

ABSTRACTS OF PAPERS PRESENTED AT THE SPRING 2001 COLLEGIATE MEETINGS

MIDDLE REGION

AUSTIN PEAY STATE UNIVERSITY
CLARKSVILLE, TENNESSEE

INVESTIGATIONS OF ANTI-CANCER COMPOUNDS FROM EXTRACTS OF *ECHINACEA* AND ST. JOHNS WORTS. *Clifton Randell**, *Wendell Fortson*, *E. Lewis Myles*, and *Deborah Long*, *Tennessee State University, Nashville, Tennessee*. Many compounds found in plants have anti-bacterial, anti-fungal, and anti-cancer activities. The herb *Echinacea purpurea* (purple coneflower) produces natural compounds that may increase the efficiency of mammalian immune systems. This investigation used extracts from the dried roots of *Echinacea*. To test for anti-carcinogenic activity, breast cancer cells (BT549) were treated with the *Echinacea* extracts. These cells were grown at 37°C with 5% CO₂ and exposed to either 100 µl of physiological saline, 100 µl of acetone, or acetone with extract. Two-dimensional gel electrophoresis was used to analyze gene expression in the control and experimental groups. The growth rates of cells in the control and acetone groups were significantly higher than in cells exposed to the extract. The extract from the roots appeared to inhibit growth of cancer cell line BT549. After 2 and 4 d of exposure, growth rates decreased 25% and 74%, respectively. Ideally, a different solvent that has no effect on the cell growth rate will better define the extract's effect. The mitotic index was not significantly different in the groups studied. Future experiments will focus on different solvents and apoptosis. These results indicate that further studies are necessary to identify anti-carcinogenic compounds in *Echinacea*. (Supported by USDA/CSREES Evans-Allen Funds & MARC Grant # 5T34MO7663)

DETERMINING THE ANTI-MICROBIAL OR ANTI-CARCINOGENIC PROPERTIES OF ST. JOHN'S WORT AND *ECHINACEA* EXTRACTS. *Shani Smith**, *Anthony Ejiofor*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

AN EXAMINATION OF THE METALLIC RESIDUE LEFT BY DANDRUFF SHAMPOOS AND CITY WATER SOURCES ON HAIR THROUGH THE USE OF ATOMIC ABSORPTION SPECTROMETRY. *Megan C. Kime** and *John Shibata*, *The University of the South, Sewanee, Tennessee*. It has been hypothesized that certain types of chemicals are readily absorbed into human hair. Scientists have proven this hypothesis by looking at many different categories of chemicals such as hallucinogenic drugs and certain polymers from shampoos. One class of chemicals often ignored when looking at surface absorbed molecules is metals. This project assessed the presence of lead,

iron, and zinc pyrithione in human hair. It is believed that iron and lead are deposited into the hair through the water source. Zinc pyrithione is the active ingredient in dandruff shampoos and may be deposited through a simple washing process. Detailed analyses of these three metals were done using human hair samples from 10 different subjects. Samples were treated with RO water, tap water from Sewanee, Tennessee, 1% zinc pyrithione solution, or Head and Shoulders dandruff shampoo. A Varian Spectra AA-10 Atomic Absorption Spectrometer was used to examine the metals. The concentration ranges examined for iron were 1-50 ppm, for lead were 1-20 ppm, and for zinc pyrithione were 0.4-1.6 ppm. These results and their implications will be discussed further.

RECENTLY DISCOVERED ANCIENT (PENNSYLVANIAN) ARTHROPOD TRACKWAYS, NORTHERN CUMBERLAND PLATEAU. *Scott R. Schoeferacker** and *Molly F. Miller*, *Vanderbilt University, Nashville, Tennessee*. Trackways consisting of two rows of linear indentations have been found in the Fentress Formation northwest of Jamestown, Tennessee. The two parallel rows of "scratch marks" are separated by a structureless medial zone 3.8 cm wide. Scratch marks are 1.6 to 1.8 cm long and oriented obliquely to the medial zone. The uniformity in mark length and orientation is striking. The trackways occur in evenly bedded sandstone with clay drapes that was deposited in a marginal marine setting. Occurrence of a fossil starfish resting burrow indicates that salinity approached marine conditions, at least at times. However, the trackway was found just under (< 5 m below) braided stream deposits (freshwater) of the Rockcastle Conglomerate. The trackway probably was produced by a large arthropod, perhaps a horseshoe crab. Horseshoe crab resting traces have been found in the same rock unit nearby. The trackway closely resembles trackways interpreted as made by horseshoe crabs found in the eastern Kentucky coalfields. However, they differ significantly from other trackways interpreted as produced by horseshoe crabs. This raises the question of what other large arthropod might have been living in this marine margin environment ~320 million years ago.

