiation exposure.

~400-FOLD NATURAL BACKGROUND RADIATION DOES NOT INDUCE DETECTABLE DNA DAMAGE *IN VIVO*

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In the event of a nuclear accident, people are exposed to elevated levels of continuous low dose-rate radiation. Nevertheless, most of the literature describes the biological effects of acute radiation. Our major aim is to reveal potential genotoxic effects of low dose-rate radiation. DNA damage and mutations are well established for their carcinogenic effects. Here, we assessed several key markers of DNA damage and DNA damage responses in mice exposed to low dose-rate radiation. We studied low dose-rate radiation using a variable low dose-rate irradiator consisting of flood phantoms filled with ^{125}Iodine-containing buffer. Mice were exposed to 0.0002 cGy/min (~400X background radiation) continuously over the course of 5 weeks. We assessed base lesions, micronuclei, homologous recombination (using fluorescent yellow direct repeat [FYDR] mice), and transcript levels for several radiation-sensitive genes. Under low dose-rate conditions, we did not observe any changes in the levels of the DNA nucleobase damage products hypoxanthine, 8-oxo-7,8-dihydroguanine, 1,N^6-ethenoadenine or 3,N^4-ethenocytosine above background. The micronucleus assay revealed no evidence that low dose-rate radiation induced DNA fragmentation. Furthermore, there was no evidence of double strand break-induced homologous recombination. Finally, low dose-rate radiation did not induce Cdkn1a, Gadd45a, Mdm2, Atm, or Dbd2. Importantly, the same total dose, when delivered acutely, induced micronuclei and transcriptional responses. Together, these results demonstrate in an in vivo animal model that lowering the dose-rate suppresses the potentially deleterious impact of radiation, and calls attention to the need for a deeper understanding of the biological impact of low dose-rate radiation.

THE IMPORTANCE OF RADIATION DOSE-RATE

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The biological and clinical impact of any dose of ionizing radiation depends on the temporal rate at which that dose was received. For generating dose limits for radiation workers, regulatory bodies and advisory agencies use the Dose Rate Effectiveness Factor (DREF) to convert risk estimates generated at the very high dose-rates received by the A-bomb survivors to risk estimates that can be used at lower dose-rates. An important question is: should the DREF also be used to evaluate risks to the general public from living in an environment contaminated with radionuclides? In evaluating available experimental data, the NCRP found a determining factor in the magnitude of the dose-rate effect to be the overall irradiation time (NCRP 64). Extending the delivery time of a given dose to a sizeable fraction of the lifespan reduced the effect of that dose compared to what would be predicated using only the DREF; this is because the DREF, evaluated through studies of shorter duration, accounts just for the effects of DNA repair. For long exposures the NCRP recommends use of the Protraction Factor (PF range 6.6-12.8, mean of 10) rather than the Dose Rate Effectiveness Factor (DREF range 1.1-10, mean of 4). Unfortunately, since risk estimates for the general public are derived directly from risk estimates for radiation workers, the DREF is universally used in evaluating low dose-rate risks in any situation. We examine the range of dose-rates evaluated in published studies of tumorigenesis and longevity in animals and humans, paying particular attention to those studies cited by regulatory bodies. The experimental difficulties involved in generating dose-effect relationships at the very low dose-rates of most importance to the public, those within a factor of ~ 100 above natural background, are discussed, along with the consequences of a lack of data in this crucial dose-rate range.

HORMESIS, GUILT AND MARKET SCIENCE

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In this paper I intend to examine the proposition that hormesis remains outside the mainstream of science not for scientific reasons, but for reasons associated with authority (guilt) and the economics of market science. Market science involves using science, reduced to it's most simplistic form, to create both unnecessary demand for products and to "pinkwash" to perform the equivalent of the magician's trick of finding the Queen. Pinkwashing refers to giving prominence to Gay Rights, to distract from the general assault on all rights, and I use it here as an analogy for the marketing of "acceptable" science. "Acceptable" science either helps control populations or creates profit, whereas "unacceptable" science is concerned with choice. Science is becoming market orientated, and so market economics help direct policy and acceptable paradigms.

BIOMEDICAL I

THE IMPORTANCE OF DOSE RESPONSE FOR THE BIOLOGY OF SYNTHETIC TRITERPENOIDS

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TESTING THE HORMESIS HYPOTHESIS OF DIETARY RESTRICTION

*Chris Hine and **Jay Mitchell**, Harvard School of Public Health, Boston, MA*

NUTRIENT OVERLOAD AND DIVERGENCE IN AADAPTIVE REDOX RESPONSES BETWEEN HEART AND SKELETAL MUSCLE

***Ethan Anderson**, East Carolina University, Greenville, NC*

MOLECULAR EVENTS IN BIOPHASIC NON-GENOMIC ESTROGEN AND ANTIESTROGEN SIGNALING

*Xintian Zhang and **Zhaoyi Wang**, Creighton University Medical School, Omaha, NE*

THE BRAIN, ENERGY METABOLISM AND HORMETIC PATHWAYS TO OPTIMAL HEALTH

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CENTRAL ROLE OF THE BRAIN IN STRESS AND ADAPTATION: ALLOSTASIS AND ALLOSTATIC LOAD

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THE IMPORTANCE OF DOSE RESPONSE FOR THE BIOLOGY OF SYNTHETIC TRITERPENOIDS

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Synthetic oleanane triterpenoids are multifunctional drugs being developed for the prevention and treatment of a variety of chronic diseases driven by chronic inflammation and oxidative stress. Low nanomolar concentrations of triterpenoids inhibit the induction of inducible nitric oxide synthase (iNOS) and other inflammatory mediators in macrophages and in other immune cells. These drugs are also among the most potent known activators of the Nrf2 transcription factor. In response to oxidative stress, Nrf2 induces numerous anti-inflammatory and cytoprotective genes that can inactive the insult. Surprisingly, the induction of the Nrf2 cytoprotective pathway and the anti-inflammatory properties of the triterpenoids are directly linked. Moreover, the ability of triterpenoids to reduce the production of reactive oxygen species (ROS) in cells challenged with hydroperoxide and to inhibit iNOS is lost in cells lacking Nrf2. The concentration of triterpenoids is important, as higher concentrations (low micromolar) of these drugs increase the production of ROS and selectively induce apoptosis in cancer cells. Because cancer cells respond differently to ROS than normal cells, it might be possible to exploit these differences during therapy. Ongoing studies in experimental lung cancer models suggest that triterpenoids can a) enhance the efficacy of radiation or carboplatin/paclitaxel treatments directed against the tumors and b) simultaneously protect normal tissue from the side effects of these treatments.

