2350 COMPARATIVE ACHE AND BCHE METHODS: THE WRAIR WHOLE BLOOD ASSAY, TEST-MATE OP SYSTEM, MICHEL (ΔPH) ASSAY, AND MICROELLMAN METHOD

J. R. Haigh[1], R. Gordon[1], L. Lefkowitz[1], B. Doctor[1], C. Clark[2], A. D'Ambrosio[2], N. Kirschten[2], B. Capacio[2], A. Brooks[2], S. Davis[2] and D. Goodman[3]. *[1]Division of Biochemistry, Department of Biochemical Pharmacology, Walter Reed Army Institute of Research, Silver Spring, MD, [2]Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD and [3]Cholinesterase Reference Laboratory Army Center for Health Promotion and Preventative Medicine, Aberdeen Proving Ground, MD.* Sponsor: M. Nambiar.

Current clinical tests to determine cholinesterases (ChE) in blood require several processing steps and specific ChE inhibitors to determine either the serum butyrylcholinesterase (BChE) or erythrocyte AChE concentrations separately. In contrast, the patented Walter Reed Army Institute of Research Whole Blood (WRAIR WB) cholinesterase assay rapidly determines AChE and BChE activities in a small amount of unprocessed whole blood, and is fully automated (using the Biomek 2000 robotic system). The WRAIR whole blood assay has been used to measure AChE and BChE in human blood that has been inhibited by pyridostigmine bromide (after a 30 mg oral dose). The assay is also currently being used to evaluate protection afforded by huperzine A, a potential anti-Alzheimer's drug, to organophosphate (OP) intoxication of both the peripheral and central nervous system, (pyridostigmine protects only the periphery). The objectives are (a) extend studies of the WRAIR WB over a range of ChE values using three OP nerve agents – GB (sarin), GD (soman), and VX; (b) development of a viable conversion database between WRAIR WB ChE values and the Test-Mate OP system, ΔpH (Michel) assay, and microEllman method. For the 4 assays, the ChE values were determined for the 3 OPs over a wide range of AChE and BChE activity (10-100% control RBC AChE and serum BChE), except for the Michel assay, where BChE is not routinely determined. Regression analyses yielded equations with high correlation, demonstrating the feasibility of a ChE conversion database between the four assays. Thus, the WRAIR assay provides a rapid, reliable, accurate technique to determine ChE inhibition in soldiers, agricultural workers (e.g., pesticide poisoning), and first responders.

2351 DETECTION OF MODIFICATIONS OF PROTEIN THIOLS IN COMPLEX TISSUE SAMPLES

L. K. Rogers[1], B. L. Leinweber[2] and C. V. Smith[1]. *[1]Pediatrics, Columbus Children's Research Institute, Columbus, OH and [2]Pharmacology and Toxicology, University of Arizona, Tuscon, AZ.*

Oxidation/reduction reactions of protein thiol groups (PSH) have been implicated in many physiological and pathological processes, but studies to date have been restricted by the limited specificities of methods available. Tissue samples were freeze-clamped, exposed to N-ethylmaleimide (NEM), iodoacetic acid (IAA) or iodoacetamide (IAM), and residual PSH determined by derivatization with monobromobimane (mBBr), electrophoresis, and quantitation by fluorescence. Initial derivatization of PSH with NEM proved to be more effective (faster, lower mol ratio of agent, and active at lower pHs) than either IAM or IAA. For reduction of protein disulfides (PSSX) and other reversible thiol modifications, dithiothreitol (DTT) and tris(2-carboxyethyl)phosphine (TCEP) were comparably effective. Best results were obtained with freeze-clamped tissues ground to powders in liquid N2 and added to ice-cold NaPO4 buffer containing 125 mol of NEM per mol of PSH for 15 min. Proteins were precipitated in 4 volumes of ice cold MEOH, centrifuged, washed 3 times with MeOH, treated with 50 mM DTT, and washed again with MeOH. The protein pellet was dissolved in 9 M urea containing 2 mM mBBr and incubated for 30 min at room temp in the dark. In-gel standard curves were generated using mBBr-derivatized proteins that were quantitated by absorbance and fluorescent intensities measured following electrophoretic separation. The in-gel quantitation was applied to liver homogenates obtained from Fischer-344 rats treated with doses of 0 to 0.125 mmol/kg of diquat and drug-dependent effects were observed in bands representing at least 4 different proteins. The techniques described were applied to 2D electrophoresis separations. Kidneys from glutathione reductase deficient and control mice exhibited differences in fluorescence intensities of some individual spots, but the differences observed were relatively subtle. We have described techniques that minimize oxidation, ex vivo, and shown these techniques to be useful in studies of PSH and PSSX status in tissues.

