

ABSTRACTS OF PAPERS PRESENTED AT THE 2005 COLLEGIATE MEETINGS

EASTERN REGION

SOUTHERN ADVENTIST UNIVERSITY
COLLEGEDALE, TENNESSEE

DEMONSTRATION OF VACUUM DISTILLATION/DEMONSTRATION OF DISTILLATION OF DEET. *Lindsay Collins and Robin Breeden, Walters State Community College, Morristown, Tennessee.* Student prepared DEET was purified by vacuum distillation. Operation of the system will be presented.

DEMONSTRATION OF LARGE SCALE FRACTIONAL DISTILLATION. *David A. Fowler, Walters State Community College, Morristown, Tennessee.* An experiment was performed to demonstrate large-scale fractional distillation. Using a mixture of cyclohexane and toluene, a distillation was conducted to model those used in industry. The fundamental chemistry relating to the cyclic process of fractional distillation was explored and applied to the operation of a large-scale fractional distillation apparatus.

DEMONSTRATION OF A REACTION WITH A MORTON FLASK. *Daniel Hammer, Walters State Community College, Morristown, Tennessee.* A Morton flask is used to more efficiently mix a homogeneous reaction mixture. Operation of the flask will be presented.

STEAM DISTILLATION. *Holly Harrell and Neelie Rapien, Walters State Community College, Morristown, Tennessee.* Steam distillation was demonstrated by removing oil from caraway seeds.

DEMONSTRATION OF TWO-DIMENSIONAL THIN LAYER CHROMATOGRAPHY. *Nse-Abasi Okon, Walters State Community College, Morristown, Tennessee.* Two-dimensional thin layer chromatography will be demonstrated using Merck silica gel TLC plates developed in various mobile phases.

SOXHLET EXTRACTION: FAT FROM FRENCH FRIES. *Laura E. Wells, Walters State Community College, Morristown, Tennessee.* This demonstration experiment involves the extraction of fat from commercial French fries by an exhaustive extraction with solvent hexanes using the Soxhlet extractor apparatus. This experiment is a method for the extraction and determination of percent fat content by weight in a food sample.

DEMONSTRATION OF RADIAL CHROMATOGRAPHY. *Dinah Sweet, Walters State Community College, Morristown, Tennessee.* Using the Analytic 6000, a demonstration of radial chromatography will be performed.

DEMONSTRATION OF PRODUCE DRYING WITH AN ABDERHALDEN APPARATUS. *La Breeska Jones, Walters*

State Community College, Morristown, Tennessee. The Abderhalden apparatus is used to quick dry a sample by heating with a refluxing solvent while the drying chamber is under vacuum operation. Operation of the device will be presented.

DEMONSTRATION OF ACE PHOTOCHEMICAL EQUIPMENT/SYNTHESIS OF BENZOPINACOL. *Sheree Pack and Josh Rowland, Walters State Community College, Morristown, Tennessee.* We found that using Ace Photochemical equipment makes the synthesis of benzopinacol much faster than using sunrays. When sunlight is used, it can take weeks to fully synthesize benzopinacol. Ace Photochemical equipment provides a more efficient way to synthesize benzopinacol.

THE FISHER ESTERIFICATION WITH A DEAN-STARK TRAP. *Amber Johnson, Walters State Community College, Morristown, Tennessee.* The Fisher Esterification by Dean-Stark Trap demonstrates the production of an ester from a carboxylic acid and an alcohol with an acid catalyst. This process removes the water from the solution and traps it in a compartment of the apparatus. This yields a purer ester.

DEMONSTRATION OF CONTINUOUS LIQUID-LIQUID EXTRACTION WITH A HEAVIER (DENSER) THAN WATER SOLVENT. *Steve Lovell, Walters State Community College, Morristown, Tennessee.* The process of extraction is based on the relative solubility of a solid in two immiscible liquids. Solubility is the chemical property that defines the maximum mass of a solid that will dissolve in a particular solvent. Two immiscible solvents will form two distinct layers (often called phases) when mixed together, and the solvent with the greatest density will form the bottom layer. This demonstration deals with an extraction solvent that has a density greater than water ("Heavier than Water Solvent"). Coffee will be the free solvent, which contains caffeine. The apparatus used in this demonstration allows us to do a continuous extraction of caffeine rather than a batch type extraction typically used in a laboratory environment. Dichloromethane is used as the extraction solvent because it is immiscible in water and has a much higher solubility for caffeine.

DEMONSTRATION OF AN ABBE REFRACTOMETER. *James Muscari, Walters State Community College, Morristown, Tennessee.* Demonstration of the operation of an Abbe Refractometer. As light passes through a transparent substance, it is refracted or bent. The Abbe Refractometer can measure the specific degree of refraction of a liquid sample.

DEMONSTRATION OF LOW PRESSURE CATALYTIC HYDROGENATION. *Robert L. Sempson, Walters State Community College, Morristown, Tennessee.* This presentation is about hydrogenation. Hydrogenation is used to reduce the double bonds of alkenes. The reduction occurs when the alkenes react

with hydrogen in the presence of a metal catalyst. The metal catalyst is important as the reaction of the alkenes and hydrogen occur on the metal surface. Thus, without it, the reaction could not take place. After the reaction is complete the alkenes are formed into alkanes and the catalyst can be recovered.

DEMONSTRATION OF LARGE SCALE PRODUCT DRYING WITH A VACUUM OVEN. *Kendal West, Walters State Community College, Morristown, Tennessee.* Operation of the Napco 585 I vacuum drying oven for larger scale product drying will be demonstrated.

THE USE OF SOLID PHASE EXTRACTION. *Kyle Cutshaw, Walters State Community College, Morristown, Tennessee.* Solid Phase Extraction (SPE) is a cheap, efficient, and timesaving method of extraction. Solid phase extraction can isolate specific types of chemical compounds faster and easier than the more classic liquid/liquid extraction methods (Separatory Funnel or HPLC Purification). With great efficiency in both results and cost, it is easy to see why SPE would be a preferred method in the extraction of chemical compounds.

DEMONSTRATION OF A LABORATORY DEVICE: CHEM-DRY APPARATUS. *Kelli Harrison, Walters State Community College, Morristown, Tennessee.* The CHEM-DRY apparatus is a quick and effective method of drying a product. The sample is placed inside a glass tube containing a glass boat. The tube is placed inside the apparatus. The device is connected to a vacuum, heat is applied, and the temperature can be adjusted by the operator. The sample dries quickly. Not only is the CHEM-DRY apparatus quick and effective, it also is very easy to use.

DEMONSTRATION OF A I2R TEMPERATURE CONTROLLER. *Scott A. Harris, Walters State Community College, Morristown, Tennessee.* This will be a demonstration of how to set-up and use a Therm-O-Watch L7-800 I²R temperature controller. This instrument, when used in conjunction with a properly calibrated thermometer, is used to regulate or maintain temperatures with an acceptable maximum sensitivity of $\pm 0.3^{\circ}\text{C}$ for temperature critical reactions. The tool also can be safely setup for use in an environment where there is possibility of contact with corrosive liquids.

MODELING CHANGES IN THE ACETYLCHOLINESTERASE GORGE IN THE PRESENCE OF INHIBITORS. *Sara Ashcraft, Jessica Bathe, and Margaret Franzen Hicks, Pellissippi State Technical Community College, Knoxville, Tennessee.* Recent attention has been focused on inhibitors of acetylcholinesterase for applications in treatment of Alzheimer's and insect pests, and their potential use as bioterrorist agents. The mechanism of enzyme action has been studied through use of crystallographic data involving irreversible inhibitors. Mouse (*Mus musculus*) and Electric ray (*Torpedo californica*) enzymes were modeled to study the structural changes within the deep gorge leading to the active site. Inhibitors bind to a tryptophan residue at the rim of the gorge, and then slide down the gorge, which is lined with fourteen aromatic residues. There is a constriction within the gorge; the omega loop of the enzyme rotates, as if on a hinge, to permit inhibitors (and acetylcholine) to squeeze past. Comparison of various inhibitors has allowed identification of gorge residues whose positions change due to the presence of the

inhibitors. At the base of the gorge is a catalytic triad that cleaves acetylcholine.

RELATIONSHIP BETWEEN FEMALE BODY SIZE, TEMPORAL AND SPATIAL ENVIRONMENTAL FACTORS, AND REPRODUCTIVE SUCCESS AS INDICATED BY INCREASED LITTER SIZE. *Karan Sharma and John R. Hisey, Lee University, Cleveland, Tennessee.* Individual fitness is related to number of offspring produced, which may be influenced by energy balance relating to the mother's body size and environmental factors that vary over space and time. We tested hypotheses on these relationships in Virginia opossums, an important species in which litter size is easily assessed, by operating two trapping grids near Memphis, Tennessee over three summers for a total of 12,000 trap-nights. Forty-six female opossums with pouch young were captured. Mean weight of mothers was 2.20 kg and mean snout-rump length was 479.6 mm. Mean number of young per litter was 8.8 (range 3–13). No significant relationships were found between number of pouch young per litter and female mass, length, trap grid habitat, or year. Wide variation in number of young may indicate, however, that differences in genetics, health, or other factors affecting individual females play a role in determining litter size.

