

dase inhibition). In contrast, inhibitors of thioredoxin reductase, auranofin and 1-chloro-2,4-dinitrobenzene, attenuated H₂O₂ removal rates in mitochondria by over 70%. Furthermore, oxidation of thioredoxin-2 via arsenic resulted in a significant decrease in H₂O₂ removal whereas copper-induced glutathione oxidation showed minimal effects. Inhibition of the thioredoxin system also exacerbated mitochondrial H₂O₂ production by oxidative-stress inducing agents such as paraquat. These data suggest that the thioredoxin/peroxiredoxin system is the major contributor to respiration-dependent H₂O₂ removal in brain mitochondria. Additionally, mitochondria pre-incubated with paraquat showed severely compromised H₂O₂ removal rates (up to 80% decrease) and inhibition of thioredoxin reductase activity in a concentration-dependent manner, suggesting dysfunction of the thioredoxin system in response to environmental neurotoxicants. Therefore, in addition to their well recognized role in the production of ROS, mitochondria may also participate in cell signaling events and/or pathological processes by serving as a potent net ROS removal system.

PS 765 **ROLE OF CYTOCHROME P450 REDUCTASE IN MEDIATING REDOX CYCLING OF 9, 10-PHENANTHRENEQUINONE.**

R. G. Udasin¹, K. C. Fussell¹, Y. Wang², D. E. Heck³, V. Mishin², P. J. Smith⁴, D. L. Laskin^{5, 1} and J. D. Laskin^{2, 1}. ¹Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ, ²Environmental and Occupational Medicine, UMDNJ-RWJMS, Piscataway, NJ, ³Environmental Health Science, New York Medical College, Valhalla, NY, ⁴BioCurrents Research Center, Marine Biological Laboratory, Woods Hole, MA and ⁵Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.

Among the most toxic components of diesel exhaust particles is 9,10-phenanthrenequinone (PAQ), a known redox cycling agent. During redox cycling, PAQ undergoes an enzymatic NADPH-dependent one-electron reduction to a semiquinone radical. Reaction of this radical with oxygen generates reactive oxygen intermediates (ROI). We found that recombinant NADPH-cytochrome P450 reductase (Supersomes™) readily mediates PAQ (0.1-30 μM) redox cycling and generation of ROI, including hydrogen peroxide (Km = 1.1 μM, Vmax = 282 nmoles/mg microsomal protein/min), and in the presence of redox active iron, hydroxyl radicals. PAQ was also found to stimulate the production of ROI by Chinese hamster ovary (CHO) cells. Lysates from CHO wild type cells (CHO WT) and cells overexpressing cytochrome P450 reductase (CHO OR) both generated hydrogen peroxide. (The Km and Vmax were respectively 0.52 μM and 3.1 pmoles/unit activity/min for CHO WT cells and 0.32 μM and 34.3 pmoles/unit activity/min for CHO OR cells). Using self-referencing microelectrodes, we found that PAQ redox cycling in intact cells was associated with increased oxygen uptake and cellular release of hydrogen peroxide. Similar amounts of hydrogen peroxide were released by both cell types. Taken together, these data indicate that cytochrome P450 reductase can mediate PAQ redox cycling; however, similarities between the CHO WT and CHO OR cells indicate that both cell types have adequate ROI detoxification despite differences in their cytochrome P450 reductase activity. Supported by NIH grants AR055073, ES004738, CA100994, CA093798, and ES05022.

PS 766 **REGULATION OF INFLAMMATORY RESPONSES: POSSIBLE REDOX SYNAPSE BETWEEN NEUTROPHILS AND MACROPHAGES.**

W. Feng¹, J. Shi², N. V. Konduru¹, H. Bayir², B. Fadeel³ and V. E. Kagan¹. ¹Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, ²Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA and ³Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

The term 'phagocytic synapse' describes the interaction between the professional phagocytic cell and its target via a complex system of receptors, bridging molecules and 'eat me' signals. We hypothesized that the phagocytic synapse is not merely a cell-cell interaction, but also a redox synapse. We predict that the apoptotic neutrophils activate macrophages by PS externalization to utilize them as donors of H₂O₂ thus satisfying high demands of neutrophils' myeloperoxidase. The provision of H₂O₂ to the neutrophils may thus lead to the enhancement of myeloperoxidase activity; it may also induce the release of oxygenated free fatty acids or resolvins from apoptotic neutrophils. The local release of oxidized free fatty acids or resolvins may function as a signal to promote the uptake and clearance of apoptotic neutrophils. To test the hypothesis, macrophages differentiated from human monocytes and differentiated neutrophils from PLB-985 cells were used. The extracellular H₂O₂ levels were monitored by Amplex red assay. Our results show that macrophages have a higher H₂O₂ generation at resting state than neutrophils, while macrophages produce lower levels of H₂O₂ than neutrophils upon activation with PMA (10nM). This suggests that macrophages are not likely to be the major

providers of H₂O₂ for activated neutrophils. In cells co-cultured in the presence of H₂O₂, PMA, or H₂O₂ plus PMA, the extracellular H₂O₂ levels were markedly decreased compared to either macrophage or neutrophil incubations alone, suggesting an enhanced consumption of H₂O₂. This consumption of H₂O₂ was not diminished by pretreatment of cells with a potent peroxidase inhibitor, NaN₃. We conclude that there is a possible synaptic interaction between neutrophils and macrophages in terms of H₂O₂ consumption. Supported by NIH HL70755, HL094488, NIOSH OH008282, Swedish Research Council.