DIELS-ALDER REACTION OF CYCLOPENTADIENE AND METHYL VINYL KETONE IN ROOM TEMPERATURE IONIC LIQUIDS: SELECTIVITY AND RATE ENHANCEMENT. *Christopher Wray**, *Carlos Lee*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

A LAKE STUDY IN GENERAL CHEMISTRY LABORATORY. *Blake Anderton**, *Jessica Bekar**, *Megan Kiraly**, *Brett Murdoch*, *Matt Nichols*, *Beki Nickens*, *Elizabeth Setty*, *Jason Smith*, *Daniel Young**, and *Kent Clinger*, *Lipscomb University, Nashville, Tennessee*.

Abstract not available.

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FIG. 1. Participants in the 2001 Middle Region, Tennessee Academy of Science Collegiate Division Meeting

A KINETIC ANALYSIS OF A DIGESTIVE ENZYME. *Jason Cobb**, *Katie Hartigan*, *Mary Langham*, *Sarah Lavender*, *Rob Nichols*, *David Peery*, *Justin Usery**, and *Kent Clinger*, *Lipscomb University, Nashville, Tennessee*.

Abstract not available.

THE STUDY OF THE HYDROLYSIS OF POLYPHOSPHATES USING P31 NMR: EFFECTS OF NORMALIZATION OF DATA AND AL³⁺. *ShaRee McIntosh** and *Ron Robertson*, *Austin Peay State University, Clarksville, Tennessee*.

Abstract not available.

TEMPERATURE AND PH EFFECTS ON LEAD LEACHING FROM CRYSTAL MUGS. *Jennifer Pollock** and *John P. DiVincenzo*, *Middle Tennessee State University, Murfreesboro, Tennessee*. Lead is the most ubiquitous toxic metal and is detectable in practically all spheres of the environment. It is a cumulative poison and about 90% of the lead retained in the body enters the bones, from which it can be remobilized. This study was designed to look at the temperature and pH effects on lead leaching from crystal mugs. Identical lead crystal mugs were filled with 150 ml of analytical-grade deionized water. Each water sample was heated to 25°C, 50°C, or 95°C. The pH of the samples was 2.0, 4.8, or 6.0. The samples were analyzed using a HACH Lead Fast Trak[®] kit and a HACH 2010[®] spectrophotometer. The mugs leached between 60–500 mg/l of lead, with the lowest pH and highest temperature releasing the most lead. A kinetic study also was carried out on one mug. The majority of the lead was found to leach out within the first 15 min (67%), with 85% leaching out within 30 min. In terms of its environmental concentration lead is much closer to the level at which overt signs of poisoning become manifest than is any other substance. Thus it is appropriate that society continues to take steps to further reduce human exposure to lead.

ANALYSIS OF C-FOS EXPRESSION IN THE NUCLEUS ACCUMBENS OF PD128907 AND METHAMPHETAMINE TREATED MICE. *Mark Owusu**, *M. Ann Blackshear*, *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

DRUG DISCRIMINATION: A PROCEDURE FOR ASSESSING THE SUBSTITUTION AND BLOCKING OF DRUG-IN-

DUCED STIMULUS PROPERTIES. *Jewell Hartwell**, *William Caul*, *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

THE STUDY OF HUMAN-RELATED RAD54 MUTATIONS IN YEAST. *Candace Jones**, *Terrance Johnson*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

THE SURVIVAL OF SALMONELLA SPECIES IN THE FOOD SAFETY DANGER ZONE. *Rosalind Johnson**, *Carolyn A. Caudle*, *Yvonne Myles*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

THE DYNAMICS OF A WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) POPULATION IN SOUTHERN HUMPHREYS COUNTY, TENNESSEE. *M. Crockett Bone** and *Wilodean D. S. Burton*, *Austin Peay State University, Clarksville, Tennessee*. A population of white-tailed deer inhabiting 425 ha (1050 acres) in southern Humphreys County was assessed during August and September 2000 using infrared photography to determine the composition and dynamics of the population. The study site was divided into seven individual plots of 61 ha, baited and photographed for ten days. The sex and age classes of the deer were recorded. The results suggest an estimated population of 32.9 animals with a density of 7.74 deer/km². The sex ratio of the population was determined to be 75 bucks/100 does. When compared to census data of 1999, the population increased 15% with fawn recruitment improving from a doe/fawn ratio of 6:1 (1999) to 3:1 (2000), indicating increased fitness among the reproductive segment of the population.