2352 PROFICIENCY TESTING OF THE UDP-GT ASSAY FOR THYROXINE IN THE MALE F344 RAT

B. D. Lusiak, J. D. Johnson, T. Deck, S. Graves and B. Burback. *Chemistry Technical Center, Battelle Memorial Institute, Columbus, OH.*

Uridine-diphosphate-glucuronosyltransferase (UDP-GT) activity for thyroxine (T_4) is increased by exposure to some xenobiotics and therefore, this effect provides further insight into changes in serum T_4 and thyroid stimulating hormone (TSH) concentrations. The present study evaluated an assay procedure for measuring UDP-GT activity for T_4 and presents assay proficiency data using commercially available 3-methylcholanthrene (MC)-induced and control liver microsomes, as well as liver microsomes from male Sprague-Dawley (SD) and Fischer 344 (F344) rats treated with corn oil (control) or MC. The assay procedure followed was described by Hood and Klaassen (2000). Briefly, ^{125}I-T_4 was purified and incubated with microsomes. The ^{125}I-T_4-glucuronide was separated using the Sephadex column and a series of elution steps (0.1 M HCl, water, ethanol/0.1 M NaOH). Analysis of UDP-GT activity for T_4 using commercially-obtained microsomes resulted in values of 6.27 ± 0.55 pmoles T_4-glucuronide/mg protein/min (%CV = 8.8) for control microsomes and 24.9 ± 0.92 pmoles T_4-glucuronide/mg protein/min (%CV = 3.7) for MC-induced microsomes. Male SD and F344 rats, 11 weeks of age, were given single daily injections of corn oil (vehicle control) or MC (50 mg/kg, 2.5 mL/kg, IM) for four consecutive days, euthanized, and the livers removed for microsome preparation. The UDP-GT activity for T_4 in the SD and F344 rats was 3.9 ± 0.5 pmoles T_4-glucuronide/mg protein/min (%CV = 12.8) and 4.9 ± 0.7 pmoles T_4-glucuronide/mg protein/min (%CV = 15.3) for the control group animals and 31.9 ± 3.1 pmoles T_4-glucuronide/mg protein/min (%CV = 9.8) and 26.2 ± 4.2 pmoles T_4-glucuronide/mg protein/min (%CV = 15.9) for the MC-treated animals. These results demonstrated that proficiency in conducting the assay was achieved and provided further information about UDP-GT activity for T_4 in the F344 male rat prior to using this species and strain in regulatory toxicology studies. (This work was supported by NIEHS Contract N1-ES-05456.)

2353 ONE-DIMENSIONAL NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY FOR THE DETECTION OF INFLAMMATORY BIOMARKERS DUE TO ENVIRONMENTAL AIR CONTAMINANTS

C. A. Hood[1, 2], R. Balhorn[2] and K. Pinkerton[1]. *[1]Center for Health and the Environment, UC Davis, Davis, CA and [2]Biological Research and Develoment, Lawrence Livermore National Laboratory, Livermore, CA.*

Metabonomics can serve as a highly sensitive measure to detect changes in physiologic and biochemical function due to exposure to a variety of environmental air pollutants. A number of environmental factors may trigger changes in gene expression leading to alterations in phenotype expression. Therefore, measurement of systemic biochemical profiles and the regulators of cellular function may serve as a method for identifying early indicators of disease. One-dimensional nuclear magnetic resonance spectroscopy (NMR) was used to examine blood samples taken every three months from young Rhesus macaque monkeys exposed to environmental tobacco smoke (ETS). A filtration method was used to allow rapid visualization of small metabolites (<30kD) normally hidden behind large protein signals. NMR analysis of plasma samples displayed individual monkeys to have unique metabolite fingerprints, which also demonstrated metabolite alterations over time. In addition, we were able to observe distinct differences in the profile of metabolites between the treatment groups. While the ultimate consequences of ETS exposure on children's health is unknown, the mapping of metabolites found in the blood following exposure to environmental pollutants could significantly advance our understanding of the relative sensitivity of children and the potential mechanisms by which adverse health effects occur, suggesting that the utilization of NMR techniques for metabonomics fingerprinting could be instrumental in predicting health outcomes.
This work was supported by the U.S. Department of Energy Lawrence Livermore National Laboratory under Contract W-7405-ENG-48 and NIH E5011634.

2354 THE DEVELOPMENT OF PROTEOMICS-BASED ACRYLAMIDE BIOMARKERS USING SURFACE ENHANCED LASER DESORPTION IONIZATION (SELDI)

K. L. Cheever. *Molecular and Genetic Monitoring, NIOSH, Cincinnati, OH.*

Acrylamide (CAS 79-06-1), a widely used industrial chemical, also formed in thermally processed food, is known to produce neurotoxicity, reproductive effects and has been classified as a probable human carcinogen. Bioactivation of acrylamide is reported to occur by CYP2E1 oxidation and GSH conjugation. Protein and DNA adducts have been reported for both Acrylamide and glycidamide (5694-00-8), its oxidative metabolite. In the current study the reaction products of acrylamide and glycidamide with human alpha- and beta-globins or albumin, obtained through in vitro incubation, were studied using Surface Enhanced Laser Desorption Ionization (SELD) with Time of Flight mass spectrometry. The study showed that the reactivity of glycidamide with albumin, alpha-globin and beta-globin was much more than measured after incubation with acrylamide. After 12 hours the glycidamide-adduct levels for albumin < alpha-globin < beta-globin, but detectable adduct formation for acrylamide was measured only for albumin. The adduct levels continued to increase during the 72-hour test period. Tryptic digests of the proteins may be utilized for specific proteomic-based biomarkers of exposure to acrylamide or glycidamide.

SOT | Society of Toxicology

The Toxicologist

Supplement to *Toxicological Sciences*

An Offical Journal of the Society of Toxicology

45th Annual Meeting and ToxExpo™

San Diego, California

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 90, Number 1, March 2006

www.toxsci.oupjournals.org

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

The abstracts are reproduced as accepted by the 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