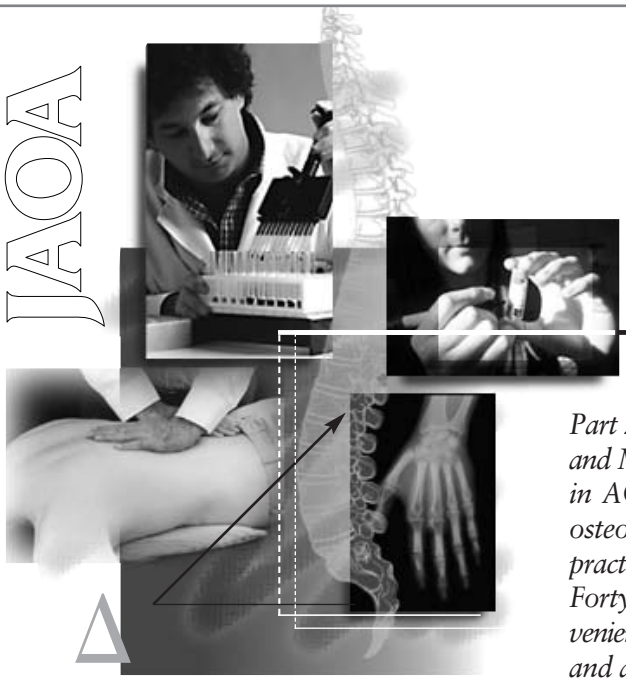

Forty-fourth Annual AOA Research Conference Abstracts, 2000: Part 2

Part 2 contains abstracts in the Poster Session on Basic Sciences and Medical Education. Part 1 (August issue) contained abstracts in AOA Research Fellowships, and poster presentations on osteopathic manipulative medicine/osteopathic principles and practice (OMM/OPP) and Clinical Studies to be presented at the Forty-fourth Annual AOA Research Conference. For the convenience of attendees, abstracts appear in their scheduled sequence and are numbered for easy reference.

Abstracts are reproduced exactly as submitted. The American Osteopathic Association and The Journal of the American Osteopathic Association *assume no responsibility for the content of the abstracts printed herein.*

Basic Sciences

B01

Human Infant Body Segment Morphology: A Strategy for Developing Locomotor and Postural Skills. J.P. Wells, Ph.D., D. Hyler-Both, D.O., A.J. Wells, MSI, D.L. Prisk, MSII, K.M. Waldron, MSII. West Virginia School of Osteopathic Medicine, Division of Structural Biology, Lewisburg, WV 24901.

Human infants and quadrupedal old world monkey infants share similar lower limb adaptations which have developed to allow for propulsive forces to be produced in the dominant lower limb. These adaptations came about in response to freeing the upper extremity in humans which led to the development of bipedalism and producing an upper extremity capable of steering and pulling in old world nonhuman primates. In both primates, development of muscle mass, concurrent with locomotor and postural skill development, occurred in the proximal portions of the thigh and leg.

This study attempts to establish a clear relationship between normal structure and function, that is, between biomechanics of the developing musculoskeletal system in human infants and the positional behavior which it permits. 167 human infants ranging in age from 0.1 to 18 months were characterized as to body segment length and shape in such a fashion that centers of mass could be determined by biomechanical structure analysis. Specific findings include: in the trunk the greatest relative lengthening occurred in the pelvis followed by the abdomen and then the thorax. The center of mass of the thigh and leg migrate away from the trunk. The leg center of mass migrates faster than the thigh and therefore, the center of mass of the thigh is more proximal.

The final result of these studies will be the production of a document which will plot the normal pattern of human infant growth and development.

The authors wish to thank the American Osteopathic Association and the WVSOM intramural research program for their generous support.