TESTING THE HORMESIS HYPOTHESIS OF DIETARY RESTRICTION

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Dietary restriction (DR) encompasses a variety of regimens resulting in a reduction of total calories and specific nutrients without malnutrition. DR is best known for extending longevity in a variety of species, but also increases metabolic fitness and resistance to acute oxidative stress. In mammals, benefits of short periods of DR include protection from surgical ischemia reperfusion injury and ionizing radiation. Previously, we and others have hypothesized that the benefits of DR are derived from adaptations to the mild stress of reduced nutrient/energy intake, thus placing DR within a mechanistic framework consistent with hormesis. If so, what is the molecular nature of this mild stress, and what are the adaptations that it engenders? Here we tested the hypothesis that reactive oxygen/nitrogen species (RONS) derived from increased oxidation of fats for energy are the source of the mild stress, and that induction of Phase II antioxidant and detoxification systems through an increase in key transcription factors such as NRF2 are required for the benefits of short-term DR. To test this, mice were fed ad libitum (AL) or restricted (50% DR) for one week prior to induction of hepatic ischemia reperfusion injury (IRI) or total body ionizing radiation (TBI). Liver damage markers were used to assess protection from IRI, while loss of bone marrow cellularity and greying of the fur were used to assess protection from TBI. Consistent with the hormesis hypothesis of DR, DR-induced protection against IRI and TBI was abolished by antioxidant treatment during the period of food restriction. However, a genetic requirement for NRF2 was only observed under limited situations, suggesting either redundancy in transcriptional control of the Phase II response or an alternate mechanism of protection induced by RONS. Finally, potential translation of these findings to clinically relevant endpoints will be discussed.

NUTRIENT OVERLOAD AND DIVERGENCE IN ADAPTIVE REDOX RESPONSES BETWEEN HEART AND SKELETAL MUSCLE

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Increased knowledge of the mechanisms by which nutrient overload (i.e. high fat, high sucrose, HFHS diet) impacts the redox environment of oxidative tissues is mandatory for understanding the causes of cardiovascular and metabolic disease. We therefore conducted a study to determine if heart and skeletal muscle experience differential adaptations in redox environment to a 12-week intervention of HFHS diet in rats. Here, we report that cardiac and skeletal muscle show contrasting redox adaptations to HFHS diet and uncover a novel role for TxnRd2 in underlying these differences. Further, using a systematic experimental approach we examined the control of mitochondrial redox status by TxnRd2 when mitochondrial respiration is supported by a variety of substrates and show that this enzyme is critical to maintaining mitochondrial redox state especially during fatty acid oxidation.

The findings of this study are novel and important for a number of reasons. First, they demonstrate that the heart is a unique organ and very different from skeletal muscle in that it can positively adapt to the oxidative stress that accompanies obesity by up-regulating endogenous antioxidant/anti-inflammatory enzyme systems, while skeletal muscle cannot. In addition, these findings uncover a novel role for TxnRd2 in underlying these differences. Since insulin resistance following nutrient overload has been linked to oxidative stress, particularly in mitochondria, the findings of this study are important because they suggest that skeletal muscle's inability to adapt to the oxidative stress may, in part, underlie this pathology.

To summarize, this study is the first to report that cardiac-specific up-regulation of TxnRd2 is a mechanism by which cardiac and skeletal muscle differentially adapt to nutrient overload, and it lays the groundwork for future studies directed towards exploiting these adaptive mechanisms to prevent and treat cardiovascular and metabolic diseases.

MOLECULAR EVENTS IN BIOPHASIC NON-GENOMIC ESTROGEN AND ANTIESTROGEN SIGNALING

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A large body of evidence indicated that the effects of mitogenic estrogen signaling exhibit a non-monotonic, biphasic or the “bell” shaped dose response curve; estrogen at low concentrations, elicits a mitogenic signaling to stimulate cell proliferation while at high concentrations, estrogen inhibits cell growth. However, the molecular mechanism underlying this paradoxical effect of estrogen on cell proliferation remains largely unknown.

Recently, we reported that ER- α36, a variant of ER-α is mainly expressed near the plasma membrane and mediates biphasic, non-genomic estrogen signaling in ER-negative breast cancer cells. We decided to investigate the molecular mechanisms underlying the biphasic estrogen signaling in ER-negative breast cancer MDA-MB-231 and MDA-MB-436 cells. We found that 17β-estradiol (E2β) at l nM induced the phosphorylation of the Src-Y416 residue, an event to activate Src, while at 5 μM failed to induce Src-Y416 phosphorylation but induced Src-Y527 phosphorylation that inactivates Src. E2β at 1 nM but not at 5 μM also induced phosphorylation of the MAPK/ERK and activated Cyclin D1 promoter activity through the Src/EGFR/STAT5 pathways. In addition, E2β at 1 nM induced phosphorylation of the cell cycle inhibitor p21/WAF1 and redistribution of p21/WAF1 from the cell nucleus to the cytoplasm while E2β at 5 μM failed to do so. These molecular events were also observed in the cells treated with different concentrations of antiestrogens such as tamoxifen and ICI 182, 780, suggesting estrogen and antiestrogen share similar non-genomic signaling pathways. Knock-down of ER-α36 expression abrogated the biphasic estrogen signaling and forced expression of recombinant ER-α36 shifted the “bell” shaped dose response curve from right to left. Our results thus demonstrated that ER-α36 mediates biphasic estrogen and antiestrogen signaling in these ER-negative breast cancer cells, and Src functions as a switch of biphasic estrogen and antiestrogen signaling through the EGFR/STAT5 pathway. The biological significance of ER-α36-mediated biphasic estrogen signaling in the osteoblast and osteoclast cells during development of osteoporosis will also be discussed.