MUSIC TEMPO: ITS AFFECT ON RESPIRATION RATE, HEART RATE, AND HAND GRIP STRENGTH. *Debby Sentana and Keith A. Snyder, Southern Adventist University, Collegedale, Tennessee.* Many studies have been done on the effects of music on human physiology. The effect of music tempo on human physiology is reported in this study. Twenty female subjects listened to a song played in three different tempos: fast (150 bpm), medium (120 bpm), and slow (90 bpm). Half of the subjects listened to descending tempo order, and the other half listened to ascending tempo order. Respiration rate, heart rate, and handgrip strength were measured before and after listening to the tempo sequence. The data obtained from this study showed no correlation between music tempo and respiration rate, heart rate, and handgrip strength.

MIDDLE REGION

TENNESSEE STATE UNIVERSITY
NASHVILLE, TENNESSEE

THE CHARACTERIZATION OF NUCLEAR CHANNELS IN HELA CELLS. *Jeremy R. Anthony and John R. Palisano, The University of the South, Sewanee, Tennessee.* The interphase nucleus of a cell may be a more dynamic organelle than once thought. Invaginations of the nuclear envelope (NE) into the nucleus that are continuous with the cytoplasm have been identified in several cell types and are known as nuclear channels (NC) or nuclear tubules. Previous research suggested that NC associate closely with centrioles and only exist during early interphase, which is identified by the presence of a midbody during cytokinesis between daughter cells. Nuclear channels were visualized in HeLa cells following immunofluorescent labeling of lamin B, a nuclear envelope-specific protein; midbodies were detected following immunofluorescent labeling of β -tubulin, a microtubule-specific protein; and centrioles were identified

following immunofluorescent labeling of γ -tubulin, a centrosomal-specific protein. Nuclear channels are typically round in shape, have a diameter of 2–3 μm and extend through the entire nucleus. They occur in 2.6% of cells and few nuclei contain more than one; they are never consistently seen in close association with centrioles. To determine if NC are associated with a specific stage of the cell cycle, cell cultures were synchronized using a standard cell synchronization protocol (Stein and Borun, 1972). The association of midbodies and NC was examined in synchronized as well as unsynchronized cultures. Data derived from unsynchronized cultures suggest that NC are associated with early interphase, while data derived from synchronized cultures were inconclusive. The frequency of NC seen in this investigation is lower than that reported for NC in other investigations. This discrepancy might be the result of the microscopy used. Most investigations use a confocal microscope, which permits visualization of serial thin sections through the entire nucleus so that small channels that do not completely traverse the nucleus can be visualized. The events leading to the formation of NC and the role of NC are discussed.

INVESTIGATIONS OF ANTI-CANCER COMPOUNDS FROM ROOT EXTRACT OF ECHINACEA. *Lakeshia N. Wright, Makeesa Dukes, Todd Gary, and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* Many compounds found in plants have anti-bacterial, anti-fungal, and anti-cancer activities. Over 25% of our common medicines contain at least some compounds obtained from plants. In the United States approximately 10% of our major drugs have plant extracts as their active ingredient. In less developed countries the World Health Organization estimates that 75–80% of the people rely on plant-based medicines for primary health care. The herb *Echinacea purpurea* (purple coneflower) and *Echinacea pallidae* produces natural compounds that may increase the efficiency of mammalian immune systems. This investigation used extracts from the dried roots of Echinacea. The extract was used on a breast cancer cell line, BT549, to determine possible anti-cancer activity. These cells were grown at 37°C with 5% CO₂ and exposed to either 7, 14 or 21 μL of DMSO or the extract. The extract was obtained by conventional methods and dissolved in DMSO. The growth rates of cells in the control and experimental groups were significantly different in that both plants showed a reduction in growth. The extract from the roots appears to inhibit growth of cancer cell line BT549. *Echinacea pallidae* reduced the growth of cell line BT549 more significantly than *E. purpurea*. These results indicate that further studies are necessary to identify anti-carcinogenic compounds in Echinacea.

INFLUENCE OF DIETARY SOY PHYTOESTROGENS ON CORTICOTROPIN RELEASING HORMONE AND AGGRESSION IN MALE SYRIAN HAMSTERS (MESOCRICETUS AURATUS). *Shani Robinson, T. O. Moore, G. Small, O. Atilola, and P. Kahlon, Tennessee State University, Nashville, Tennessee (SR) and Clark Atlanta University, Atlanta, Georgia (TOM, GS, OA, PK).* Phytoestrogens are non-steroidal, estrogen-like compounds found in soy containing products, such as flaxseed and soybeans. Phytoestrogens bind to estrogen receptors; thus studies have examined their effects on brain structure, function and behavior. Previous research suggests that phytoestrogens have anxiolytic effects, and therefore may have

an effect on aggressive behavior. The brain hormone that regulates anxiety related behavior is corticotropin releasing hormone (CRH). Corticotropin releasing hormone coordinates behavioral, autonomic and hormonal responses to stress. We examined the influence of dietary soy on aggressive behavior in castrated Syrian male hamsters. A phytoestrogen diet (PE) or a phytoestrogen free diet (PE free) was used to determine if phytoestrogens had any effect on CRH neurons in the brain of Syrian male hamsters (*Mesocricetus auratus*). The hamsters were pre-tested for aggression, castrated, and placed on a PE diet or PE free diet for four weeks. During week five, the hamsters were exposed to a group housed, non-aggressive opponent in a neutral cage and a resident intruder paradigm. Behavioral testing included the latency to attack, duration of aggression, and number of attacks. Immunohistochemistry was utilized to examine the brain for CRH neurons. We found no significant behavioral differences in the duration of aggression and the number of attacks between PE fed and PE free fed hamsters. However, a One-Way ANOVA ($P < 0.05$) revealed statistical differences in the latency to attack among the groups. Immunohistochemistry revealed visual differences in paraventricular thalamus and cerebellum CRH neurons. An independent t -test ($P < 0.05$) revealed a greater number of CRH neurons in the paraventricular thalamus of the PE free fed hamsters. These findings may reflect the anxiety-reducing effects of phytoestrogens, and support previous research suggesting reduced anxiety leads to aggressive behavior. Therefore, phytoestrogens may affect aggression and anxiety in male Syrian hamsters.

SYNTHESIS OF SEMICONDUCTOR PBS/SIO₂ AND PBTE/SIO₂ NANOCOMPOSITES. *Demetria Griffin, J. Moore, M. Karim, and P. Kahlon, Tennessee State University, Nashville, Tennessee.* With this project, our main goal is the formation of metal oxide (ceramic) networks from the solution phase. This can be accomplished through the hydrolysis of a metal (or nonmetal) alkoxide precursor followed by condensation of the hydrolysis products. Because sol-gel chemistry is possible for all alkoxide complexes the nanocomposite that will be used is PbS/SiO₂ because it is a fairly simple nanocomposite to synthesize.

PREVENTING RNA DETERIORATION IN CAENORHABDITIS ELEGANS. *Natoya Hopkins, Carla Coleman, Yvonne Myles, Todd Gary, and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* Sydney Brenner suggested (in the early 60s) that nematodes would serve as a good model organism to perform molecular studies. The nematode worm *Caenorhabditis elegans* would become that experimental model to begin a comprehensive study of animal development and the underlying principles of nervous system function in simple animals. *Caenorhabditis elegans* is a translucent, organism found in the soil. It is approximately 1 mm in length and lives for approximately two weeks. This animal model offers several experimental advantages. It is very easy to grow in the laboratory and gives birth to a large number of progeny (approximately 300) by self-fertilization. Our lab is studying gene expression in extreme environments such as Space exploration. To do these kinds of studies, it would be necessary to preserve the macromolecules for examination. RNA later is a compound used to preserve RNA when proper refrigeration is not available. In our studies we found that RNA later preserved RNA longer than 2 weeks at room temperature.

STUDY OF THE ENZYMATIC ACTIVITY OF HYALURONAN LYASE USING GEL PERMEATION CHROMATOGRAPHY WITH PHOTODIODE ARRAY DETECTION. *Jeanita S. Pritchett and Koen P. Vercruyse, Tennessee State University, Nashville, Tennessee.* Combining gel permeation chromatography and on-line UV-VIS absorbance measurements at 232 nm we studied the kinetics and mechanism of the action of *Streptomyces hyalurolyticus* and *Streptococcus pneumoniae* hyaluronan lyase enzymes. For both enzymes we provided evidence that in the initial stages of the degradation process they act in a random endolytic fashion. The reported processive exolytic activity of the *S. pneumoniae* hyaluronan lyase enzyme was not observed until the hyaluronan substrate was degraded to a molecular mass average of about 100 kDa. In addition, we studied the enzymatic activity of various *S. pneumoniae* hyaluronan lyase mutants.

ANTI-TUMORGENIC PROPERTIES OF PHYTOCHEMICALS. *Makeesa Duke, Lakeshia Wright, Lakeisha Woods, Todd Gary, and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* Casualty that results from the common forms of cancer is still inappropriately high. Regardless of many beneficial advances in the understanding of the processes in malignant tumors, overall mortality statistics are unlikely to change until there is a change in the use of natural products as new phytochemical agents. Natural compounds may be used to block, reverse, or prevent the development of invasive cancers. Cellular proliferation forms the biologic basis for the identification of preventive products, the assessment of their activity, and ultimately the success or failure of a therapy. Medicinal plants have long been used as anticancer agents. Promising evidence has demonstrated that many phytochemicals isolated from plant sources possess anti-tumor properties. Apoptosis, a definite form of programmed cell death, plays an important role in development, growth regulation, and other degenerative disorders, as well as tumor development and therapy. We used a crude extract of Echinacea to determine its anti-tumor properties on the Breast cancer cell line BT549. One thousand cells were plated in six well plates and grown in soft agarose containing RPMI1640 media with 10% Fetal Bovine Serum. The experimental group was exposed to three different concentrations of Echinacea's crude extract. All concentrations showed tumor inhibition when compared to the control group.