B02

Modulation of Estrogenic Effects in *Candida albicans* as a Means of Augmenting Antifungal Activity of Azole-Type Compounds. Bryan Larsen, Ph.D. and Michael Essmann, B.S. Des Moines University Osteopathic Medical Center, Office of University Research. Des Moines, IA 50312

Previous work in our laboratory (J. Infect. Dis. 181:1441, 2000) indicated that 17β-estradiol is a pleiotropic regulator of *Candida albicans* virulence and fitness under conditions of stress. This finding led us to hypothesize that estrogen may also increase resistance of fungal pathogens therapeutic drugs and as a corollary, anti-estrogens may enhance susceptibility. We tested this hypothesis with a panel of clinical yeast isolates inoculated into a series of serial two-fold dilutions of miconazole (5ug – 0ug) with various additives. We measured turbidity and did viable plate counts at 6 and 18 hours after inoculation. We found that the addition of estradiol shifted the antimicrobial endpoint in the direction of lesser susceptibility to miconazole. Fetal calf serum is able to substitute for estradiol in these experiments. When these studies were repeated with nafoxidine and tamoxifen, the MIC values were shifted in the direction greater susceptibility. Evidence showing that estradiol upregulates ABC-transporters in yeast suggests a possible mechanism for the augmented resistance in the presence of estradiol. The effects of nafoxidine and tamoxifen are encouraging as drug entities that may enhance antifungal resistance. Future studies will investigate larger panels of fungal pathogens and additional estrogen receptor modifying agents to determine how widespread this phenomenon is among pathogenic yeast.

B03*

A non-invasive method for monitoring the re-epithelialization of a wound *in vivo*
GJ Koenig, M.S. and CH Greene, Ph.D.
Philadelphia College of Osteopathic Medicine
Department of Biomedical Sciences
Philadelphia, PA 19131

The purpose of this study was to develop a reproducible, non-invasive method for demonstrating the restoration of an intact epithelial barrier after wounding.

Thirty-two male Hartley Albino guinea pigs received two, midline, full skin-thickness, excisions on the back using a 5-mm biopsy punch. An occlusive chamber was applied to each wound and replaced daily until re-epithelialization occurred. The chamber allowed the maintenance of a moist wound environment and daily monitoring of the wound physiology *in vivo* by sampling chamber fluids without disturbing the wound. The end point of healing was determined as the time elapsed from wounding until the protein concentration in the chamber returned to pre-wounding levels.

The mean healing time for the sites was 10.1±1.0 days. When compared to values reported by other investigators, the chamber treated wounds healed faster than air-exposed wounds. Furthermore, the histological results obtained at day fifteen were comparable to those reported for air-exposed wounds at day twenty.

Previous studies to evaluate the rate of wound healing focused on qualitative methods or were dependent on biopsy material. Qualitative methods are imprecise and the collection of biopsy material disturbs the healing process. The use of this chamber model enabled the rate of wound healing to be measured in a reliable manner without disrupting the healing process. The versatile design allows a standardized method for assessing wound healing with different modalities and therapies.

Funding for this study was provided by the Philadelphia College of Osteopathic Medicine.

B04

Differential Processing of Beta –Amyloid Following Infection of Monocytes, Astrocytes, and Epithelial Cells With *C pneumoniae*: Implications for Alzheimer's Disease.
[1]C.S. Little, PhD , [1]A. MacIntyre, MS, [2]C. Hammond, BS, [2]B.J. Balin, PhD, and [1]D.M. Appelt, PhD. [1]Department of Biomedical Sciences, [2] Department of Pathology /Microbiology & Immunology, Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131