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THE BRAIN, ENERGY METABOLISM AND HORMETIC PATHWAYS TO OPTIMAL HEALTH

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The electrochemical activity of neurons in the brain controls our cognitive processes and behaviors, including energy intake and expenditure which are fundamental determinants of health and longevity. Unfortunately, the recent epidemic of sedentary and gluttonous behaviors is resulting in sharp increases in obesity, diabetes and associated diseases including cardiovascular disease, cancers, stroke and Alzheimer's disease. Our research has elucidated the cellular and molecular mechanisms responsible for the adverse effects of high energy diets and lack of exercise on brain function and vulnerability to AD, PD and stroke (Mattson MP. *Cell Metabolism*, 2012 Nov 14. doi:pii: S1550-4131(12)00402-0). On the other hand, dietary energy restriction, particularly intermittent energy restriction/fasting (IER), can protect neurons against dysfunction and degeneration in experimental models of AD, PD and stroke. Energy restriction and exercise activate adaptive cellular stress response signaling pathways (i.e., hormesis pathways) in neurons resulting in the production of neurotrophic factors, protein chaperones, DNA repair enzymes, and proteins such as PGC-1alpha that are critical for mitochondrial biogenesis (Cheng et al. *Nature Commun.* 2012 Dec 4;3:1250). Among these factors, brain-derived neurotrophic factor (BDNF) appears to be particularly important in enhancing synaptic plasticity, neurogenesis and cognitive performance. Excessive energy intake, particularly in combination with a sedentary lifestyle reduces the activation of adaptive cellular stress response pathways thereby rendering neurons vulnerable to dysfunction and degeneration. More recently, we have discovered that BDNF signaling in the brain improves cardiovascular function and peripheral glucose metabolism, and may mediated the beneficial effects of exercise and IER on overall health and risk for cardiovascular disease and diabetes. Our societies are therefore faced with a major challenge: implement hormesis-based IER- and exercise-based prescriptions for brain health beginning in young adulthood, or endure a continuing epidemic of poor general health and neurodegenerative disorders for the foreseeable future.

CENTRAL ROLE OF THE BRAIN IN STRESS AND ADAPTATION: ALLOSTASIS AND ALLOSTATIC LOAD

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The brain is the central organ of stress because it interprets what is stressful and also determines behavioral and physiological responses. Besides major life events, the aggravations of daily life elevate activities of physiological systems so as to cause some measure of wear and tear. We call this "allostatic load", and it reflects not only the impact of life experiences but also of genes; individual life-style habits reflecting items such as sleep quality and quantity; diet, exercise and substance abuse; adverse early life experiences that set life-long patterns of behavior and physiological reactivity; and exposure to toxic agents in the environment. Hormones associated with stress and allostatic load protect the body in the short-run and promote adaptation ("allostasis"), but in the long run overuse and dysregulation of allostasis causes changes in the body and brain that lead to disease ("allostatic load/overload"). Mediators of allostasis include autonomic nervous system activity, glucocorticoids and pro- and anti-inflammatory cytokines and these operate as a non-linear network, with each mediator capable of biphasic effects and regulating the other mediator systems. The brain is a target of stress and stress and stress hormones produce both adaptive and maladaptive effects on the brain throughout the life course. The amygdala is important in fear and strong emotions and the prefrontal cortex is involved in attention, executive function and working memory while hippocampus mediates spatial and episodic memory. Hippocampal and medial prefrontal cortical neuron dendrites become shorter and less branched and dentate gyrus neurogenesis is suppressed by repeated stress, whereas amygdala and orbitoprefrontal cortical neurons show hypertrophy after repeated stress. Repeated stress as well as circadian disruption, as in jet lag and shift work, promote structural remodeling of brain circuits and impair cognitive function as well as causing systemic allostatic load.

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BIOMEDICAL II

ASTROCYTE PLASTICITY REVEALED BY ADAPTATIONS TO SEVERE PROTEOTOXIC STRESS

***Rehana Leak**, Amanda M. Titler, Jessica M. Posimo, Duquesne University, Pittsburgh, PA*

NON-MONOTONIC DOSE RESPONSES IN STUDIES OF ENDOCRINE DISRUPTING CHEMICALS: BISPHENOL A AS A CASE STUDY

***Laura Vandenberg**, Tufts University, Medford, MA*

ANTIBIOTIC-INDUCED BIOFILM FORMATION

***Jeffrey Kaplan**, American University, Washington DC*

APPLICATION OF THE DISREGULATED ADAPTIVE HYPERPLASIA (DAH) TUMORIGENESIS MODEL TO ESTIMATE LOW-DOSE DIBENZO[A,L]PYRENE TUMOR RISK

***Kenneth T. Bogen**, Exponent, Oakland, CA*

ASTROCYTE PLASTICITY REVEALED BY ADAPTATIONS TO SEVERE PROTEOTOXIC STRESS

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Neurodegeneration is characterized by an accumulation of misfolded proteins in neurons. It is less well appreciated that glial cells often also accumulate misfolded proteins. However, glia are highly plastic and may adapt to stress more readily. Such endogenous adaptations to stress can be measured by challenging stressed cells with a second hit and then measuring viability. For example, exposure of astrocytes to subtoxic stress protects them against subsequent challenges. This phenomenon, termed preconditioning, is consistent with hormetic responses to sublethal stress. However, it is not known whether *toxic* stress that kills half the population can elicit adaptations in the cells that manage to survive. Glia, with their resilient nature, offer an ideal model in which to test this new hypothesis. In the present study, primary astrocytes were challenged with two hits of severe stress from the proteasome inhibitor MG132. Astrocytes surviving one LC_{50} hit were rendered resistant to a second LC_{50} hit. ATP loss in response to the second hit was also prevented. MG132 caused compensatory rises in stress-sensitive heat shock proteins. However, stressed astrocytes exhibited an even greater rise in ubiquitin-conjugated proteins upon the second hit, illustrating the severity of the stress and verifying the continued impact of MG132. Despite this stress, MG132-pretreated astrocytes were completely prevented from losing glutathione in response to the second hit. Furthermore, inhibiting glutathione synthesis rendered astrocytes sensitive to the second hit, unmasking the cumulative impact of two hits by removal of an endogenous adaptation. These findings suggest that stressed astrocytes become progressively harder to kill by virtue of thiol defenses. Glial plasticity may permit stressed astrocytes to better fulfill their neurosupportive roles. Similar adaptations to severe stress in the human brain may explain the delayed onset and protracted nature of neurodegenerative diseases.