SELF-DIFFERENTIATION AS A PREDICTOR OF WELL-BEING. *Aisha Smith**, *Peter Millet*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

LOCALIZATION OF THE DOPAMINE D3 RECEPTOR FOLLOWING CHRONIC METHAMPHETAMINE ADMINISTRATION USING IMMUNOHISTOCHEMISTRY. *Jacinta Tibbs**, *M. Ann Blackshear*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

RECOVERY OF THE CYTOTOXIC FUNCTION OF HUMAN NATURAL KILLER CELLS FOLLOWING A BRIEF EXPOSURE TO TRIBUTYL TIN. *Tarrah Williams**, *Margaret Whalen*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.

THE INFLUENCE OF QUERCETIN ON IRON-INDUCED LIPID PEROXIDATION. *Shareen Lacy**, *William Y. L. Boadi*, and *Prem S. Kahlon*, *Tennessee State University, Nashville, Tennessee*.

Abstract not available.



FIG. 2. Participants in the 2001 Western Region, Tennessee Academy of Science Collegiate Division Meeting

WESTERN REGION
UNIVERSITY OF MEMPHIS
MEMPHIS, TENNESSEE

XENOTRANSPLANTATION OF PORCINE ISLETS INTO SCID MICE. *Cina Ali and Ivan Gerling, Christian Brothers University, Memphis, Tennessee, and University of Tennessee, Memphis, Memphis, Tennessee.* Xenotransplantation of porcine tissue into human recipients may be a way to alleviate the current organ shortage. One possible disease that would benefit is diabetes. Transplantation of islet cells to replace malfunctioning cells would aid the recipient. This study investigated the use of human or pig islets transplanted into immune deficient mice. These transplants were then exposed to the same species or different species immunocompetent leukocytes by injecting them into the mice carrying islet grafts. We used immunohistochemical methods to determine the presence of insulin if the graft was functional, and CD45 (a leukocyte common antigen) if the immune cells were actively infiltrating and destroying the islet grafts. We compared these results with appropriate controls for every group. Our results show that human and porcine islet rejection appears to be a species-specific response. Therefore the potential exists for successful cross species cellular transplants without rejection. (Support by NIH DK-55263)

EXPRESSION OF SPECIFIC CYTOKINES IN RESPONSE TO INFECTION OF *PLASMODIUM BERGHEI*. *D. Arrington, H. C. van der Heyde, and T. Brown, Christian Brothers University, Memphis, Tennessee (DA), and Louisiana State University Health Sciences Center, Shreveport, Louisiana (HCH, TB).* Cerebral Malaria (CM) is one of the leading causes of morbidity in tropic and subtropical regions of the world, little is known about why CM is so fatal. The specific aim of this study was to characterize and distinguish cytokine activities in response to infection with *Plasmodium berghei*, the principle cause of CM. Cytokines are involved in this study because information is needed on which cytokines are expressed due to *P. berghei* infection and why these expressions are relevant in regards to CM. It is necessary to understand the cytokine response so that potential treat-

ments can be formulated for future studies. Techniques involved were the infection of C57/BL6 mice with *P. berghei*, homogenization and isolation of mRNA from the tissues (brain, spleen and lung), reverse transcription (RT), and polymerase chain reactions (PCR) using ABI Prism 7700 Sequencer with PE Biosystem's sequence detection software for analysis of ABI results. These techniques will be used to elucidate which day (2, 4, or 6) a specific tissue has a greater cytokine response to the infection. The studies conducted will further our knowledge of which cytokine plays a key role during infection of *P. berghei* and possibly how science can one day slow or stop the effects of this rapid infection. It was found that cytokines IFN- γ , TNF- α , and Interleukin-12 had the highest percentage of change, whereas IL-10, -4, and -2 had little expression. (Supported by NIH)