BRIEF EXPOSURE TO PENTACHLOROPHENOL CAUSES PROGRESSIVE LOSS OF CYTOTOXIC FUNCTION IN HUMAN NATURAL KILLER CELLS. *Telpriore Tucker, Margaret Whalen, and P. Kahlon, Tennessee State University, Nashville Tennessee.* Pentachlorophenol (PCP) is used predominantly as a fungicide in wood preservatives. Pentachlorophenol also has been used as a bactericide and fungicide to protect many products such as adhesives, paper, paints, and textiles. Natural killer (NK) lymphocytes are central in immune defense against virus infection and tumor formation. In a previous study we found that exposure of NK cells to 10 μ M PCP for 24 h, 48 h, or 6 days caused about a 90% decrease in their tumor-cell-lysing function, while a 1 h exposure caused no consistent loss of function. This study investigated the effects of a brief exposure (1 h) to 10 μ M PCP followed by 0 h, 24 h, 48 h and 6 day incubations in PCP-free media on the tumor-cell-lysing and tumor-cell-binding functions of human NK cells. NK cells exposed to PCP for 1 h followed by 0 h or 24 h in

PCP-free media showed no significant decreases in either lytic or binding function. NK cells that were given 6 d in PCP-free media following a 1 h exposure showed as much as an 80% loss of lytic function but no accompanying loss of binding function.

THE EFFECT OF HYPERGRAVITY ON MUSCLE PROTEIN IN CAENORHABDITIS ELEGANS. *Kelly Steward, Yvonne Myles, Todd Gary, P. Kahlon, and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* *Caenorhabditis elegans* is a transparent, free-living, soil nematode 1 mm in length with a maximum diameter of 80 μ m. Many distinct cell types associated with complex functions in mammals/humans can be recognized and identified in *C. elegans*. *Caenorhabditis elegans* have a simple anatomy. However, the 959 somatic cells of the adult represent most major differentiated tissue types, including muscles (111 cells), neurons (302 cells), intestine (34 cells) and epidermis (213 cells) (Ahringer, 1997). *Caenorhabditis elegans* have 40% homology with the human genome (Ahringer, 1997). A Western Blot procedure was performed to determine the effect of hypergravity on dystrophin. Hypergravity experiments that involve centrifuging samples 4 to 20 times gravity were used to assess effects on muscle activity. The dystrophin levels in the control and experimental groups were compared. Two Western Blots were performed, the first blot contained dystrophin only and the second blot contained actin as an internal control. With the first blot, only one control and one experimental group appeared faintly. Thus, after exposure to hypergravity (10 \times gravity) for 48 h dystrophin decreased. Although this was a preliminary experiment and must be repeated, the results indicate that dystrophin decreases with hypergravity.

WESTERN REGION

CHRISTIAN BROTHERS UNIVERSITY
MEMPHIS, TENNESSEE

MONITORING REPRODUCTIVE STATUS IN THE FEMALE GIANT ANTEATER (*MYRMECOPHAGA TRIDACTYLA*) BY FECAL HORMONE ANALYSIS FOR IMPROVED BREEDING MANAGEMENT. *Jennifer L. DeBeauchamp, Jennifer Hudgins, Heather DeCaluwe, Andy J. Kouba, Peter Riger, Carrie K. Vance, Memphis Zoo, Memphis, Tennessee (JDL, AJK, CKV), Nashville Zoo at Grassmere, Nashville, Tennessee (JH, HD, PR), and University of Memphis, Memphis, Tennessee (JLD, CKV).* The stability of the captive population of giant anteaters (*Myrmecophaga tridactyla*), in United States zoological institutions is questionable due to difficulties in the management of male-female pairs during reproductive cycles and pregnancy as well as the high neonatal mortality rate. To establish improved breeding management for the giant anteater regular examination of female reproductive status was determined by non-invasive fecal hormone analysis and coupling hormone cycles to external behavioral indicators. A protocol for an enzyme linked immunosorbent assay (ELISA) was developed for the detection of the steroid hormone derivatives of progesterone (P_4) and estradiol glucuronide (E_1G) present in extracts of giant anteater feces. Four traditional wet fecal extraction methods were tested. Optimal anteater hormone levels were obtained by solvating 0.5 g of fecal material in 5 mL of 80%

EtOH solution for 20 h. Data obtained from 12 months of samples from five individual giant anteaters suggest a cycle varying in length from 45–60 days in agreement with results obtained from radioimmunoassay data. One pregnancy occurred during the duration of the project giving an approximate pregnancy term length of 180 days. (Supported by Conservation Action Network of the Memphis Zoo)

ASSESSING BONE DENSITY WITH ULTRASONIC BACKSCATTER. *John A. Janeski and Brent K. Hoffmeister, Rhodes College, Memphis, Tennessee.* There is increasing interest in developing ultrasonic backscatter techniques for detecting changes in bone density caused by diseases like osteoporosis. Objective: To measure two ultrasonic backscatter parameters, apparent integrated backscatter (AIB) and the frequency slope of apparent backscatter (FSAB) using a broadband 7.5 MHz system. AIB represents the frequency averaged power in the backscattered signal and FSAB represents the slope of the frequency dependence of this power. Methods: Cubes of cancellous (spongy) bone with side lengths of 15 mm were prepared from the heads of 10 human femurs (7 donors). Data were collected by mechanically scanning an ultrasonic transducer over each bone specimen and acquiring the backscattered signals. These were post-processed to obtain a single value of AIB or FSAB for each specimen. In addition, the mass density of each specimen was measured by allowing the specimen to air dry for 24 h and then dividing the mass of the specimen by its volume. Results: AIB and FSAB both demonstrated highly significant linear correlations with density ($P < 0.001$ and $P < 0.001$). Conclusion: AIB and FSAB are sensitive to changes in bone density, and may provide a useful new clinical technique for detecting and monitoring osteoporosis. (Supported through a MRI/RUI research grant from the National Science Foundation)

SYNTHESIS AND CHARACTERIZATION OF CROSS-LINKED POLY(MMA-CO-EGDMA). *Anne R. Tamer and Richard D. Redfearn, Rhodes College, Memphis, Tennessee.* Often the amount of cross-linking comonomer in commercial polymers is very small compared to other components of a polymer product. The amounts of ethylene glycol dimethacrylate (EGDMA) in poly(methyl methacrylate) (PMMA) can range from 30–40% by weight in contact lens acrylic polymer down to the tenths of a weight percent in plastics used in automotive or bathtub/hot tub plastics. However, only a few hundred ppm of EGDMA in a copolymer with MMA can render the polymer insoluble and intractable for the purposes of obtaining good spectroscopic data by ^1H and ^{13}C NMR, for example. Pyrolysis GC-MS has the advantage of providing a quantitative determination of EGDMA incorporated into the polymer, even at low levels down to 200 ppm (0.02 wt% or approximately 0.01 mol% based on total polymer) in poly(MMA-co-EGDMA) copolymers. Also, "gel state" NMR can be effective in showing tacticity of the copolymer with significant levels of cross-linking. This poster will illustrate the reproducible synthesis of poly(MMA-co-EGDMA) polymers and their characterization by "gel state" NMR and pyrolysis GC-MS.

NUCLEAR MAGNETIC RESONANCE STUDY OF THE HOST-GUEST COMPLEXATION OF A COMMERCIAL CHLORDANE SAMPLE WITH BETA-CYCLODEXTRIN AND BETA-CYCLODEXTRIN TRIACETATE. *Rachel M. Methvin*

and Richard D. Redfearn, Rhodes College, Memphis, Tennessee. One long-term threat to urban environmental health and safety is the occurrence of persistent organic pollutants (POPs) in urban soil and water. Some of these compounds are associated with acute and chronic human toxicity. In Memphis, there has been recent awareness of the occurrence of polychlorinated pesticide residues in the soil along Cypress Creek in Midtown and North Memphis neighborhoods. These POPs are artifacts of the production of the pesticide chlordane by the chemical company Velsicol, although production was halted in the late 1980s. One possible route to remediation of Cypress Creek soil is the possible phytoremediation scheme of allowing the organochlorine POPs to be translocated up into the native plants growing on the banks. A previous study in our laboratories ruled out any significant phytoremediation from the growth of three common weed species, most likely due to very low water solubility of the POPs involved. A way that phytoremediation could be enhanced might be to increase the water solubility by encapsulating the soil-bound organochlorine POPs into cyclodextrin complexes. This preliminary study tests this hypothesis by attempting to create chlordane-cyclodextrin host-guest complexes and gauging the efficacy of the complexation by ^1H and ^{13}C NMR.