NON-MONOTONIC DOSE RESPONSES IN STUDIES OF ENDOCRINE DISRUPTING CHEMICALS: BISPHENOL A AS A CASE STUDY

Laura N. Vandenberg, *Center for Regenerative & Developmental Biology, Tufts University, Medford, MA USA*

Non-monotonic dose response curves (NMDRCs) have been demonstrated for natural hormones and endocrine disrupting chemicals (EDCs) in a variety of biological systems including cultured cells, whole organ cultures, laboratory animals and human populations. There are several mechanisms responsible for these NMDRCs, most of which are related to the interactions between the ligand (hormone or EDC) and a hormone receptor. In spite of the hundreds of examples of NMDRCs in the EDC literature, there are still claims that they are not reproducible. Here, we chose bisphenol A (BPA), a well-studied EDC, to examine the non-monotonic responses that have been reported. We analyze the types of endpoints that produce NMDRCs *in vitro*, the strength of the U- and inverted U-shaped curves, and the mechanisms that have been proposed for each example. Our results indicate that NMDRCs are relatively common in the BPA literature, that there are several reproducible examples of non-monotonicity, and that there are often explanations available when studies fail to identify NMDRCs in endpoints that have been implicated in other studies. Taken together, these results provide strong evidence for NMDRCs in the EDC literature, and question the current risk assessment practice where ‘safe’ low doses are predicted from high dose exposures.

ANTIBIOTIC-INDUCED BIOFILM FORMATION

***Jeffrey B. Kaplan**, Department of Biology, American University, Washington DC*

Surface-attached colonies of bacteria known as biofilms play a role in the pathogenesis many chronic infections. Biofilm colonies are notorious for their resistance to high concentrations of antibiotics. We found that low concentrations of some antibiotics can act as agonists of bacterial biofilm formation in vitro. The fundamental nature of the dose response is biphasic and is characterized by low-dose stimulation of biofilm formation and high-dose inhibition. Some antibiotics can act as antagonists of biofilm formation at low levels, agonists at higher levels, and antagonists at still higher levels. These U-shaped and multiphasic dose response relationships are characteristic of many chemicals, drugs, hormones, biological molecules and physical stressors. Low-dose stimulation of bacterial biofilm formation may help explain the recalcitrance of some bacterial infections to antibiotic treatment in clinical settings and the evolution of antibiotic-resistant bacteria in agricultural settings.

APPLICATION OF THE DISREGULATED ADAPTIVE HYPERPLASIA (DAH) TUMORIGENESIS MODEL TO ESTIMATE LOW-DOSE DIBENZO[*A*,*L*]PYRENE TUMOR RISK

***Kenneth T. Bogen**, Exponent, Health Sciences, Oakland, CA 94612, Tel: 925-268-5048, Fax: 925-268-5099, Email: kbogen@exponent.com*

Linear no-threshold (LNT) risk extrapolation for chemical carcinogens is consistent with multistage somatic mutation cancer theory—e.g., the Moolgavkar-Venzon-Knudson (MVK) risk model—but not necessarily with theories positing that other types of tumorigenic events are critical, such as aberrant DNA methylation or (most recently, based on rapidly expanding experimental and clinical evidence) microRNA dysregulation (MRD). MRD theory in particular suggests that most current theories are consistent with a new, dysregulated adaptive hyperplasia (DAH) theory, which predicts that: (i) all tumors arise most efficiently under stress conditions that induce and sustain either of two specific states of adaptive hyperplasia (AH), protective or regenerative; and (ii) if mutagenically dysregulated, these AH states may yield benign or malignant tumors, respectively (*Med Hypotheses* 2013; 80(1):83–93). The MVK and DAH risk models each provide excellent fits to markedly nonlinear ED_{001} study data on increased liver and stomach tumor incidence among >40,000 ~2-month-old trout fed 0–225 ppm dietary dibenzo[*a*,*l*]pyrene (DBP) for 4 weeks, followed by 0 ppm for 9 months. The MVK fit obtained to each tumor type implies that DBP is a pure (non-mutagenic) promoter, which is surprising because DBP is one of the most potent chemical mutagens known. Nevertheless, each MVK fit implies an LNT increase in risk for each tumor type induced by low-dose DBP. In contrast, the DAH fits obtained each imply a corresponding increased low-dose risk that is $(1/q)$-fold less than that predicted by the corresponding MVK fit, where $0 \le q \le 1$ and q is proportional to the effective DBP potency for increasing the rate of the single, critical mutation assumed by the DAH model. Because the true value of q cannot be estimated reliably from ED_{001}-type data, improved mechanistic data and theory will clearly be pivotal to more accurate risk extrapolation for chemical carcinogens.

POSTERS

Origin of the Linearity-No Threshold (LNT) Dose Response Concept
***Edward J. Calabrese**, University of Massachusetts, Amherst, MA*

Low Dose Radiation Therapy (LD-RT) is Effective in the Treatment of Arthritis: Animal Model Findings
***Edward J. Calabrese**, University of Massachusetts, Amherst, MA, Vittorio Calabrese, University of Catania, Catania, Italy*

The Historical Use of Radiotherapy in the Treatment of Sinus Infections
***Edward J. Calabrese**, Gaurav Dhawan, University of Massachusetts, Amherst, MA*

Application of Radiation Hormesis to Resolve Metastatic Disease in Late-Stage Cancer Patients
***Mohan Doss**, Fox Chase Cancer Center, Philadelphia, PA*

Towards a Mechanistic Understanding of Hormetic Responses and their Regulation in Arabidopsis
***Thomas Eulgem**, University of California at Riverside, Riverside, CA, Melinda-Rodriguez-Salus, University of California at Riverside, Riverside, CA, Yasemin Bektas, University of California at Riverside, Riverside, CA, Trang Vu, University of California at Riverside, Riverside, CA*

A role for Annexin II and Ca^{2+} Signalling in Human Epithelial Cells by Low Doses of X-ray Radiation in a Radiation-Induced Bystander Effect
***Hayley Furlong**, Dublin Institute of Technology, Dublin, Ireland, Richard Smith, McMaster University, Hamilton, ON, Canada, Jiaxi Wang, Queen's University, Kingston, ON, Canada, Colin Seymour, McMaster University, Hamilton, ON, Canada, Carmel Mothersill, McMaster University, Hamilton, ON, Canada, Orla Howe, Dublin Institute of Technology, Dublin, Ireland*

Association of Hypertension Mortality Rates with Concentrations of Chiropractors and Medical Doctors in the U.S., 2007-2009
***John F. Hart,** Sherman College of Chiropractic, Department of Research, Spartanburg, SC*

EPA's Stage 2 Disinfection Byproducts Rules: Scientific Efficacy and Economic Impact for Northern Kentucky
***Hugh Henry**, Northern Kentucky University, Highland Heights, KY*

Antibiotic-induced Biofilm Formation
***Jeffrey Kaplan**, American University, Washington DC*

Astrocyte Plasticity Revealed by Adaptations to Severe Proteotoxic Stress
***Rehana Leak**, Amanda M. Titler, Jessica M. Posimo, Duquesne University, Pittsburgh, PA*