POSSIBLE AUTONOMIC NERVOUS SYSTEM DYSFUNCTION IN POST-ABDOMINAL SURGERIES. *A. M. Cox and H. Rashed, Christian Brothers University, Memphis, Tennessee, and University of Tennessee, Memphis, Memphis, Tennessee.* Autonomic nervous system (ANS) dysfunction is characterized by a variety of symptoms including dizziness, sweating, tachycardia, and gastrointestinal problems. Any abdominal manipulation, such as partial gastrectomy, cholecystectomy, and hysterectomy, can cause such vasomotor instability. All patients referred to the Autonomic Function Lab in the period of 1998–2000 were included in this study. Using both tilt table and cold stress tests, subjects with autonomic functional disorders were studied. Two groups were observed in this study, including both those with a history of abdominal surgery and those with no history of abdominal surgery. Although patients in both groups showed an equal amount of symptoms, our results show a correlation between autonomic nervous system dysfunction and surgical history. Patients with three or more surgeries were statistically different from the controls. In the future physicians can be prepared to encounter ANS as a possible complication post-surgery. (Supported by Univ. Tennessee, Memphis)

CHARACTERIZATION OF ULTRA-HIGH MOLECULAR WEIGHT POLYETHYLENE FOR JOINT PROSTHESES. *Ben Evans, Ann Viano, and Sanjay Mishra, Rhodes College, Memphis, Tennessee (BE, AV), and The University of Memphis, Memphis, Tennessee (SM).* Ultra-high molecular weight polyethylene (UHMWPE) is a material commonly used in medical applications such as joint prostheses. Repetitive abrasion over time, such as occurs at the surface of the material in a prosthetic joint, may lead to the formation of wear particles and cause medical complications. UHMWPE is comprised of crystalline regions (lamellae) embedded in an amorphous background; by studying this structure, it may be possible to pinpoint the causes and reduce the formation of these free-floating particles. Several treatments of UHMWPE were studied by transmission electron microscopy (TEM) and atomic force microscopy (AFM) to quantify various properties of UHMWPE such as lamellar length, orientation, and crystallinity. We report investigations of UHMWPE from two different processing techniques, highlighting visible differences in lamellar length.

ATYPICAL PROTEIN KINASE C INHIBITION IS REQUIRED FOR PHOSPHOLIPASE D ACTIVATION IN RAT-1 FIBROBLASTS EXPRESSING α_{1A} ADRENERGIC RECEPTORS. *Gautam K. Gandhi, Jean-Hugues Paramentier, and Kafait U. Malik, Christian Brothers University, Memphis, Tennessee (GKG),*

and University of Tennessee, Memphis, Memphis, Tennessee (BE, AV). Phospholipase D (PLD) is a widely distributed regulatory enzyme that catalyzes the hydrolysis of phosphatidylcholine to phosphatidic acid and choline. PLD operates in transduction of extracellular signals, regulation of changes in cell morphology and motility, and control of protein trafficking and secretion in a variety of tissues. Several receptor subtypes, including the α - and β -adrenergic receptors, promote activation of PLD. The regulation of PLD has been shown to involve members of the protein kinase C (PKC) superfamily. PKC ζ is an atypical PKC, which comprises a subgroup of protein kinases that are calcium and diacylglycerol independent and phorbol ester insensitive. PKC ζ is ubiquitously expressed and is involved in mitogenesis, but little is known about its mechanism of activation or actions. The aim of this study was to determine the contribution of atypical PKC ζ to the activation of PLD by phenylephrine (PHE), a selective α_1 adrenergic receptor (AR) agonist, in rat-1 fibroblasts expressing the α_{1A} AR. PLD activity was measured by [3 H] phosphatidylethanol production after addition of ethanol to cells pre-labeled with [3 H] oleic acid. PHE (2 M) caused translocation of PKC ζ to the membrane fraction as determined by western blot analysis, suggesting coupling of PKC ζ to α_{1A} AR. PKC ζ activity in response to PHE was measured using a kinase assay at 0, 2, 5, 10, and 15 minutes. PKC ζ activity was reduced at 2–5 minutes and returned to a sub-basal level within 10–15 minutes. Over-expression of a kinase inactive PKC ζ in rat-1 fibroblasts potentiated PHE-induced increase in PLD activity by two fold. Over-expression of constitutively active PKC ζ decreased basal and PHE-induced increase in PLD activity by 35–40%. Transfection efficiency was measured by western blot analysis using a monoclonal anti-FLAG antibody. These data suggest that PHE-induced PLD activity is regulated by PKC ζ in rat-1 fibroblasts expressing the α_{1A} adrenergic receptor. (Supported by USPHS-NIH grant 19134–25 (KUM), Am. Heart Assoc. Southeast Affiliate (JHP), and Univ. Tennessee Grad. Health Sci. Ctr. (GKG)).