A MICROSTRUCTURAL STUDY OF ULTRAHIGH MOLECULAR WEIGHT POLYETHYLENE USING TRANSMITTING ELECTRON MICROSCOPY AND PYROLYSIS GAS CHROMATOGRAPHY-MASS SPECTROMETRY. *M. Andrew Scott, Terese A. Holm, Karyn E. Spence, Carl W. Carlson, Ann M. Viano, Richard D. Redfearn, and Asit K. Ray, Rhodes College, Memphis, Tennessee (MAS, KES, AMV) and Christian Brother's University, Memphis, Tennessee (TAH, CWC, RDR).* Ultrahigh molecular weight polyethylene (UHMWPE) is a commonly used material in human joint prostheses. The combination of its non-reactivity in the body and its mechanical properties make UHMWPE ideal for cartilage replacement in artificial knee and hip joints. A drawback is the material's production of submicron wear particles, which can cause adverse biological reactions. Post processing of the material with gamma-irradiation and annealing has been shown to reduce the production of these wear particles. While the effects of these treatments on the macroscopic properties of UHMWPE have been investigated, few researchers have studied their effects on the molecular level of structure. We have used transmission electron microscopy to visualize the crystalline (lamellar) and amorphous regions of the polymer, and measured a "stacking parameter" that quantifies the degree of lamellar freedom in the polymer. This method was combined with a chemical analytical technique, pyrolysis coupled with gas chromatography-mass spectrometry, which identifies the types and extent of crosslinking. Together, these two methods have provided a determination of the microscopic structure of UHMWPE due to post-manufacturing treatments. The results should provide insight into future material processing to reduce wear in this important artificial joint material.

SYNTHESIS AND CHARACTERIZATION OF A SERIES OF IRON CHLORIDE LAYERED PEROVSKITES. *David Tran, Doinita Neiner, Xiao Zhang, Chris McArdle, Elisha Josepha, and John B. Wiley, Christian Brothers University, Memphis, Tennessee (DT) and University of New Orleans, New Orleans, Louisiana (DN, XZ, CM, EJ, JBW).* $(\text{FeCl})\text{LaTa}_2\text{O}_7$ and $(\text{FeCl})\text{-Ca}_2\text{Nb}_3\text{O}_{10}$ were synthesized for the first time by using ion exchange with the Dion-Jacobson double and triple layer

perovskites. The Dion-Jacobson double and triple layer perovskites, $\text{RbLaTa}_2\text{O}_7$ and $\text{RbCa}_2\text{Nb}_3\text{O}_{10}$ were formed by conventional solid-state method. Lithium analogues were prepared by an ion exchange method. The compounds were then exchanged with FeCl_2 at different temperatures under vacuum. The compounds were characterized by X-ray powder diffraction (XRD) and elemental analysis was done by energy dispersive spectroscopy (EDS). $(\text{FeCl})\text{LaTa}_2\text{O}_7$ appears to be isostructural with $(\text{FeCl})\text{LaNb}_2\text{O}_7$. The interlayer space increased in the new compounds from host compounds. Elemental analysis confirmed the presence of both iron and chlorine in the new compounds. (Supported by NSF Summer Research Program in Solid State and Materials Chemistry (DMR-0303450) and Dr. Hwu)

MANED-WOLF ECOLOGY AND CONSERVATION IN EMAS NATIONAL PARK. *Jennifer Longo, Sara Lynch, Leandro Silveira, and Anah Jacomo, Christian Brothers University, Memphis Tennessee (JL, SL) and Emas National Park, Emas Brazil (LS, AJ)*. The maned-wolf, *Chrysocyon brachyurus*, is an endangered resident species of Emas National Park, located in the southwest corner of Goias, Brazil. From June 4 to July 18, 2004, data on home-range and diet of maned-wolves was collected in cooperation with a larger, long-term project. The purpose of the study was to broaden our understanding of maned-wolf ecology and raising awareness for the conservation of this species. During this study a total of seven maned-wolves were captured 27 times for radio-collaring and collection of biological data. Nine additional animals had been collared previously. Average home-range size of the 16 individuals was 1,247.34 hectares (7,616.04 acres), and their food habits were consistent with a generalist carnivore, where animal and plant matter was consumed regularly. Data will be presented describing their habitat and the surrounding agricultural area and the need for conservation of these carnivores. (Supported by NIH and Fogarty International, Minority International Research Training Program (TW 00123-S2) and Conservação International Brazil)

RESPIRATION STUDY OF THE MEMPHIS ZOO'S GIANT PANDAS. *Jennifer M. Bernard and Meghan M. Carr, Christian Brothers University, Memphis, Tennessee and the Memphis Zoo, Memphis, Tennessee*. The objective of this study was to analyze the respiration rates of resting giant pandas in order to understand metabolic rates. The higher an animal's metabolism is the more oxygen it will use. Since a metabolic chamber cannot be used to measure oxygen consumed in the Memphis Zoo's giant pandas, respiration rates were used to indicate the metabolic rate of the subjects. The female showed relatively consistent respiration rates throughout the study while the male showed a tendency to decrease average respiration rates 60 min after feeding. The causes of the differences were not determined, but could be linked to various factors, such as age, health, size, or sex. (Supported by The Assisi Foundation, Biodiversity Fellowship)

COMPARING DATA COLLECTION PROTOCOLS OF MULTI-WEEKLY ACTIVITY BUDGETS OF MEMPHIS ZOO'S MALE PANDA FOR WINTER 2005. *Sally Lynch and Meghan Carr, Christian Brothers University, Memphis, Tennessee and the Memphis Zoo, Memphis, Tennessee*. The Giant Panda is one of many endangered species being housed and studied at the Memphis Zoo. Currently, the male individual living at the zoo

(M466) is under careful watch for reasons concerning breeding season and diet studies. This animal's daily activity budget is of great interest to researchers who are monitoring hormone levels and dietary intake and output. Data collection protocols for these daily budgets is subject to change based on the following comparison of the current protocol, which requires that 10-min scans be conducted to gather specific activity occurrences, and a newly suggested protocol that collects data based on random scans over an 11-h period. The following comparison is based on four weeks (January 3–30, 2005) of data that were collected using both protocols via a camera-recording system. Statistical analyses were conducted using the Cochran-Mantel-Haenszel Statistics for a Stratified Table and chi-square table. (Supported by The Assisi Foundation, Biodiversity Fellowship)

ADRENERGIC MODULATION OF THE RAT'S AVOIDANCE RESPONSE IN THE ELEVATED PLUS MAZE. *Kelley Ward, C. Anzini, and Antonio P. Carobrez, Christian Brothers University, Memphis, Tennessee (KW) and Universidade Federal de Santa Catarina, Florianopolis, Brazil (CA, APC)*. The elevated plus maze (EPM) is an animal model of anxiety based on the spontaneous behavior of the rat in a single 5 min trial. However, when re-evaluating rats on a Trial 2 session (24 h after) in the EPM, a previous learned experience from Trial 1 will increase the avoidance response (AR). To test the hypothesis that the experience in the EPM Trial 1 would be mediated by the activation of β -adrenergic receptors, two experiments were conducted. In Experiment 1, Wistar rats were injected (IP) with propranolol (PROP; 5, 10 or 20 mg/kg) prior to Trial 1 and were retested (Trial 2) undrugged in the EPM. PROP-treated rats showed an increased open arm entries (OAE) and time (OAT), suggesting an anxiolytic-like (anti-aversive) effect in Trial 1. In Trial 2 the rats from PROP group performed equal to experimental naïve control rats, suggesting a blockade of the AR elicited by PROP. In Experiment 2, naïve rats were injected with PROP 20 mg/kg prior to Trial 1. Trial 2 they were treated (IP) with either saline or midazolam (MDZ; 0,25 mg/kg) 30 min before submission into the EPM. In trial 2, MDZ-treated rats showed a reliable anxiolytic-like effect shown by an increased OAE and OAT, when compared to saline-treated rats. Therefore, PROP reinstated the anxiolytic properties of the MDZ, known to be blocked in saline-treated subjects. The above results suggest that the PROP blockade of ascending stress-related information, in Trial 1 impairs the rat to acquire and retain the AR characteristic of the EPM task. (Supported by NIH and Fogarty International, Minority International Research Training Program (TW 00123-S2))

THE ROLE OF INTERLEUKIN-16 IN MOTOR LEARNING. *Bradley W. Petkovich, Jay A. Blundon, and Catherine P. Fenster, Rhodes College, Memphis, Tennessee*. Neuronal interleukin-16 (NIL-16) is expressed specifically in the hippocampus and cerebellum, brain regions associated with certain forms of learning. NIL-16 can be enzymatically cleaved to result in neuronal secretion of IL-16; thus, NIL-16 is a precursor of IL-16, a cytokine associated with immune system functions. We believe that in the brain, IL-16 may be a signaling molecule that regulates learning processes. The purpose of this study was to investigate the possibility that IL-16 plays a role in motor learning. To address the role of IL-16 in motor learning, we used an accelerating rotarod test to compare motor learning for IL-16 knock-out and wild-type mice. Both groups were initially

exposed to the rotarod (constant speed of 20 RPMS) for seven days. During the learning phase (two trails per day for four days), the duration that mice remained on the accelerating rotarod (from 0 to 50 RPMs) was compared. For the memory phase, the duration that mice remained on the accelerating rotarod following a week of non-exposure was compared. No significant differences were observed between knock-out and wild-type mice on accelerating rotarod tests; however, we are currently performing additional behavioral tests to explore potential effects of IL-16 on motor learning.