PS 767 **HIGH SATURATED-FAT DIET AND DEFICIENT NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE ARE CONTRIBUTING FACTORS TO MITOCHONDRIAL DYSFUNCTION IN C. ELEGANS.**

S. Bryner¹, C. Thomas¹ and L. Carnell². ¹Chemistry, Central Washington University, Ellensburg, WA and ²Biology, Central Washington University, Ellensburg, WA.

Type 2 diabetes is a worldwide epidemic affecting over 246 million people, but the cellular mechanisms that initiate the disease are still unclear. Recent research indicates that there may be a link between mitochondrial dysfunction and decreased insulin, which is a risk factor for the development of type 2 diabetes. The control of insulin secretion is dependent on the energy state of the pancreatic beta cells making it susceptible to disruption of mitochondrial function and ATP levels. The aim of this work was to investigate the role of a mitochondrial enzyme, Nicotinamide Nucleotide Transhydrogenase (NNT), in maintaining mitochondrial function in animals exposed to high-fat diets enriched with stearic, oleic or linoleic acid. These studies were performed using the nematode, *Caenorhabditis elegans*, and included the examination of both wild-type nematodes and two different deletion strains lacking the *C. elegans*' ortholog of NNT, *nnt-1*. The different strains were grown on high-fat and normal diets and the function of mitochondria in these nematodes was compared using oxygen consumption to measure respiratory rate and fluorescent imaging techniques to indicate mitochondrial reactive oxygen species (ROS) production and membrane potential. Our results show that, when grown on normal diets, the *nnt-1* mutant nematodes had lower respiratory rates and lower mitochondrial membrane potentials as compared to wild type, suggesting a defect in mitochondrial function. In addition, our results show increased mitochondrial dysfunction and reactive oxygen species production in nematodes grown on a high saturated-fat diet as compared to normal and unsaturated-fat diets. The results suggest that NNT plays a role in maintaining mitochondrial respiratory function and that a high saturated-fat diet increases mitochondrial dysfunction via increased ROS production and decreased energy production.

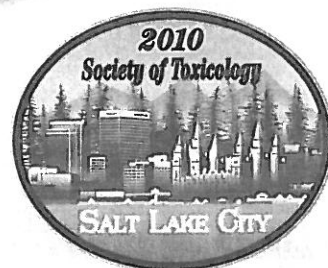
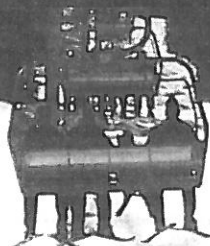
PS 768 **TREATMENT WITH AN ETC COMPLEX III INHIBITOR STIMULATES APICAL CHOLINE TRANSPORT IN PRIMARY CULTURES OF CHOROID PLEXUS.**

A. R. Villalobos and R. K. Young. *Nutrition & Food Science, Texas A&M University, College Station, TX.*

This laboratory reported for both intact choroid plexus and primary cultures that low concentrations cadmium elicited significant increases in the apical transport of the nutritive organic cation choline. In cadmium-treated primary cultures of choroid plexus, the antioxidant N-acetyl cysteine (NAC) attenuated induction of oxidative stress and stress proteins, as would be expected. However, NAC also attenuated up-regulation of choline transport by cadmium, suggesting that the induction of oxidative stress was requisite to the observed increases in choline transport. As first approach to investigate regulation of choline transport in response to oxidative stress in the absence of heavy metals, we examined regulation of apical 3H-choline transport by antimycin-A that, like cadmium, inhibits complex III of the electron transport chain (ETC). Primary cultures of choroid plexus were treated with 5 microM antimycin-A for 6 h. The mitochondrial membrane potential was monitored using the potential-sensitive fluorescent probe TMRM and epi-fluorescence microscopy; antimycin-A reduced fluorescence of TMRM, indicating depolarization of mitochondrial membrane potential. The ETC inhibitor also increased total cellular accumulation of reactive oxygen species (ROS), as per increased fluorescence in cells loaded with fluorescein diacetate. Increased ROS and mitochondrial membrane depolarization were consistent with induction of oxidative stress. Thus, 30-minute apical uptake of 10 microM 3H-choline then was assayed in the absence of the ETC inhibitor and compared in non-treated and antimycin-A treated cells. As compared to controls, 3H-choline uptake was stimulated by nearly 60% following antimycin-A treatment. These preliminary data suggest that oxidative stress may regulate choline transport by choroid plexus. Possible modulation of stress regulation of transport by antioxidants and the essential metal zinc is under investigation.

The Toxicologist

Supplement to *Toxicological Sciences*



*An Official Journal of the
Society of Toxicology*

SOT | Society of
Toxicology

Creating a Safer and Healthier World
by Advancing the Science of Toxicology

**49th Annual Meeting
and ToxExpo™**

SALT LAKE CITY, UTAH

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 49th Annual Meeting of the Society of Toxicology, held at the Salt Palace Convention Center, March 7–11, 2010.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 473.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 496.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

Copies of *The Toxicologist* are available at \$45 each plus \$5 postage and handling (U.S. funds) from:

**Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190**

www.toxicology.org

© 2010 Society of Toxicology

All text and graphics are © 2010 by the Society of Toxicology unless noted. For promotional use only. No advertising use is permitted.

This abstract book has been produced electronically by ScholarOne, Inc. Every effort has been made to faithfully reproduce the abstracts as submitted. The author(s) of each abstract appearing in this publication is/are solely responsible for the content thereof; the publication of an article shall not constitute or be deemed to constitute any representation by the Society of Toxicology or its boards that the data presented therein are correct or are sufficient to support the conclusions reached or that the experiment design or methodology is adequate. Because of the rapid advances in the medical sciences, we recommend that independent verification of diagnoses and drug dosage be made.