PREMOTOR ANATOMY OF ORBICULARIS OCULI MOTONEURONS IN RATS. **Melissa L. Greene and Mark S. LeDoux**, Christian Brothers University, Memphis, Tennessee, and University of Tennessee, Memphis, Memphis, Tennessee. The motor circuitry controlling eyelid movement is crucial for normal visual function and for the prevention of ocular injury. Eyelid motor dysfunction in the form of blepharospasm (involuntary spasms of the eyelid muscles), apraxia of the eyelid opening (inability to voluntarily open the eyes), and apraxia of eyelid closure (inability to voluntarily close the eyes) is present in many patients with movement disorders. Normal eyelid motor function depends on the neurons that innervate the orbicularis oculi (OO) muscles that close the eyes during blinks. Neural structures premotor to eyelid motoneurons may be dysfunctional in eyelid movement disorders. However, the current understanding of eyelid premotor networks lacks all the information necessary to develop treatments for eyelid movement disorders. Therefore we investigated the premotor projections to the OO in rodents. Injection of a retrograde tracer, the pseudorabies virus, was conducted and the brain was investigated to determine the projection neurons. Labeled neurons were photographed and drawn to get a better understanding of the motor pathways. Improved understanding of eyelid motor networks may contribute to the development of more effective treatments for eyelid motor dysfunction. (Supported by NIH)

ISOLATION OF *ASPERGILLUS NIDULANS* MUTANTS SHOWING HYPERSENSITIVITY TO CALCOFLUOR WHITE. **Terry W. Hill, Darlene M. Loprete, Mridula Bagrodia, Jennifer A. Livesay, Amit Mirchandani, and Michelle Momany**, Rhodes College, Memphis, Tennessee (TWH, DML, MB, JAL, AM), and University of Georgia, Athens, Georgia (MM). The hyphal wall is the cell structure most responsible for mediating the varied interactions between the fungal cell and its environment. The architectural relationships between the numerous polysaccharides and glycoproteins of the wall are little known, as are the steps by which the complex fabric of the wall is assembled and modified. We have isolated 52 strains from an *Aspergillus nidulans* mutant collection that are deficient in cell wall assembly, determined by hypersensitivity to the chitin synthase inhibitor Calcofluor White at concentrations having little effect on wild type strains. The phenotype appears as reduced hyphal growth rate, blocked or delayed conidium germination, and aberrant hyphae. The screening strategy is based upon the premise that mutants with already-weakened walls will be less able to endure an additional disturbance from a sublethal concentration of the inhibitor. We have identified 6 single-gene mutations, assigned them to 6 complementation groups, and determined some phenotypic characteristics.

AN INVESTIGATION OF THE MAJOR URINARY PROTEINS OF *MICROTUS OCHROGASTER*. **Candice L. Johnson, Charles J. Biggers, and Melvin L. Beck**, University of Memphis, Memphis, Tennessee. Rodents unlike other mammals excrete proteins in their urine. Components of the urine can be identified by electrophoretic separation and staining the proteins in the gel. The proteins can then be analyzed by their relative positions. The objectives of this research are: (1) to investigate the major urinary protein (MUP) components of vole urine as separated by electrophoresis, (2) to determine if one can distinguish between the electrophoretic urine pattern of male and female voles, (3) to compare vole urine and plasma, and (4) to compare vole and *Mus musculus* urine electrophoretic patterns. In addition, these results will be compared to those of previous work performed in the lab on *Dipodomys ordii*. The status and the significance of the research will be discussed.

GLYCOGEN CONCENTRATIONS IN FRESHWATER MUSSELS. **Seth Jones, Ross Cascio, and David Kesler**, Rhodes College, Memphis, Tennessee. We measured glycogen concentration of freshwater mussels from three Rhode Island Ponds to test the hypothesis that glycogen concentration is related to growth and is a measure of physiological stress. Reciprocal transplant experiments have shown that *Eliptio complanata* grows much less in Worden Pond compared to nearby Yawgoo and Tucker Ponds. Mean glycogen concentrations of *E. complanata* for Worden, Tucker, and Yawgoo Ponds, expressed as either mg glycogen/g fresh mass or dry mass did not differ among ponds ($P > 0.05$). However, while mean glycogen concentration, expressed as mg/g ash-free-dry mass did differ among ponds ($P = 0.001$), mean Worden Pond mussel glycogen concentration was not significantly lower than that from Yawgoo or Tucker Ponds ($P > 0.05$). Glycogen reserves therefore did not reflect the low growth of mussels in Worden Pond.