DOSE NOT EXPOSURE CONCENTRATION MAKES THE POISON – AQUATIC TOXICITY UNDER CHANGING WATER CONCENTRATIONS

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HORMETIC REACTION PATTERN OF MICROGLIA

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REGULATION OF AUTOPHAGY BY AMPK IN ENDOTHELIAL CELLS

***Katrin Spengler**, Jena University Hospital, Jena, Germany, Regine Heller, Jena University Hospital, Jena, Germany*

PHYSIOLOGY FROM THE PERSPECTIVE OF CONTROL: WHY EXPECT HORMESIS: HOW TO INTEGRATE DIVERSE EXPOSURES

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ORIGIN OF THE LINEARITY-NO THRESHOLD (LNT) DOSE RESPONSE CONCEPT

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This paper identifies the origin of the linearity at low dose concept [i.e., linear no Threshold (LNT)] for ionizing radiation induced mutation. After the discovery of X-ray induced mutations, Olson and Lewis (1928) proposed that cosmic/terrestrial radiation-induced mutations provide the principal mechanism for the induction of heritable traits, providing the driving force for evolution. For this concept to be general, a LNT dose relationship was assumed, with genetic damage proportional to energy absorbed. Subsequent studies suggested a linear dose response for ionizing radiation induced mutations (Hanson and Heys 1929; Oliver 1930), supporting the evolutionary hypothesis. Based on an evaluation of spontaneous and ionizing radiation induced mutation with *Drosophila*, Muller argued that background radiation had a negligible impact on spontaneous mutation, discrediting the ionizing radiation based evolutionary hypothesis. Nonetheless, an expanded set of mutation dose response observations provided a basis for collaboration between theoretical physicists (Max Delbruck and Gunter Zimmer) and the radiation geneticist Nicolai Timoféeff-Ressovsky. They developed inter-related physical science based genetics perspectives including: a biophysical model of the gene, a radiation induced gene mutation target theory and the single hit hypothesis of radiation induced mutation, which, when integrated, provided the theoretical mechanism and mathematical basis of the LNT model. The LNT concept became accepted by radiation geneticists and recommended by national/international advisory committees for risk assessment of ionizing radiation induced mutational damage/cancer from the mid 1950s to the present. The LNT concept was later generalized to chemical carcinogen risk assessment and used by public health and regulatory agencies worldwide.

LOW DOSE RADIATION THERAPY (LD-RT) IS EFFECTIVE IN THE TREATMENT OF ARTHRITIS: ANIMAL MODEL FINDINGS

***Edward J. Calabrese**, Ph.D., School of Public Health and Health Sciences, Department of Public Health, Morrill I, N344, University of Massachusetts, Amherst, MA 01003, Tel: 413-545-3164, Fax: 413-545-4692, Email: edwardc@schoolph.umass.edu, Vittorio Calabrese, Ph.D., University of Catania, Department of Chemistry, Viale Andrea Doria, 95100 Catania, Italy, Tel: 39-95-738-4067, Email: calabres@unict.it*

Purpose: This paper provides a critical assessment of the hypothesis that low doses of ionizing radiation may be potentially effective in the treatment of inflammatory conditions, with particular focus on arthritis.

Materials and Methods: A critical review of the biomedical literature was undertaken to assess whether low doses of ionizing radiation may affect the progression of experimentally induced arthritis using multiple animal models.

Results: The findings indicate that low doses of ionizing radiation were effective in alleviating the occurrence of clinical symptoms of arthritis in five complementary experimental models of arthritis.

Conclusions: Consistent findings by multiple research groups indicate that low doses of ionizing radiation can be highly effective in reducing a broad range of arthritic changes in multiple animal models in a manner quantitatively similar to that of well known pharmaceutical agents.

The Historical Use of Radiotherapy in the Treatment of Sinus Infections

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The aim of this paper is to assess the historical role of radiotherapy in the treatment of sinus infections. This assessment involved a literature based review of how radiotherapy was used to treat sinus infections in the first half of the 20th century. Low doses of x-rays were used with considerable success to treat nearly 3,000 patients of sinus infection in a span of 12 years with these cases being reported in leading medical journals as case studies. The mechanism of x-ray induced reduction of inflammation and increased tissue repair is uncertain but appears to be related to the development of a multifactorial and integrative anti-inflammatory phenotype.

APPLICATION OF RADIATION HORMESIS TO RESOLVE METASTATIC DISEASE IN LATE-STAGE CANCER PATIENTS

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The occasional unexpected cure of distant untreated metastatic lesions, known as abscopal effect, has been reported in case reports of radiation therapy (RT) of primary tumors. The abscopal effect has been shown to be associated with increased immune response. Since high dose radiation (HDR) is known to suppress the immune system, and low dose radiation (LDR) elevates it, the increased immune response from RT is most likely due to the incidental LDR to parts of body during RT of the primary tumor. The rarity of the abscopal effect may be because of the small volume of the body usually subjected to LDR and the usual dominance of the immune-suppressing effect of HDR during RT. This indicates total-body or half-body LDR that is known to elevate the immune system response may induce the abscopal effect more reliably and lead to the resolution of the metastases. Other methods of elevation of immune system are known to have a cancer preventive or curative effect. Vigorous exercise, which is known to stimulate the immune system, is associated with reduced metastases and cancer mortality in patients diagnosed with several types of cancers. Remission of metastatic lesions has been reported in patients after acute infections that triggered immune response. Clinical trials are needed to confirm the effectiveness of LDR in resolving metastatic disease in late-stage cancer patients. Treatment of such patients with LDR would be much preferable to the presently used chemotherapies which can have adverse side effects. If LDR is able to resolve the metastases, it may result in reducing cancer mortality rates, since metastasis is known to be the cause of about 90% of the cancer deaths. Success in these clinical trials can debunk the widespread myth of carcinogenicity of LDR and naturally lead to the acceptance of LDR for cancer prevention.

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TOWARDS A MECHANISTIC UNDERSTANDING OF HORMETIC RESPONSES AND THEIR REGULATION IN ARABIDOPSIS

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Hormesis is as common among plants as it is among animals. In the vast majority of cases "growth" or "metabolic rate" were found to be endpoints affected by low-doses of hormetic agents in plants. Consequently, plant hormesis has been considered to be useful for the enhancement of crop yield in agriculture. Despite its potential significance for commercial crop production, however, the mechanistic basis of plant hormesis is completely unclear. The vast majority of plant hormesis-related phenomena have been reported in crop species and no systematic studies addressing the mechanistic basis of hormesis seem to have been performed using the main plant model system *Arabidopsis thaliana* (Arabidopsis). Arabidopsis is a highly developed molecular genetics/functional genomics model system. It is easy to transform and a large set of functional genomics-related tools are available for this species, such as genome-wide sequence indexed T-DNA mutant collections and a full sequenced genome.