We previously uncovered an association of *Chlamydia pneumoniae* and sporadic Alzheimer's disease (AD). Microglia and astroglia in the CNS are host cells for the bacterium. RT-PCR analyses indicated that the bacteria were metabolically active and were concentrated in areas of AD-neuropathology containing tau and beta-amyloid (Aβ1-40) deposits. The present study was designed to investigate the hypothesis that *C. pneumoniae* infection of human monocytes (THP-1), astroglial (CCF-STTG1), and epithelial (HEp 2) cell lines results in differential processing of beta-amyloid thereby further implicating this infection in Alzheimer's Disease. To analyze this relationship, we infected cells with isolates of *C. pneumoniae* from AD brain and characterized them by immunocytochemistry, Western analysis, and electron microscopy. We have demonstrated that certain *C. pneumoniae* infected cell lines as compared to uninfected lines display differences in immunolabeling profiles with antibodies to the amyloid precursor protein (APP), Aβ1-40, and *C. pneumoniae*. Western analysis indicates that processing of APP is influenced by the bacterial infection in the cell lines. Electron microscopy confirmed the presence of *C. pneumoniae* in all infected cells. Our data suggest the following possibilities: (1) increased or abnormal processing of the APP in infected cell lines, (2) an association of the organism with the beta amyloid peptide, and/or (3) antigenic homology with *C. pneumoniae* peptides/proteins. Results of this study will significantly increase our understanding of this pathogen as it may contribute to the neuropathogenesis characteristic of late-onset Alzheimer's Disease. Supported by NIH AI44055, Foundation for Research Into Diseases of Aging, and National Foundation for Infectious Diseases.

B05

Implications of *Chlamydia pneumoniae* infection in human monocytes and brain endothelial cells: Changes in Beta-amyloid production and monocyte migration.
[1]A. MacIntyre, MS, [2]C. Hammond, BS, [1]C.S. Little, PhD, [1]D.M. Appelt, PhD and [2]B.J. Balin, PhD. [1]Department of Biomedical Sciences, [2] Department of Pathology /Microbiology & Immunology, Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131

We are developing an *in vitro* blood brain barrier model using human brain microvascular endothelia (HBMECs) and monocytes (THP-1) to test their responses to *C pneumoniae*. Previously, we demonstrated that THP-1 cells are susceptible to infection with *C pneumoniae* isolated from Alzheimer brains, and that infection can be propagated and maintained *in vitro*. We now hypothesize that infection with *C pneumoniae* enhances monocyte migration and alters Aβ production/processing.

Changes in Aβ do not appear to differ between infected and uninfected HBMECs *in vitro*; in contrast, the infected monocytes show increased production/processing of Aβ. Infected monocytes traversing the barrier may result in an increased Aβ burden on the abluminal side of the vasculature. Our data indicate that *C pneumoniae*-infected THP-1 cells traverse an uninfected HBMEC monolayer in far greater numbers than uninfected THP-1s. HBMEC monolayer infection facilitates enhanced migration of uninfected monoctyes. Consequently, infection of both HBMECs and THP-1s demonstrates the highest number of migrating monocytes traversing this barrier. These data suggest that our blood brain barrier model with HBMECs is useful for analyzing potential entry of intracellular pathogens into the central nervous system (CNS). In addition, this model may demonstrate a selective vulnerability at the level of the blood brain barrier, resulting in access of the organism to the CNS. Therefore, the organism may gain access through the blood brain barrier indirectly within monocytes as well as directly through infection of the HBMECs. Supported by NIH AI44055, Foundation for Research Into Diseases of Aging, and National Foundation for Infectious Diseases.

B06*

Is Gentamycin Effective in Eliminating Bacteria from SIS Graft Material.
M.T. Gostkowski, BS, B.J. Bromke, PhD, C.H. Greene, PhD
Philadelphia College of Osteopathic Medicine
Department of Biomedical Sciences
Philadelphia, PA 19131

This study investigated a preservation procedure for swine intestinal submucosa (SIS) in a gentamycin/saline solution for its anti-bacterial efficacy.

Two SIS graft specimens were prepared from swine intestine procured from a slaughterhouse. One specimen was placed in a 10.0% gentamycin/normal saline solution and the other specimen was placed in a normal saline solution only. Both solutions were refrigerated at 10°C. The solutions were changed daily. Tissue samples were collected at standard intervals over a two-week period using a 5mm-biopsy punch. These samples were macerated in sterile saline and plated on blood-agar plates. The remaining solutions from the containers also were collected at each of the corresponding intervals and centrifuged. The supernatant was decanted and the remaining pellet was diluted with sterile saline and plated on two blood-agar plates. One plate was cultured aerobically and the other was cultured anaerobically. A protocol was devised to identify any subsequent growth.