MINERAL DEFICIENCY SYMPTOMS IN *BRASSICA RAPA*. **Matthew Keogh and Charles Stinemetz**, Rhodes College, Memphis, Tennessee. *Brassica rapa* (Wisconsin Fast Plant) has re-

cently seen increased use as both a physiological research tool and pedagogical model due to both a rapid reproductive life cycle (38 days) and ease of cultivation in a limited laboratory space. Although *B. rapa* has been used extensively to demonstrate the importance of minerals in plant growth and development, previous physiological studies on both uptake and symptoms of mineral deficiency are limited in both design and scope. This study attempts to analyze individual macronutrient deficiencies as well as the lack of micronutrients. In this study, *B. rapa* was grown on acid-washed sand to eliminate the nutrients from the soil. Seeds were germinated and supplied with macronutrient deficient solutions or a solution lacking all micronutrients. Images of these nutrient deficiency symptoms will be presented.

APPLYING MEDICAL ANTHROPOLOGY TO UNDERSTAND PERCEIVED EFFICIENCY OF MEDICAL TREATMENT IN DIFFERENT CULTURES. **Christopher Laurent and Teri Mason**, *Christian Brothers University, Memphis, Tennessee*. From the study of Medical Anthropology, we know the perception of health and illness differs between cultures, an important point in effective medical treatment. The first objective of this study was to look at differences in perception of health, illness, and treatment in two different cultures in order to gain understanding of how these perceptions can vary. The second point of the study was to look at perception of efficacy of treatment in Western versus traditional Vietnamese culture, since potentially, this may affect the treatment itself. Interviews of 10 Vietnamese subjects in Memphis, Tennessee, revealed a very different idea of health and illness between the two cultures. In Vietnam, a concept of balancing forces in the universe (Qigong) is an important component of much of Chinese-based Asian medicine, along with the wide use of herbal remedies. The majority of the interviewees felt that Western medicine is much faster and more efficient than traditional Vietnamese medicine. Traditional Vietnamese treatment, though is regarded as cheaper, has fewer side effects, and is superior in treating broken bones. In summary, this group of immigrants seems to incorporate what they see as the best of medical treatment from each culture. However, in any research in this area, a factor to be considered is belief in a treatment system in appraising its efficacy.

N-BENZYLADRIAMYCIN-14-VALERATE (AD198) INDUCES THE RELEASE OF MITOCHONDRIAL CYTOCHROME C DESPITE THE EXPRESSION OF BCL-2. **Jessie Morgan, Leonard Lothstein, and Tina Barrett**, *Christian Brothers University, Memphis, Tennessee (JM), and University of Tennessee, Memphis, Tennessee (LL, TB)*. Apoptosis is a form of cell death in which cells are destroyed into sub-cellular particles that are phagocytized by surrounding cells. The release of cytochrome *c* from a cell's mitochondria aids in this process. In some cancerous cells, there is an over expression of the mitochondrial protein Bcl-2, and this protein halts the apoptotic process, contributing to cancer growth. Recently, AD198 has been shown to bypass the anti-apoptotic effects of Bcl-2. It was unknown if AD198 could direct the release of cytochrome *c* from the mitochondria. Two lines of 32D mouse myeloid leukemia cells were studied: 32D/C3, which were transfected with constitutively-expressed human Bcl-2 cDNA and 32D/C1, which were non-transfected. The cell lines were treated with either the drug AD198 in DMSO or DMSO alone, homogenized, and pelleted until only the cytosol remained. The samples were subjected to SDS-PAGE, immunoblot analysis was conducted, and anti-cytochrome *c* generated

chemiluminescence was detected by X-ray film development. The results show that cytochrome *c* is directly released from the mitochondria as a result of cell exposure to AD198. This drug is the first of its kind to have the ability to bypass the over expression of Bcl-2 in cancerous cells. This may allow AD198 to kill cancer cells in later stages, when the cells are rapidly and aggressively dividing, and when chemotherapy and radiation may no longer be affective.