We observed that low doses of synthetic plant defense elicitors trigger strong hormetic effects in Arabidopsis resulting in strongly enhanced growth of roots, while high doses of these compounds inhibit root growth. Synthetic elicitors are drug-like compounds that activate plant immune responses and are structurally distinct from natural plant defense inducers. Using mRNA-seq we are planning to profile transcriptional responses triggered by two distinct hormetic agents in Arabidopsis. Transcriptional signatures uncovered by these analyses are likely to uncover regulatory processes possibly controlling plant hormesis. Candidate genes representing such hypothetical control mechanisms will be tested for their significance in plant hormesis using Arabidopsis T-DNA mutants.

A ROLE FOR ANNEXIN II AND CA^{2+} SIGNALLING IN HUMAN EPITHELIAL CELLS BY LOW DOSES OF X-RAY RADIATION IN A RADIATION-INDUCED BYSTANDER EFFECT

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It is thought that the radiation-induced bystander effect is due to a bystander factor secreted in the medium post irradiation. This research project attempts to identify the key proteomic changes when a human skin cell line is exposed to low dose irradiated cell conditioned media (ICCM) from directly radiated Fish, both of which are good reporters of the bystander effect. The ICCM contains the radiation –induced bystander factors produced from the *in vivo* response in the fish model used. Explant cultures generated from the exposed Rainbow Trout were exposed to low doses of X-ray radiation (Mothersill et al, 1990). Proteomic methods using 2D gel electrophoresis and mass spectroscopy were employed to screen for proteins which were significantly over-or under–expressed in the recipient HaCaT cells. From the proteomic screen the results revealed an increase of expression of Annexin II, a Ca^{2+} dependent phospholipid binding protein associated with reduced apoptosis and is increased in the majority of cancers. There was a decrease in expression of Rho-GDP-dissociation inhibitor II which is known for its protective properties against reactive oxygen damage in cells. The proteomic changes reported here indicate immediate damage and no long term protection to subsequent radiation exposure. Further to the discovery of key proteins involved, we have uncovered critical and novel information detailing the signalling events that occur in the indirect response of low dose radiation in HaCaT's.

ASSOCIATION OF HYPERTENSION MORTALITY RATES WITH CONCENTRATIONS OF CHIROPRACTORS AND MEDICAL DOCTORS IN THE U.S., 2007-2009

***John F. Hart**, Sherman College of Chiropractic, Department of Research, P.O. Box 1452, Spartanburg, SC 29615, Tel: 864-578-8770, Email: jhart@sherman.edu*

As concentration (dose) of health care providers increases, health outcomes (responses) are expected to be favorable (e.g., decrease in mortality rates). Accordingly, this data-driven, ecological study compares concentrations of two types of health care practitioners, doctors of chiropractic (DC) and medical doctors (MD) in 2007 in the U.S. to 2007-2009 hypertension mortality rates by state. The condition of hypertension was selected because there is some evidence that improvement in hypertension morbidity may occur following chiropractic care, presumably by way of neurological pathways in the spine. Practitioner concentrations were calculated by dividing their total practitioner numbers by total population numbers by state (including District of Columbia) in 2007, and then multiplying this number by 10,000 to arrive at a practitioner ratio per 10,000 population. The 51 DC ratios and 51 MD ratios were separately compared to the hypertension mortality rates for each state using Spearman correlation and linear regression (for statistically significant correlations). DC ratios revealed a stronger inverse (beneficial) association with hypertension death rates ($r = -0.397$, $p = 0.0040$) compared to MD ratios ($r = 0.178$ with an observed outlier and $r = 0.132$ without the outlier, both correlations not statistically significant). Linear regression revealed that an average national decrease of 13.8 (95% CI = 6.7 to 20.8) hypertension deaths per 100,000 population would be expected with a national increase of one DC per 10,000 population within the range in this study (1.0 to 5.22 DCs per 10,000 population). Limitations to the study are its ecological design, where populations rather than known individuals are studied. Since this is an observational study, causal inference is not claimed. The study is intended as a first step for further, more rigorous research.

EPA'S STAGE 2 DISINFECTION BYPRODUCTS RULES: SCIENTIFIC EFFICACY AND ECONOMIC IMPACT FOR NORTHERN KENTUCKY

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EPA's Stage 2 Disinfection Byproducts Rules (DBPR) will cause a water rate increase of over 25% in Northern Kentucky while providing miniscule (if any) benefit. Hence an economic and scientific analysis was undertaken in light of President Obama's "Scientific Integrity" directive and his call to eliminate costly and unnecessary government regulations.

The rules will reduce concentrations of nine chlorine disinfection byproducts (DBPs), but the cost far outweighs any benefit. The maximum estimated benefit is to eliminate ~0.49% of new bladder cancers, which is too small to measure and hence impossible to confirm – yet is almost certainly overstated. Using EPA's standards, failure to implement the rules will not cause "unreasonable risk to health" (URTH). The rules will cost Northern Kentuckians ~$100/household annually – 100 times EPA's estimated average cost (<$1.00/household). The cost to implement is ~200 times the maximum treatment cost savings.

EPA fails to establish a dose-response link between chlorinated water and cancer, and there are problems with the underlying science for Stage 2 DBPR. EPA's epidemiological data are inconsistent and contradictory, probably skewed toward "positive" results, and suggestive of different cancer sites than animal studies. In their analysis, EPA threw out data showing no cancer risk from chlorinated water. EPA also set allowable levels almost 60% below the sum of levels EPA considers safe for individual DBPs. Two international agencies disagree with EPA's conclusions.

EPA may be using the wrong dose response model. Stage 2 DBPR is based on the Linear No Threshold (LNT) model, but LNT has been proven wrong with one of the nine regulated DBPs, 83% of EPA's epidemiological data show a statistical possibility that DBPs cumulatively might follow the threshold or hormetic models, and LNT in general is not as accurately predictive in the low-dose zone as other dose response models.