The results from the blood-agar cultures showed no appreciable bacterial growth in either the experimental or control group over the two-week observation period.

The results of this research demonstrated that a 10.0% gentamycin /saline solution changed daily is a practical and effective preservation procedure for SIS with the time frame of the two-week experimental period. It appears that either the acellular nature of the SIS or the gentamycin solution prevents bacterial growth on the graft substrate, while the gentamycin prevents bacterial growth in the storage solution.

Funding for this research was provided by the Philadelphia College of Osteopathic Medicine.

*Student presentation competition.

B07*

Replacement of the Infra-renal Aorta Using a Small Intestine Submucosa (SIS) Allograft in the Rabbit.

Smith, S., MS, Lambert, M., Graf, S., Greene, CH., Ph.D., DeMartino, A. DO, Glover, R., Costa, L., Rosnig, G., Leach, W., Rosenblatt, R., MS, Treaves, J., Tsaventsano, J., White, M.
Philadelphia College of Osteopathic Medicine
Department of Biomedical Sciences
Philadelphia, PA 19131

The purpose of this investigation was to develop a graft material for replacement or repair of aortic pathology by regeneration of the native tissue.

A graph was created using fresh porcine SIS (small intestine submucosa) obtained from a commercial source. The material was processed and manufactured into a tubular graph. The process of graft manufacturing involves isolating the SIS material and then forming of a tubular graft using aforementioned techniques. Certification of potency of the graft material and manufacturing process was completed prior to intra-operative placement of the graft. Laparotomy was then performed under sterile conditions on the research model and the infra-renal aorta was identified. Proximal and distal control were gained and then aortotomy was performed. The graft was sutured in place under optical imagery and the anastomosis was completed. At 28 days postoperatively the graft was harvested and histological, immunocytochemical and microscopic studies were performed to determine the level of tissue regeneration.

Tissue re-growth of the SIS circumferential graft in-situ has shown complete revascularization and recanalization of the implanted graft. Additionally other cellular components such as fibroblasts and myocytes have been demonstrated.

Funding has been provided by the Philadelphia College of Osteopathic Medicine. This investigation is a collaborative study involving medical students, surgical residents, and clinical and basic science faculty.

B08*

ANDROGEN REGULATION OF ANDROGEN RECEPTOR IN THE THYMUS. Poloyi F, B.S., Virelli S, Ph.D. Midwestern University, Chicago College of Osteopathic Medicine, Department of Biochemistry, 555 31st Street, Downers Grove, IL 60515

Androgen induction of thymic involution is well known. However, the biochemical mechanism of androgen action in the thymus has not been well characterized. The focus of our project has been to elucidate the effect of androgens upon the expression of androgen receptor and the phosphorylation of tyrosine residues of the androgen receptor protein. We began with an organ culture model using thymic lobes from castrate male mice that were incubated in the presence of sodium only, sodium orthovanidate (Na_3VO_4, 1µm) a tyrosine phosphatase inhibitor, dihydrotestosterone (DHT, 1µm), or sodium orthovanidate (Na_3VO_4, 1µm) with dihydrotestosterone (DHT, 1µm). Cell lysates were made from thymic lobes and immunoblotting was done to assess androgen receptor expression. We observed that with the subsequent treatments, androgen receptor expression was unchanged. Next we used an in vivo model where castrate male mice were treated with dihydrotestosterone for 2 hours or 24 hours prior to sacrifice. Thymuses were harvested and a 47% decrease in thymic cell number was observed upon exposure to androgens. The cell lysates were immunoprecipitated with anti-androgen receptor antibody and subjected to immunoblotting with antibody to tyrosine-phosphorylated proteins. We observed that the androgen receptor in the thymus of the untreated castrate mouse was tyrosine phosphorylated, and that phosphorylation of androgen receptor appeared to decrease with increased exposure time to androgen. Our data strongly suggest that either androgen are a tyrosine phosphatase inhibitor altered the protein expression of androgen receptor in the thymus, but that androgens did reverse the castration-induced thymic hypertrophy. Also, our evidence strongly suggests that tyrosine residues within the androgen receptor protein are phosphorylated, with a qualitative decrease in tyrosine phosphorylation with increased androgen exposure.