CHARACTERIZATION OF NUCLEOSIDE AND DNA ADDUCTS FORMED BY S-(1-ACETOXYMETHYL)GLUTATHIONE AND IMPLICATIONS FOR DIHALOMETHANE-GLUTATHIONE CONJUGATES. **Brent J. Morris, Glenn A. Marsch, and F. Peter Guengerich**, *Union University, Jackson, Tennessee*. S-(1-Acetoxyethyl)glutathione was synthesized and used as a model for the reaction of glutathione (GSH)-dihaloalkane conjugates with nucleosides and DNA. Previously S-[1-(N2-deoxyguanosinyl)methyl]GSH had been identified as the major adduct formed by GSCH₂OAc and deoxyguanosine. GSCH₂OAc was incubated with the remaining deoxyribonucleosides to identify other possible adducts. Adducts to all three remaining nucleosides were found using electrospray ionization mass spectrometry (ESI-MS). The adduct of GSCH₂OAc and deoxyadenosine was identified as S-[1-(N7-deoxyadenosinyl)methyl]GSH. The thymidine adduct also was formed, but lability prevented its structural identification by ¹H-NMR. On the basis of the available UV spectra, we propose the thymidine to have the structure of S-[1-(N3-thymidinyl)methyl]GSH. Because adduct degradation occurred most rapidly at alkaline and neutral pH values, an enzymatic DNA digestion procedure was developed for the rapid hydrolysis of DNA to deoxyribonucleosides at acidic pH. Analysis of the hydrolysis products by HPLC-ESI-MS indicated the presence of the thymidine adduct.

ANGIOTENSIN II-INDUCED TRANSACTIVATION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND HYPERTROPHY IS MEDIATED BY CALCIUM/CALMODULIN KINASE II IN RABBIT VASCULAR SMOOTH MUSCLE CELLS. **Binh Nguyen, M. M. Muthalif, and Kafait U. Malik**, *Christian Brothers University, Memphis, Tennessee (BN), and The University of Tennessee, Memphis, Memphis, Tennessee (M, KUM)*. Angiotensin II (Ang II) is an important hormone involved in numerous physiological functions and has been linked to many cardiovascular diseases, including high blood pressure, myocardial hypertrophy, heart failure, and arteriosclerosis. An understanding of the cellular signaling that occurs after the binding of Ang II to its receptor AT₁ could potentially lead to the development of better drugs to treat cardiovascular diseases. Ang II has been shown to activate epidermal growth factor receptor (EGFR) complex via the AT₁ receptor. Moreover, activation of EGFR can lead to cell proliferation, which can result in myocardial hypertrophy. Therefore, investigation of the cellular mechanism of the transactivation of EGFR by Ang II should further our understanding by which this peptide hormone promotes vascular hypertrophy. Previous studies have shown that Ang II increases calcium influx, which by interacting with calmodulin increases calcium/calmodulin kinase II (CaMK II) activity in rabbit aortic smooth muscle cells (VSMC). These observations and the ability of Ang II to cause activation of EGFR led to the hypothesis that CaMK II is involved in Ang II-induced transactivation of EGFR and hypertrophy in VSMC. To test this hypothesis, we investigated the effect of Ang II on EGFR transactivation and

hypertrophy in the presence and absence of KN-93, an inhibitor of CaMK II. VSMC after treatment with these agents were lysed with RIPA solution. EGFR protein was immunoprecipitated with polyclonal EGFR antibody and subjected to electrophoresis. The phosphorylated EGFR was probed with monoclonal phosphotyrosine antibody and detected using ECL system. VSMC hypertrophy was determined by incorporation of ³H-leucine into cell proteins. Ang II caused EGFR transactivation and increased ³H-leucine incorporation in VSMC. In the presence of KN-93, activation of EGFR and ³H-leucine uptake elicited by Ang II was decreased. These data suggest that Ang II-induced EGFR transactivation and hypertrophy of VSMC is mediated by CaMK II.

THE ROLE OF GAMMA AMINO BUTYRIC ACID IN SYNAPTOGENESIS BETWEEN HORIZONTAL CELLS AND PHOTORECEPTOR CELLS IN THE MAMMALIAN RETINA. *Lewis Pearson, D. A. Johnson, and M. E. C. Fitzgerald, Christian Brothers University, Memphis, Tennessee (LP, MECF), and University of Tennessee, Memphis, Memphis, Tennessee (DAJ).* Recently there has been evidence that the neurotransmitter gamma amino butyric acid (GABA) may have some role as a developmental signal in the formation of retinal circuitry. The current investigation postulates that GABA is a developmental signal in synaptogenesis between horizontal cells and photoreceptor cells in neonatal rabbit retina, from postnatal days 1-7, and that calcium is a second messenger involved in this process. Another hypothesis included in this investigation is that horizontal cell process growth in culture conditions is dependent upon numbers of photoreceptor cells synapsed to the process. Dissociated cultures of neonatal rabbit retina, treated with one of four treatments, were cultured and analyzed to investigate these hypotheses. The four treatments included one control treatment, and three other treatments, to which were added exogenous GABA, exogenous Verapamil (a calcium-channel blocker), and exogenous GABA and Verapamil. (Supported by Crane Vision Research Fellowship (LP) and NIH NEI grant (DAJ))