ELECTROPHYSIOLOGICAL INVESTIGATION OF THE EFFECTS OF NEURONAL INTERLEUKIN-16 ON LONG TERM POTENTIATION IN MOUSE HIPPOCAMPAL SLICES. *Jessica A. Devitt, Catherine P. Fenster, and Jay A. Blundon, Rhodes College, Memphis, Tennessee.* The purpose of this experiment was to determine the effects of neuronal interleukin-16 (NIL-16) on long term potentiation (LTP) in the mouse hippocampus. Previous studies have reported that NIL-16 is found in the hippocampus, a brain region associated with learning and memory. We believe that NIL-16 may play a role in LTP and the learning process. The C-terminus of NIL-16 is identical to the cytokine pro-interleukin-16. IL-16 knock-out mice show spatial memory deficits compared to their wild type siblings. Therefore, to test whether IL-16 secretion influences LTP, we performed electrophysiological tests on hippocampal slices from wild type and IL-16 knock-out mice. Stimulating electrodes were placed in the CA3-Schaffer collateral pathway and the recording electrode was placed in the CA1 dendrite region. After baseline low frequency (0.033 Hz) postsynaptic potentials (PSPs) were recorded, a frequency tetanus stimulation was administered, followed by low frequency stimulation for 60 min. If the slope of the post-tetanus PSP was significantly greater than the slope of the pre-tetanus PSP, we concluded that LTP had occurred. Although we had hypothesized that LTP would be reduced with the absence of IL-16, we found no significant differences in LTP between the wild type and IL-16 knock-out mice.

USING THE MONTE CARLO TECHNIQUE TO SOLVE TOUGH INTEGRALS. *Paul Sinclair and Shubo Banerjee, Rhodes College, Memphis, Tennessee.* Research into the condensation of ionic systems requires calculating a six dimensional integral that relates the number of free ions to the number of neutral molecules. Regular methods of integration, such as using the software *Mathematica* prove inadequate. Instead we use Monte Carlo Integration that uses random sampling of the integrand to estimate its average. Multiplying by the limits of integration for each variable then yields an estimate of the integral with accuracy that depends on the number of sampled points. The process is easily expanded to multiple dimensions and gains in accuracy over other methods (for the same computation time) as the number of dimensions increase. The error is estimated by looking at the variation in the final answer for different trials. We wrote a C++ program to evaluate our integral. To generate random numbers for sampling we used a random number generator called the *Mersenne Twister*. Due to the severely "spiked" nature of the integrand, we developed weighting methods for each variable to get a better sampling of the spikes and thus reduce sampling errors. Running the final program for 125,000,000 sampled points and 100 trials at each value of temperature, we achieved accuracy to within 0.2%.

ENVIRONMENTAL EDGE AND RESOURCE GRADIENTS IN PLANTS. *Karla L. Gage, Melissa B. Lee, and Maciej Biernacki, University of Memphis, Memphis, Tennessee.* Traditionally edge effect is a concept related to qualitative differences between core environments. However, where two core environments meet, there are quantitative graded changes in the most basic resources that plants require, including light, temperature, and soil moisture. Resource availability changes nonlinearly over space as the two core environments blend. Temporal dynamic changes occur as a result of diurnal and seasonal cycles. Plants will respond to changes in resource gradients and may be used as bioindicators of the availability of resources along the edge by analyses of the timing of phenological stages. Bioindicators may allow quantification of the width of gradients between two core environments. Changes in gradients of resource availability over time may be measured quantitatively. In a controlled study, three treatment groups were established to represent the continuum of resource availability in edge systems. Plant response was evaluated in each. Preliminary data will be presented.

PLANT RESOURCE ALLOCATION: PROJECTED SURFACE AREA. *Melissa B. Lee, Karla Gage, and Maciej Biernacki, University of Memphis, Memphis, Tennessee.* The well-being of a plant may be assessed by a variety of quantitative measures. Recent studies suggest that patterns of resource allocation may be better reflected by measures of plant leaf and root surface areas rather than traditional measures of biomass. Surface areas reflect the area of interaction a plant has with the surrounding environment. Comparative studies have indicated that evaluation of the projected surface area is an accurate and precise method of measuring plant performance. The objective of the present study was to quantitatively evaluate plant performance using several measures including above-ground biomass, belowground biomass, projected leaf surface area, leaf surface area, and root surface area. Plants were evaluated periodically using both destructive and nondestructive techniques. Statistical analyses identified significant relationships among quantitative parameters of plant growth. There were significant relationships found between leaf surface area and other plant growth parameters. Leaf and root surface areas were ranked in most analyses as the most responsive plant traits. Statistically significant relationships among quantitative plant characteristics allow for development of nondestructive, species specific procedures to evaluate nondestructively plant resource allocation patterns, plant health, productivity, and allow for objective inter-study comparisons.

ESTERASE ISOZYMES OF *THYRIDOPTERYX EPHEMERA-AEFORMIS*: DEVELOPMENTAL ONTOGENY AND TISSUE SPECIFICITY. *Cheri Kimes, Jamie Walker, Melvin Beck, and Charles Biggers, University of Memphis, Memphis, Tennessee.* The objective of the present study was to characterize the general esterases of *Thyridopteryx ephemeraeformis* on the basis of their tissue and ontogenetic developmental distribution. Native polyacrylamide gel electrophoresis with α - and β -naphthyl acetate as substrates revealed six general esterase isozymes (Esterase 1-6) in whole body homogenates of larvae, pupae, and adults and tissue extracts (alimentary canal, fat body, Malpighian tubules, and salivary glands) of *T. ephemeraeformis*. Variations in isozyme patterns occurred during developmental stages, indicating differential gene expression and/or epigenetic

modification of the isozymes to meet the metabolic needs of the different developmental stages. There appeared to be some isozyme variation within the same developmental stage. The isozyme pattern in larvae was more complex than that of the pupae or adults. Each larval tissue showed a characteristic esterase pattern that is probably correlated with the functional role of the isozymes. The midgut and hindgut of the larval alimentary canal exhibited the most complex esterase profile, which would indicate that some of the esterase isozymes have a digestive function.

COMPARISON OF THE DEVELOPMENTAL ONTOGENY OF ISOZYMES IN *TRIBOLIUM CONFUSUM* AND *TRIBOLIUM CASTANEUM*. *Victoria K. Lo and Melvin L. Beck, University of Memphis, Memphis, Tennessee.* The objective of this study was to compare the developmental ontogeny of α -glycerophosphate dehydrogenase (α -GPD), esterase (EST), and leucine aminopeptidase (LAP) isozymes in *Tribolium confusum* and *T. castaneum*. Polyacrylamide gel electrophoresis was performed on whole body homogenates of larvae, pupae, and adults of each species followed by appropriate histochemical staining. A single zone of α -GPD activity occurred in larvae and pupae of both species. No detectable α -GPD was found in adults of either species. Differences were observed in the esterase electrophoretic profiles between the two species and among the developmental phases for *T. castaneum*, indicating gene activity related to specific developmental stages. *Tribolium confusum* did not display ontogenetic changes for esterases. The esterase activity was dramatically lower in all developmental stages of *T. castaneum*, when compared to similar stages in *T. confusum*. We found differences in gene activity among developmental stages for LAP for both species, but the electrophoretic profiles for LAP were more similar between the two species than for either α -GPD or esterases.

ARTIFICIAL FERTILIZATION AND HORMONAL INDUCTION OF SPAWNING IN THE ENDANGERED WYOMING TOAD (*BUFO BAXTERI*). *Robert K. Browne, Jessica Seratt, Carrie K. Vance, and Andy J. Kouba, Memphis Zoo, Memphis, Tennessee (RKB, AJK), Mississippi State University, Starkville, Mississippi (JS), and University of Memphis, Memphis, Tennessee (CKV).* Endangered Wyoming toad hormonal induction of male and female gametes is administered as a single dose of one hormone. Female spawning (fertilization) is often low or absent with low viability. Using female Wyoming toads, we tested a combination of human chorionic gonadotrophin (hCG) and luteinizing hormone releasing hormone analogue (LHRHa) administered in zero, one, or two priming doses. Females were spawned separate from males, and oocytes were fertilized in vitro with hormonally induced sperm from several males. Unprimed females produced no oocytes. Spawning of females occurred with 70% with one priming and 88% with two primings. Over the total spawning period (1.5–23 h post-induction) higher spawn numbers occurred with two primings (25,721), versus one (13,320). During the period with fertile oocytes (11.5–17 h post-induction), spawn numbers were 2871 ± 154 and 1439 ± 175 , respectively. During the fertile period the percent fertilizations were not significantly higher in toads receiving two primings vs. one ($12.7 \pm 1.2\%$ vs. $6.4 \pm 1.4\%$). Nevertheless, lower mortality from two primings resulted in a significantly greater ($P < 0.05$) percent neurulation ($9.5 \pm 1.0\%$; $1.9 \pm 0.5\%$) and swim-up ($6.9 \pm 0.8\%$; $0.82 \pm 0.04\%$), resulting in 84 tadpoles with one

priming, and 2300 tadpoles with two. (Supported by Conservation Action Network of the Memphis Zoo)