ANTIBIOTIC-INDUCED BIOFILM FORMATION

Jeffrey B. Kaplan, *Department of Biology, American University, Washington DC*

Surface-attached colonies of bacteria known as biofilms play a role in the pathogenesis many chronic infections. Biofilm colonies are notorious for their resistance to high concentrations of antibiotics. We found that low concentrations of some antibiotics can act as agonists of bacterial biofilm formation in vitro. The fundamental nature of the dose response is biphasic and is characterized by low-dose stimulation of biofilm formation and high-dose inhibition. Some antibiotics can act as antagonists of biofilm formation at low levels, agonists at higher levels, and antagonists at still higher levels. These U-shaped and multiphasic dose response relationships are characteristic of many chemicals, drugs, hormones, biological molecules and physical stressors. Low-dose stimulation of bacterial biofilm formation may help explain the recalcitrance of some bacterial infections to antibiotic treatment in clinical settings and the evolution of antibiotic-resistant bacteria in agricultural settings.

ASTROCYTE PLASTICITY REVEALED BY ADAPTATIONS TO SEVERE PROTEOTOXIC STRESS

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Neurodegeneration is characterized by an accumulation of misfolded proteins in neurons. It is less well appreciated that glial cells often also accumulate misfolded proteins. However, glia are highly plastic and may adapt to stress more readily. Such endogenous adaptations to stress can be measured by challenging stressed cells with a second hit and then measuring viability. For example, exposure of astrocytes to subtoxic stress protects them against subsequent challenges. This phenomenon, termed preconditioning, is consistent with hormetic responses to sublethal stress. However, it is not known whether *toxic* stress that kills half the population can elicit adaptations in the cells that manage to survive. Glia, with their resilient nature, offer an ideal model in which to test this new hypothesis. In the present study, primary astrocytes were challenged with two hits of severe stress from the proteasome inhibitor MG132. Astrocytes surviving one LC_{50} hit were rendered resistant to a second LC_{50} hit. ATP loss in response to the second hit was also prevented. MG132 caused compensatory rises in stress-sensitive heat shock proteins. However, stressed astrocytes exhibited an even greater rise in ubiquitin-conjugated proteins upon the second hit, illustrating the severity of the stress and verifying the continued impact of MG132. Despite this stress, MG132-pretreated astrocytes were completely prevented from losing glutathione in response to the second hit. Furthermore, inhibiting glutathione synthesis rendered astrocytes sensitive to the second hit, unmasking the cumulative impact of two hits by removal of an endogenous adaptation. These findings suggest that stressed astrocytes become progressively harder to kill by virtue of thiol defenses. Glial plasticity may permit stressed astrocytes to better fulfill their neurosupportive roles. Similar adaptations to severe stress in the human brain may explain the delayed onset and protracted nature of neurodegenerative diseases.

EMISSION OF ULTRAVIOLET RADIATION FROM HUMAN KERATINOCYTES IS A POTENTIAL BYSTANDER STRESS MECHANISM

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The bystander effect is a phenomenon of particular importance at low radiation doses, although the various mechanisms by which it occurs are not yet fully understood. The objective of elucidating potential bystander stress mechanisms, therefore, has become the primary focus of this research venture. Upon exposure of human keratinocyte cells to low-LET beta radiation, ultraviolet A (UVA) photon emission from irradiated cells was quantified using a single photon counting apparatus. The quantity of UV photon emission from cells was observed to be significantly greater than those emitted at background level. A lack of correlation between the cell density and the quantity of UV photon emission was observed. It is interesting to note that the independence of the UV emission signal from the cell density corresponds with results found in bystander experiments where clonogenic survival was also observed to be independent of the cell density after a threshold of approximately 100 000 cells had been reached. Based upon this initial observation and the knowledge of ultraviolet radiation's ability to induce DNA damage, it is hypothesized that UV emission subsequent to primary cell irradiation may be a culprit in inducing damage in neighboring cells that were not directly traversed by the primary beta particles. A number of endpoints will be assessed to validate our hypothesis. Among those endpoints will be appreciation of reactive oxygen species – detection of reactive oxygen species within the cell population will be representative of UV-induced damage as UVA incidence upon cells has been known to produce reactive oxygen species; UV quantification in unirradiated cell populations following medium transfer; and micronucleus identification to assess damage induced in the exposed cell population. The significance of these experiments is seen as a prospective pathway to uncovering a novel bystander stress mechanism.

DOSE NOT EXPOSURE CONCENTRATION MAKES THE POISON – AQUATIC TOXICITY UNDER CHANGING WATER CONCENTRATIONS

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Exposure concentrations that change with time are the norm for field exposures and for studies attempting to simulate field conditions. However, this can create ambiguity in defining the actual dose (the toxicant concentration at the site of action). Using theoretical and case studies, we review dynamic contaminant exposures to aquatic organisms to investigate variables important for interpreting toxicity. Because the magnitude and timing of peak absorbed concentrations change with exposure dynamics, interpreting the toxic response is generally limited to a specific experiment. Extrapolation to other experiments with different exposure dynamics, or to the field where exposure dynamics can vary, requires substantial information about the toxicokinetics and the exposure dynamics along with temporal threshold data for extrapolation between studies. This is particularly true for mixture exposures, where the concentration and composition and, therefore, the timing and magnitude of exposure to individual components of different potential potency can vary. For the short term, initial water concentration can be considered a conservative measure of exposure, although the extent of conservatism will vary with the dynamics of exposure and the toxicokinetics of the chemicals of interest. A better metric for interpreting toxicity is the peak absorbed dose, although this neglects toxicodynamics and requires repeated measures of accumulated dose over time so that the peak concentration can be determined. Aquatic toxicology studies are needed that develop temporal thresholds for absorbed toxicant doses. Such studies would allow for better extrapolation between conditions of dynamic exposure. Improved experimental designs are also needed, including high quality temporal measures of both the exposure and the absorbed dose.