MORPHOLOGICAL INVESTIGATION OF NEUROSPHERES. *Rachel Rome, Eric Laywell, Dennis Steindler, and Malinda Fitzgerald, Christian Brothers University, Memphis, Tennessee (RR, MF), and University of Tennessee, Memphis, Memphis, Tennessee (EL, DS).* A neurosphere is a cluster of cells derived via extraction of a single neural stem cell from the subependymal zone (SEZ) of the brain. These cells are capable of giving rise to new cells of each of the three different neural lineages: neurons, astrocytes, and oligodendrocytes. If culture conditions promote growth of one cell type over another this would be advantageous to advance this research. We therefore investigated cell morphology and expression of surface receptors using SEM and antibody techniques to determine if all were similar. Each neurosphere appeared fairly uniform with cellular processes, migrating cells, and possible pseudopodia. The surface receptors were visualized with immunogold labeling. The antibodies used were PK7 and L1, adhesion molecules expressed in young glial and neuronal cells, respectively. The L1, opposed to the PK7, was observed to attach to more surface markers. Both neural and glial cell properties have been observed from plated neurospheres, and transplanted neurospheres have been observed to migrate and differentiate into diverse neural cells. These observations have

opened a new vista on the potential of regeneration of brain neurons. (Supported by NIH-NINDS 37556)

ETHNOMEDICINAL STUDIES IN ALTERNATIVE THERAPIES. *Manika Sharma, University of Tennessee, Martin, Martin, Tennessee.* According to the *New England Journal of Medicine*, Americans spent \$25 billion on "alternative" remedies in 1997 and the trend is to seek alternatives to allopathic medicine. The medicinal practices of indigenous people in remote regions of the world must no longer be allowed to remain shrouded in the unknown vaults of knowledge. The present investigation deals with the ethnomedicinal studies with the indigenous alternative therapies practiced in the remote area of Ladakh bordering Tibet. The Ayurveda, Unani, Sidha, and Amchi systems of medicine represent some of the oldest therapies in use in this isolated region of the world. Furthermore, homoeopathy also is practiced by a few. Cultural antiquity, geographic isolation, and remoteness of Ladakh have deterred scientific evaluation of the medicinal potential of these ancient ways of healing. Therefore, it is imperative that we look into these ancient emporia of medicinal wealth to cure dreaded diseases of today.

TEMPORAL CHANGES IN THE SPATIAL DISTRIBUTION OF RUBUS, LONICERA, AND SOLIDAGO. *Matthew H. Watson, The University of Memphis, Memphis, Tennessee.* A replicate block study has been set up to examine the effects of disturbance intensity and frequency in an old-field at the Meeman Biological Station. One additional factor affecting the successional system is the spatial structure of the colonizing vegetation. We used the data from an array of plots in the old-field to determine effects of colonizing structure. Data suggests for the studied taxa, *Lonicera*, *Rubus*, and *Solidago* that the forest around the old-field influences the spatial distribution of colonizing plants. This results in the development of a patchy distribution of plant colonies found in the old-field over time, which elucidates the importance of studying spatial structure in field experiments.

A COMPARISON OF BIOLOGICAL AND CHRONOLOGICAL AGE IN THE MORBIDLY OBESE: OBESITY AS AN ACCELERATED STATE OF AGING. *Laura Williamson and Cynthia K. Buffington, Christian Brothers University, Memphis, Tennessee, and University of Tennessee, Memphis, Memphis, Tennessee.* The decrease in physiological functions as humans age includes metabolic, hormonal, immunological, and psychological aberrations. This research is based on the idea that morbid obesity may actually be an accelerated state of aging. To test this hypothesis, we ran a battery of 12 physiological tests known to deteriorate with aging. The study population included 18 morbidly obese patients with an average age of 29.94 years and an average weight of 296 lbs. The 12 physiological tests were run on a computer program called the H-SCAN Analysis, measuring each subject's sensory, motor, cognitive, and pulmonary capabilities. A biological age was calculated based on each individual's performance. Within the morbidly obese group, there was a notable difference between the average biological and chronological ages. Out of all 12 tests, the morbidly obese subjects only scored higher than the 50th percentile in 3 such tests. Therefore, these results suggest that obesity could be considered an accelerated state of aging. (Supported by Nat. Ctr. Toxicol. Res.)