EXAMINATION OF THE ROLE OF GROWTH FACTOR RECEPTORS AND THE INTEGRIN $\alpha 5\beta 1$ IN CD9 MEDIATED CELL MOTILITY. *Whitney M. Appling, Jayaprakash Kotha, and Lisa K. Jennings, Christian Brothers University, Memphis, Tennessee (WMA) and University of Tennessee Health Science Center, Memphis, Tennessee (JK, LKJ).* Overexpression of tetraspanin CD9 in Chinese Hamster Ovary (CHO) cell expression model system promotes haptotactic cell migration to the extracellular matrix protein fibronectin (FN). In the current report, we investigated the possible role of serum-containing growth factors and a CD9 molecular partner, integrin $\alpha 5\beta 1$, in CD9 promoted cell migration. CD9 expressing CHO cells (A6) retained their characteristic pro-migratory phenotype even after culturing in 1% serum media for 18 h, suggesting that CD9 promoted migration is due to CD9 influence on adhesion molecules rather than growth factor receptor activity. Integrin $\alpha 5\beta 1$ mediates adhesive functions of cells such as cell migration on FN. Altered expression or activity of $\alpha 5\beta 1$ dramatically affects the phenotypic properties of cells on FN. Since $\alpha 5\beta 1$ is expressed on CHO cells and serves as the major FN receptor, we investigated possible regulation of $\alpha 5\beta 1$ function by CD9 in promoting cell migration. Flow cytometry analysis revealed that cell surface density of integrin $\alpha 5\beta 1$ was not significantly altered because of CD9 over-expression. An anti- $\alpha 5\beta 1$ monoclonal antibody (PB1), earlier shown to inhibit the functions of $\alpha 5\beta 1$ on FN, selectively inhibited CD9 promoted cell migration. These data suggest that CD9 positively modulates $\alpha 5\beta 1$ interactions with the ECM ligand FN. (Supported by the American Heart Association, Southeast Affiliate to LKJ)

STRUCTURAL EVALUATION OF THE N-TERMINAL REGION OF CHOLINE BINDING PROTEIN A, THE MAJOR ADHESIN OF *STREPTOCOCCUS PNEUMONIAE*. *Ross W. Hilliard and Richard W. Kriwacki, Rhodes College, Memphis, Tennessee and St. Jude Children's Research Hospital, Memphis, Tennessee.* *Streptococcus pneumoniae* (pneumococcus) remains a significant health threat worldwide, especially to the young and old. While some of the biomolecules involved in pneumococcal pathogenesis are known and understood in mechanistic terms, little is known about the molecular details of bacterium/host interactions. Our work is focused on understanding how the major adhesin, a protein called CbpA, binds to and causes pneumococcal invasion of human cells. Here we report the results of experiments aimed at determining the 3D structure of the N-terminal domain of CbpA. We are optimizing CbpA-N for study using nuclear magnetic resonance (NMR) spectroscopy using biochemical methods. We previously identified significant unstructured regions using NMR in an N-terminal construct comprised of CbpA residues 39–174. These unstructured regions increase the level of difficulty in determining the protein structure using NMR. To reduce such interference we used proteases to trim away unstructured amino acids, leaving only the folded core of CbpA-N. We used mass spectrometry and computer methods to identify disordered regions of CbpA-N and then designed an optimized construct. We report here the results of these studies along with the work currently underway as a result of the structural determinations. (Supported by the American Lebanese Syrian Associated Charities, The Rhodes College/St. Jude Summer Plus Program (RH), sponsored by Robert and Ruby

Priddy Charitable Trust), the National Cancer Institute (RWK), and a Cancer Center (CORE) Support Grant CA 21765 (SJCRH))

GROUP B STREPTOCOCCUS INDUCES CASPASE-MEDIATED APOPTOSIS OF RESPIRATORY EPITHELIAL CELLS.

Monica Huerta, Glen Ulett, and Elisabeth Adderson, Rhodes College, Memphis, Tennessee (MH) and St. Jude Children's Research Hospital, Memphis, Tennessee (GU, EA). Group B Streptococcus (GBS) is an important cause of neonatal pneumonia and sepsis. Infection of respiratory epithelial cells by GBS induces cell injury, a process that contributes to bacterial invasion. To identify the nature of this cytotoxicity, we characterized cell death pathways activated by GBS infection using a serotype III strain, 874391, and its isogenic beta-hemolysin-deficient mutant, CylE-. A549 respiratory epithelial cells were infected at a ratio of 5 : 1 (bacteria : cell) for 4 h. Numbers of eukaryotic cells undergoing apoptosis were determined by fluorescence-activated cell sorting analysis using AnnexinV-FITC and propidium iodide stains in the presence or absence of 5% glycine and with increasing concentrations of the caspase-3 inhibitor Ac-DEVD. Infection of A549 cells with wild type GBS 874391 resulted in a reduction in numbers of viable cells within 4 h. The percentage of viable A549 cells infected with the beta-hemolysin deficient strain CylE did not differ from that of uninfected controls. Pretreatment of epithelial cells with glycine to inhibit necrosis did not increase the viability of GBS-infected cells. However, Ac-DEVD treatment reduced the number of cells undergoing apoptosis in a dose-dependent manner. Infection of A549 respiratory epithelial cells with serotype III GBS induces rapid cell death. As previously suggested, most cytotoxicity is attributable to the expression of GBS beta-hemolysin. Cell death induced by GBS infection is caspase-3 dependent and involvement of the intrinsic pathway of apoptosis is suggested. Inhibition of cell death pathways induced by GBS may be a novel strategy to reduce the morbidity and mortality of neonatal pneumonia.

ENGINEERING A C-1 MUTATION IN THE PRESTIN GENE OF OUTER HAIR CELLS.

Manish Patel, Jiangang Gao, and Jian Zuo, Christian Brothers University, Memphis, Tennessee (MP) and St. Jude Children's Research Hospital, Memphis, Tennessee (JG, JZ). Outer hair cells (OHCs) of the mammalian cochlea actively change their cell length in response to changes in membrane potential. This electromotility, thought to be the basis of cochlear amplification, is mediated by a voltage sensitive motor molecule recently identified as the membrane protein prestin. The cell loss and reduction in soma length of OHCs seen in prestin-knockout mice suggests that other active mechanism(s) (such as that conferred by the active movements of hair bundles observed in lower vertebrates) might have been compromised. To provide definitive evidence that no active forces other than OHC electromotility exist in OHCs, we will generate and characterize prestin-knockin mice in which a crucial mutation (C1) is introduced in the endogenous prestin gene. The C1 mutation in the prestin gene results in a reduced or non-motile yet structurally intact protein in vitro. We will determine whether this mutation abolishes OHC electromotility and cochlear amplification without causing cell loss and reduction of soma length of OHCs, to confirm that prestin-mediated OHC electromotility is the only active mechanism by which cochlear amplification is generated. In this research the first stages of the

knock-in procedure was accomplished in which a stem cell clone was produced with the C1 mutation. This mutation was accomplished by stem-cell transfection of an engineered vector with the C1 mutation. Genomic southern analysis confirmed the knock-in procedure. (Supported by 5 R25 CA23944 and P30 CA-21765 from the National Cancer Institute and by the American Lebanese Syrian Associated Charities)

AMPA-TYPE GLUTAMATE RECEPTORS IN THE OCULOMOTOR NUCLEI OF RAT AND PIGEON BRAIN.

Adriane D. Wilkinson, Claudio A. B. Toledo, Raquel S. Pires, Malinda E. C. Fitzgerald, and Anton J. Reiner, Christian Brothers University, Memphis, Tennessee (ADW, MECF), City University of São Paulo, São Paulo, Brazil (CABT, RSP), and University of Tennessee, Memphis, Tennessee (MCEF, AJR). Glutamate has been identified as the primary excitatory neurotransmitter within the central nervous system, and it plays an important role in the mediation of synaptic transmission. It may be involved in activation of the cranial motor nuclei to promote rapid responses, as required in many motor reflexes. These actions can be performed through AMPA-type glutamate receptors, which are ionotropic and endorse fast neural reaction. Immunohistochemistry and in situ hybridization were performed using pigeon and rat brains to investigate the localization of the subunits which constitute the tetrameric structure of these receptors within the oculomotor nuclear complex (CNIII). We used antibodies against subunits GluR1, GluR4, and a third (GluR2/3), which recognizes a common epitope of the GluR2 and GluR3 subunits. We also used specific complementary RNA (cRNA) probes to chick subunits of the AMPA-type receptors to perform the in situ analysis. The immunohistochemical investigation showed very few labels for the GluR1 and GluR2/3 subunits at the CNIII area. Results for the GluR4 subunit, however, did display positive immunoreactivity with many neurons at the CNIII clearly expressing GluR4. In situ hybridization results agreed with our statement above indicating positive labeling of the four-glutamate subunits (GluR1-4) with intensities ranging from light to intense. These results suggest that subunits of the AMPA-type ionotropic receptor are expressed at neurons in the CNIII. The data also suggest that glutamate mediates synaptic transmission within this region of the central nervous system, and this transmission is mainly a result of the receptor type holding the GluR4 subunit. (Supported by NIH and Fogarty International, Minority International Research Training Program (TW 00123-S2), EY-05298 (AJR) and FAPESP, Brazil (CT))