CROSSTALK BETWEEN BYSTANDER AND DIRECTLY IRRADIATED CELLS: EVIDENCE FOR BYSTANDER CELL CONDITIONED MEDIUM (BCCM) MEDIATING A RESPONSE IN HUMAN KERATINOCYTES HACAT CULTURES

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It has been widely accepted that the production of bystander signals from irradiated cells leads to considerable changes in clonogenic survival within nearby non-irradiated cells. In the present study, our primary objective was to investigate whether bystander cells reciprocate a similar fate onto neighbouring irradiated cells. Such an effect is similar to biological crosstalk between common signalling proteins or candidates used in different signal transduction pathways. From mechanistic studies, there is data suggesting the involvement of several signalling candidates and signalling pathways, such as mitogen-activated protein kinase (MAPK) pathways, for bystander effects. For this study, a medium transfer clonogenic assay was developed and used to detect colony-forming changes between bystander and irradiated cells. Donor flasks with human keratinocytes HaCaT cells were irradiated (0.5 Gy) using the Cs-137 source at McMaster University. Irradiated cell conditioned medium (ICCM) was transferred to recipient HaCaT cells 1 hour post-irradiation. To test whether bystander cells emit signals to directly irradiated cells, two sets of directly irradiated flasks—control and crosstalk flasks—were set-up and irradiated with the Cs-137 source. Bystander cell conditioned medium (BCCM) was transferred to crosstalk flasks (directly irradiated cells) after the ICCM was exposed to the bystander cells for 30 minutes and 2 hours. ICCM and BCCM resulted in a significant reduction in clonogenic survival for the recipient (bystander cells) and crosstalk flasks (directly irradiated cells), respectively. Our findings suggest there may be a crosstalk between bystander and directly irradiated human keratinocytes. Future work will examine whether such a phenomenon occurs with various tumor and normal cell lines. Further investigations may have implications for understanding the magnitude of bystander effects *in vivo*—by studying whether healthy tissues emit bystander signals back onto tumor tissues.

HORMETIC REACTION PATTERN OF MICROGLIA

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Phosphoinositide 3-kinases (PI3K) belong to the family of lipidkinases, which play an important role in the control of several cellular processes like migration, proliferation and differentiation. Microglia, the macrophages of the brain execute important functions during inflammatory processes in the central nervous system. Here we attempted to investigate the role of the PI3K species PI3Kγ in inflammatory functions of microglial cells. These processes are triggered by PAMPs (pathogen associated molecular pattern molecules) or DAMPs (damaged associated molecular pattern molecules) and lead to the activation of microglia cells in a dose dependent manner. As a consequence they change their morphology, produce cytokines, express their phagocytic function and release reactive oxygen species.

First we performed *in vitro* comparative studies of phagocytotic activity of primary microglia from wild type, PI3Kγ *knockout* and PI3Kγ *knockin* (kinase-inactive) mice. Our data indicate that microglia express the signaling protein PI3Kγ in a dose dependent manner after PAMP- or DAMP-stimulation (Lipopolysaccharide-LPS, Uridindiphosphate-UDP). We could also show that LPS and UDP in a dose dependent manner affect the phagocytotic activity and generation of reactive oxygen species of wild type microglia contrasting to the functional pattern of microglia prepared from PI3Kγ *knockout* and PI3Kγ *knockin* (kinase-inactive) mice.

The signaling protein PI3Kγ plays also an essential role in the control of proliferation. After stimulation with low doses of LPS only wild type microglia showed an increased proliferation and vitality in contrast to high LPS-doses or untreated cells. PAMP-treatment of PI3Kγ *knockout* and PI3Kγ *knockin* (kinase-inactive) microglia did not affect proliferation. Together these data reveal a hormesis like reaction pattern of microglial proliferation, phagocytosis and ROS production induced by increasing doses of PAMPS and DAMPS. Our data indicate a central function of PI3Kγ as an essential mediator of all these processes.

REGULATION OF AUTOPHAGY BY AMPK IN ENDOTHELIAL CELLS

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5'AMP-activated protein kinase (AMPK) is an important intracellular energy sensor. Once activated it inhibits ATP-consuming processes while ATP-generating pathways are stimulated. AMPK is also involved in cellular stress protection by reducing oxidative stress (inhibition of NADPH oxidase, upregulation of antioxidative defense enzymes) and attenuation of inflammatory and ER stress (inhibition of NFkB, activation of SERCA). In several cell types AMPK has been shown to be involved in the regulation of autophagy which plays a crucial role in stress protection and intracellular homeostasis. The present study was aimed at investigating the role of AMPK in the regulation of autophagy in endothelial cells.

Experiments were performed in human umbilical vein endothelial cells (HUVEC). The extent of autophagy was analysed using the autophagic marker LC3B which is conjugated upon activation of autophagy and accumulates at autophagosomal membranes. In addition, processes known to initiate autophagy such as phosphorylation of ULK1 at serine 555 and inhibition of the mTOR pathway were studied. Our data show that AMPK activation by three different approaches (metabolic stress induced by 2-deoxyglucose, AICAR, which mimics AMP effects, and A769662, a novel AMPK activator) induces a transient activation of LC3B conjugation as measured in Western blots and immunofluorescence analyses. In addition, phosphorylation of ULK1 and a decreased phosphorylation of p70S6 kinase, a downstream target of mTOR, were observed in response to all three stimuli. These data indicate that AMPK is able to activate autophagic pathways, which may contribute to maintain the intracellular homeostasis in stress situations in endothelial cells. However, the stimulation of autophagy in response to AMPK agonists was transient and at later time points an inhibition of autophagy was seen. Thus, AMPK may also have counterregulatory roles in this process thereby protecting cells from overstimulation of autophagy and thus autophagic cell death.

PHYSIOLOGY FROM THE PERSPECTIVE OF CONTROL: WHY EXPECT HORMESIS: HOW TO INTEGRATE DIVERSE EXPOSURES

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Many organisms exhibit hormesis, where a low dose of an agent detrimental at higher doses is not only tolerated, but increases phenotype vigor. Hormesis is the necessary and expected emergent behavior of all organisms with capacity to respond to the environment and for all environmental influences that elicit any response at any dose. This behavior follows from control system stability requirements (the control system that adapts phenotype to environment). This necessary stability results in hormesis in the low dose range. If hormesis is not observed, then the dose is not in the low dose range.

Hormesis is not a property of a compound or an exposure, hormesis is a property of physiology.

The underlying control system is non-monotonic and non-linear, therefore perturbations to that non-linear system produce non-linear effects. To the extent that different physiological pathways are coupled and are working together "in sync", there can be only one synchronizing control signal. It is effects on this singular control signal that produce the cross-talk observed in physiology which results in cross-tolerance between different stressors.

This poster suggests that this singular synchronizing signal is NO/NOx also known as nitric oxide in its myriad forms and defined as "compounds containing N coordinated to O". Because NO/NOx is already in the active control range, perturbations to the background NO/NOx level produce changes in outcomes with no threshold.

Organisms have limited capacity to cope with detrimental agents. Integrating the effects (observed and unobserved) of all agents, xenobiotics, ionizing radiation, psychosocial stress, genomic stress, and infection stress requires understanding the effects on final common pathway(s) that integrate these effects, suggested to include NO/NOx. It is very likely that stresses at or above the low-dose hormetic range will produce multi-generational epigenetic effects.