THE GLUTAMIC-384 AND GLUTAMINE-384 VARIANTS OF THE BETA-1 ADRENERGIC RECEPTOR EXHIBIT CONSTITUTIVE ACTIVITY AND DIFFERENTIATE COUPLING OF G-PROTEIN. **Amar Bhula, Sulieman Bahouth, Noel Delos Santos, and Lidia Gardener, Christian Brothers University, Memphis, Tennessee (AB) and University of Tennessee Center for Health Sciences, Memphis, Tennessee (SB, NDS, LG).** The Beta-1 adrenergic receptor is a major mediator of catecholamine effects in the human heart, including heart rate and contractility. Stimulation of the Beta 1 adrenergic receptor by catecholamines activates the heterotrimeric G-protein, which, in turn activates the enzyme adenylyl cyclase, and promotes cyclic AMP production. Increased cyclic AMP production provides a greater risk of heart failure due to increases in heart rate and contractility. Beta-1 adrenergic receptor blockade has proved to be effective in

the treatment of chronic heart failure. Mutating the receptor, through site directed mutagenesis, allows a better understanding of variant receptor activity. Two variants of the beta receptor were tested in this experiment and were compared to the wild type receptor by measuring the accumulation of the second messenger, cyclic AMP. Compared to the wild type receptor, which has arginine at the 384 position, both types of variants, the glutamic acid and glutamine receptors, both demonstrated characteristic features of constitutively active receptors. Both variant types increased adenylyl cyclase activity compared to the wild type receptor. The glutamic acid variant showed similar amounts of cyclic AMP as did the wild type prior to receptor stimulation. The glutamine variant showed a much higher increase in enzyme activation compared to the wild type. In addition, adenylyl cyclase activity had increased prior to the addition of agonist. Mutating the beta adrenergic receptor with the respective variants suggests that arginine at the receptor 384 position holds a prominent role in receptor-G protein interactions. By mutating the wild type receptor at the 384 position from a basic residue to an acidic or neutral residue of similar mass results in an increase in downstream signaling events.

DETERMINING NEURONAL INTERLEUKIN-16 AND KV4.2 INTERACTION AND REDUCING NEURONAL INTERLEUKIN-16 VIA SMALL INTERFERING RNA. *Sinifunanya E. Nwaobi, Jay A. Blundon, and Catherine P. Fenster, Rhodes College, Memphis, Tennessee.* Neuronal Interleukin-16 (NIL-16) is a cytosolic protein found exclusively in the cerebellum and hippocampus. Previous studies have shown that NIL-16 contains PDZ domains that selectively interact with several neurotransmitter-gated and voltage-gated ion channels. It is hypothesized that the interaction of NIL-16 with these ion channels may serve to regulate channel function and/or localization. A direct and concise approach to investigate this hypothesis would be to observe the functional properties of these ion channels in neurons with normal or reduced NIL-16 expression. We are currently developing a strategy for reducing expression levels of NIL-16 that utilizes small-interfering RNA (siRNA). This novel technique allows for specific gene silencing by targeting messenger RNA molecules containing an identical sequence for degradation. The degraded message is no longer functional in translation and the corresponding gene is silenced. Using immunocytochemistry, we demonstrate that siRNA targeting NIL-16 can effectively reduce levels of artificially expressed NIL-16 protein in human-embryonic cells. Future studies will include demonstrating silencing of NIL-16 in neurons and investigating the functional role of NIL-16 protein interactions in neurons.

INTERVENING FOR PREVENTION: A TEST-BASED APPROACH FOR SEXUALLY TRANSMITTED DISEASE AWARENESS. *Leonard I. Bloom and Angela D. Phillips, University of Tennessee Health Science Center, Memphis, Tennessee.* The purpose of this study was to increase sexually transmitted disease (STD) awareness in adolescents ages 14-19, particularly females. A presentation was constructed and performed at an area high school and church youth group. The session consisted of a pretest to gauge preexisting STD knowledge, a PowerPoint slide presentation giving the facts about gonorrhea, chlamydia, and human papilloma virus, and a posttest consisting of the same questions as the pretest, to determine how much information the students were able to retain. The results showed an increase (9.4%) in the number of

correct answers from the pretest to the posttest. The presentation was effective in that the students were able to recall the information presented to them and answer questions correctly.

FREQUENCY OF REPORTING OF CONCOMITANT SUBSTANCE ABUSE IN PATIENTS WHO ABUSE COCAINE IN PREGNANCY. *Erica Hayes, Jay Bringman, Risa Ramsey, Robert Egerman, Lu Kao, Lucinda Delmar, Christian Brothers University, Memphis, Tennessee (EH), University of Tennessee Health Science Center, Memphis, Tennessee (JB, RR, RE, LK, LD), and Regional Medical Center, Memphis, Tennessee (JB, RE, LK, LD).* We conducted a retrospective analysis of 100 pregnant patients whose infants, upon delivery, had a positive urine drug screen for cocaine. We sought to determine the incidence of reporting concomitant drug use in this group of individuals. The National Institute on Drug Abuse has reported that women who admit to using cocaine admitted to using other drugs as well. Drugs frequently used were alcohol and tobacco. The purpose of this study was not only to determine the occurrence of associated drug use, but also to treat cocaine users appropriately. This is essential because cocaine causes patients to present with similar symptoms that present as a result of toxemia due to pregnancy. For example, tachycardia, fever and high blood pressure are all congruent complications of pregnancy and cocaine abuse alike. Because of these comparable symptoms, cocaine toxicology most often goes undiagnosed and thus untreated or perhaps mistreated. From this study we anticipate obtaining a more efficient way to screen for cocaine toxicology. (Supported by Dept. Maternal and Fetal Research, University of Tennessee Health Science Center, Memphis, Tennessee.)

RENAL DISEASE AFFECTING PREGNANCY COMPLICATED BY LUPUS. *Carrie McIvor, Robert Egerman, Risa Ramsey, Lu Kao, and Lucinda Delmar, Christian Brothers University, Memphis, Tennessee (CM), University of Tennessee Health Science Center, Memphis, Tennessee (RE, RR, LK, LD), and Regional Medical Center, Memphis, Tennessee (RE, RR, LK, LD).* Systemic Lupus Erythematosus (SLE) is an autoimmune disease with deleterious effects on many organ systems. Renal dysfunction and long term impairment are significant hazards from SLE. The purpose of this study was to evaluate renal complications in pregnancies with a diagnosis of SLE at a large urban hospital. We reviewed the charts of gravida who had a diagnosis of SLE, seeking to describe the frequency of renal complications including hypertensive disease in these patients, as well as describe the clinical characteristics of this population. Sixty-eight pregnancies with a diagnosis of SLE were studied retrospectively. Of the patients identified with SLE, 30 patients presented with hypertensive disease and 38 SLE patients were normotensive. The average maternal age of the SLE pregnant patients presenting with a hypertensive disorder was 24 years, compared to the normotensive SLE pregnant patients of 26 years. Creatinine levels in patients with hypertensive disorders were elevated compared to creatinine levels in SLE patients without (1.059 mg/dL and 0.677 mg/dL), respectively, reflecting a dual insult on renal function. Platelet counts in pregnant patients with SLE and hypertensive disease were similar compared to platelets of normotensive patients with SLE, 199.8/L and 199.7/L, respectively. It is common for patients with SLE to have concomitant hypertensive disease. Recognition can prompt therapies to reduce the decline in renal function in pregnant women with SLE at risk. (Supported by Dept.

Maternal Fetal Research, University of Tennessee Center for Health Sciences, Memphis, Tennessee)

ROLE OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN IN TESTOSTERONE TRANSPORT. *Matthew D. Cain, Rachel Bowden, Mark Leslie, John D. Schuetz, Rhodes College, Memphis, Tennessee (MDC) and St. Jude Children's Research Hospital, Memphis, Tennessee (RB, ML, JDS).* The multidrug resistance-associated protein, MRP4 is an ATP-dependent binding cassette (ABC) transporter that belongs to a family of transporters linked to the efflux of various organic anions and nucleotides. MRP4 is linked to the efflux of various molecules including cAMP, cGMP, and the steroid conjugate, estradiol 17- β -D-glucuronide (E₂17 β G). In regard to drug resistance, MRP4 has been shown to efflux various chemotherapeutic agents, including topotecan, an anticancer agent, and 6-mercaptopurine and 6-thioguanine, two anticancer purine analogs. Although MRP4 is expressed in several tissues including the kidney, liver, brain, testes, and various others, little is known about the biological function of MRP4 in vivo. Studying MRP4-deficient mice, it was observed that, prepubescently, these mice exhibited a decreased level of circulating testosterone. However, post-pubescently, these mice exhibited normal testosterone levels. We set out to discover the mechanisms by which testosterone levels normalized in these MRP4-deficient mice and discover MRP4's role in the transportation of testosterone. We discovered an increase in the expression of several related transporters and various steroidogenic proteins, indicating a compensation in both testosterone production and transportation in the absence MRP4. (Supported by the Rhodes College/St. Jude Summer Plus Program and funded by Robert and Ruby Priddy Charitable Trust)

IRINOTECAN PHARMACOGENETIC STUDIES IN PEDIATRIC CANCER PATIENTS. *Christen N. Gregory and Kristine R. Crews, Christian Brothers University, Memphis, Tennessee and St. Jude Children's Research Hospital, Memphis, Tennessee.* Irinotecan is an anti-cancer agent with efficacy against numerous pediatric tumors, yet its use is complicated by its significant gastrointestinal toxicity. Irinotecan is a prodrug metabolized in vivo to the active metabolite, SN-38, which is glucuronidated via the UGT1A1 enzyme to SN-38 glucuronide. It is speculated that variable interindividual UGT1A1 activity causes disparity in SN-38 glucuronidation, which may produce toxicity in pediatric cancer patients. Single nucleotide polymorphisms (SNPs) within the ABCG2 gene also may increase the bioavailability and efficacy of irinotecan, since these SNPs have been associated with prolonged SN-38 exposure in tumor cells. This research examined the effect of UGT1A1 and ABCG2 genotypes ($n = 60$ and 21 , respectively) on single-agent oral irinotecan pharmacokinetics in patients. Patient DNA samples were analyzed following PCR amplification and DNA sequencing. Statistically significant differences were discovered in irinotecan clearance, irinotecan AUC, and total and lactone SN-38 glucuronide:SN-38 AUC ratio values between wild-type patients and patients with a polymorphism within the UGT1A1 gene. Discernable, yet non-significant, trends were discovered involving ABCG2 SNPs and irinotecan bioavailability. Throughout the course of this research, significant associations were discovered between UGT1A1 genotype and the irinotecan metabolic pathway. Our results suggest that including a larger sample size in future studies may reveal a stronger association between ABCG2 and

irinotecan oral bioavailability. Ultimately, pre-treatment UGT1A1 and ABCG2 genotyping may be utilized to maximize the efficacy of irinotecan-based chemotherapeutic therapies. (Supported by 5 R25 CA23944 and P30 CA-21765 from the National Cancer Institute and by the American Lebanese Syrian Associated Charities)

A STUDY OF THE PHARMACOKINETIC EFFECTS OF CYCLOPHOSPHAMIDE ON MEDULLOBLASTOMA PATIENTS. *Andrew Kovacs and Burgess Freeman, Christian Brothers University, Memphis, Tennessee and St. Jude Children's Research Hospital, Memphis, Tennessee.* Medulloblastoma is a malignant solid brain and spinal tumor that is most commonly found in the posterior fossa, which accounts for 20% of childhood brain tumors. Cyclophosphamide is an anti-tumor prodrug that is currently used for the treatment of solid tumors. This drug must be broken down into the active metabolite hydroxycyclophosphamide in the liver by hepatic enzymes, cytochrome P450 CYP3A5 and CYP2C9. Polymorphisms in the CYP3A5 and CYP2C9 subfamilies affect cyclophosphamide breakdown. Specifically, the polymorphisms CYP3A5*3 and *6 cause almost no enzyme to be produced and CYP2C9*2 causes a decreased production of enzymes, while the *1 genotype (wild-type) allows for normal enzyme productivity. In this study, St. Jude Children's Research Hospital patients' ($n = 39$) genotype were analyzed using polymerase chain reaction (PCR) and DdeI and AvaII restriction analysis. The genotypic allele frequencies were compared using a chi square test to previously existing CYP3A5 and CYP2C9 studies. There was no statistical difference in P values when the allele frequencies were compared to these other CYP3A5 and CYP2C9 studies meaning that our genotypic results are consistent for a given population. In future studies, this genotypic information will be correlated with pharmacogenetic information regarding cyclophosphamide treatment-based protocols for these patients. By knowing each patient genotype in regard to the cytochrome P450 hepatic enzymes CYP3A5*3 and *6 and CYP2C9*2 polymorphisms, cyclophosphamide dosage may be specifically altered on a patient to patient scale.

SUPRANUCLEAR CONNECTIVE TISSUE LAMELLAE OF THE HUMAN INTERVERTEBRAL DISC. *Shannon M. Winfrey and Eldridge F. Johnson, University of Tennessee Health Science Center, Memphis, Tennessee.* Ten adults and one newborn human lumbar intervertebral discs were examined by light microscopy in order to determine the connective tissue fiber organization of the annulus fibrosus, their relationship to the hyaline cartilage end-plate and the nucleus pulposus interface, and to elucidate the possible impact of this organization on differential diffusion rates of nutrients into the intervertebral disc. The more deeply situated annular lamellae abruptly change orientation from an initially vertical direction to a more horizontal incidence at the interface of the annulus fibrosus and the hyaline cartilage end-plate. Such lamellae were organized as horizontal stacks of supranuclear lamellae between the nucleus pulposus and the overlying hyaline cartilage end-plate. A given supranuclear lamella was arranged 90 degrees to those in contiguous lamellae. Supranuclear lamellae were not observed in more central regions of the nucleus pulposus and hyaline cartilage end plate interface. Although supra nuclear connective tissue fibers were observed in both the newborn and adult intervertebral discs, connective tissue fibers of a given lamella

presented a less densely organized, arching or arcing configuration in the newborn in contrast to the parallel, more radially and compact organization of the adult.

THE EFFECTS OF STEROID AND XENOBIOTIC RECEPTOR RNA EXPRESSION IN CD4+ CELLS. *Cynthia Caceres, Lorraine Sutton, Michael L. Vetter, Maria Pia De Pasquale, and Richard T. D'Aquila, Christian Brothers University, Memphis, Tennessee (CC) and Vanderbilt University Medical Center, Nashville, Tennessee (LS, MLV, MPDP, RTD).* Successful anti-retroviral therapy for HIV has been unable to be accomplished due to many intervening factors including drug resistance. Effectiveness of anti-retroviral drugs can be limited by virus-encoded drug resistance. In addition, HIV protease inhibitors (PIs) often fail without mutations in the virus protease and it is hypothesized that this may be caused by PIs being expelled out of host cells for HIV CD4+ lymphocytes. Such cellular resistance may be mediated by P-glycoprotein, or PgP, a drug transporter that can efflux drugs including PIs out of cells. PgP is encoded by the MDR-1 gene, which is in turn activated by the orphan nuclear receptor Steroid and Xenobiotic Receptor (SXR). The SXR is a member of the superfamily of nuclear receptors and has been described as a broad specificity sensing receptor. Because of SXR's role in activating the transcription of the MDR-1 gene and thus the amount of cell membrane PgP, we hypothesized that CD4+ cells with high levels of PgP should express high levels of SXR. We developed an assay to test for the presence of SXR RNA in CD4+ lymphocytes from PI-treated, HIV-infected patients using Real-Time PCR in order to start testing this hypothesis. Primers, probe, and plasmid to generate a standard curve of defined copy numbers were designed and tested. Preliminary results show that CD4+ cells with high levels of PgP do not necessarily express high levels of SXR RNA. Therefore we concluded from these results that there are mechanisms other than SXR that increase levels of PgP in CD4+ lymphocytes of HIV-infected patients.

ROLE OF MEMBRANE SYNTHESIS IN MACROPHAGE FUNCTION. *Christopher Sage and Suzanne Jackowski, Christian Brothers University, Memphis, Tennessee and St. Jude Children's Research Hospital, Memphis, Tennessee.* Macrophages are a type of white blood cell that is part of the first-line response to an infection. Bacteria and other foreign bodies are ingested and broken down into peptides that are presented as antigens by specialized molecules on the cell's surface. With phagocytosis within the macrophage, there is a need for phospholipids within the cell membrane, due to the development of phagosomes, vesicles that contain the foreign body in the macrophage. Two mammalian genes encode isoforms of CTP:phosphocholine

cytidyltransferase (CCT), a key rate-controlling step in membrane phospholipid biogenesis. The CCT α is the most ubiquitously-expressed and well-studied, while the CCT β form is present at significantly lower levels. We investigated the role(s) of the CCT α in macrophages by generating knockout mice. The CCT α gene knockout macrophages had a slower proliferation rate compared to the wild-type macrophages, which doubled in 7 days. The CCT α gene knockout macrophages also were limited in the amount of bacteria they were able to ingest. These data suggest that replication and phagocytosis were impaired, but not absent, from the CCT α -deficient cells. Therefore, the data show a definite reduction in macrophage activity due to the decrease in phosphatidylcholine from the CCT α -deficient cells. (Supported by GM45737, 5 R25 CA23944, and P30 CA-21765 from the National Institute of Health, the National Cancer Institute, and the American Lebanese Syrian Associated Charities)

MITOCHONDRIAL GENERATION OF OXYGEN RADICALS BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN A MODEL OF CHRONIC HEART FAILURE. *Vaishali Patel and Robert A. Ahokas, Christian Brothers University, Memphis, Tennessee and University of Tennessee Center for Health Sciences, Memphis, Tennessee.* The usefulness of finding the source for the generation of reactive oxygen species (ROS) will provide a greater understanding about the role of aldosterone in the neuroendocrine-immune interface in chronic heart failure. This will lead to a better understanding of the pathophysiology of chronic heart failure. ROS are both free radicals and reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them. Examples are hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite. ROS are primarily responsible for myocardial cell apoptosis. Peripheral Blood Mononuclear Cells (PBMC) treated with aldosterone, a mineralocorticoid hormone, generate increased levels of ROS, primarily H₂O₂, and invade the heart causing lesions similar to those seen in patients with chronic heart failure. Since previous research has shown that PBMC mitochondria of aldosterone/salt treated rats generate increased ROS levels, we hypothesized that the intracellular source of the ROS are the PBMC mitochondria. To test this hypothesis we used the mitochondrion specific probe Mito-Tracker Red (CM-H₂XROS) assay to assess mitochondrial ROS production by PBMC from aldosterone/salt treated rats compared to age/gender-matched untreated control rats. The PBMC fluorescence was then analyzed by single cell flow cytometry using a FACS cytometer. The results of the study show direct evidence for the increased generation of ROS by the PBMC mitochondria.