B09*

ESTROGEN RELATED EFFECTS ON TUMOR SUPPRESSOR GENE P53 IN BREAST AND BONE.

Karen Lin & Nalini Chandar, Department of Biochemistry, Chicago College of Osteopathic Medicine, Midwestern University, 555, 31st Street, Downers Grove, IL 60515.

Declining estrogen levels after menopause cause several important changes including bone loss leading to osteoporosis and increased risk of fractures. Tamoxifen and more recently raloxifene have been used as an estrogen agent as well as a cancer preventive agent in women predisposed to breast cancer. In addition to reducing breast cancer incidence by almost half, tamoxifen reduced the number of bone fractures of the hip, wrist, and spine. Our laboratory has established a role for p53 tumor suppressor gene in bone differentiation. Recent studies have suggested a role for estrogen in the regulation of p53 gene expression. These studies were undertaken to examine the effect of estrogen and different estrogenics on bone and breast cells to determine the effect on p53 gene expression and activity. Preliminary studies show that physiological level of 8-estradiol produces changes in p53 functional activity in bone cells. During bone differentiation, estrogen receptor levels are also altered. Estrogen antagonist, tamoxifen is also able to upregulate p53 activity at different concentrations in bone and breast cells and the significance of these observations will be discussed.

B10*

MOLECULAR CHANGES DURING TRANSDIFFERENTIATION OF OSTEOBLASTS TO ADIPOCYTES. S. Jun, B.S. and N. Chandar, Ph.D. Chicago College of Osteopathic Medicine, Department of Biochemistry, Midwestern University, 555, 31st Street, Downers Grove, IL 60515

An association between an increase in bone marrow adipose tissue and osteopenia has been reported with increasing age and in some pathological conditions. It has been shown that the volume of marrow adipose tissue is increased and that of bone decreased in the elderly compared with young individuals and in osteoporosis at all ages. An increase in the volume of marrow fat is also associated with the development of disuse osteoporosis, glucocorticoid osteoporosis and osteoporosis induced due to estrogen deficiency. Our laboratory is interested in studying molecular changes during osteoblast differentiation and the role of p53 gene in this process. This study was undertaken to gain an understanding of molecular changes during transdifferentiation of osteoblasts to adipocytes and the effect of estrogen on the conversion process. We studied tumor suppressor gene p53 and a p53 regulated oncoprotein mdm-2 during this process along with markers of osteoblast and adipocyte differentiation. Assays included histochemical staining for adipocytes and osteoblasts and gene expression analyses by RT-PCR. Preliminary studies show that cloned osteoblasts differentiate into adipocytes under normal conditions. Specific markers of osteoblast and adipocyte differentiation were expressed when exposed to either differentiation media. Specific changes in mdm-2 and p53 will be discussed in the context of bone and adipocyte differentiation.

*Student presentation competition.

B11*

THE MARINE PSEUDOPTEROSINS MODULATE RAT MICROGLIA SUPEROXIDE AND THROMBOXANE GENERATION. S. Oh, M.S.[1], M.E. Mallorca[2] and A.M.S. Mayer, Ph.D.[1] (1) Chicago College of Osteopathic Medicine, Pharmacology Department and (2) Chicago College of Pharmacy, Midwestern University, Downers Grove, IL 60515

A growing body of research supports the involvement of brain microglia (BMΦ) in brain pathologies caused by meningitis, septic shock, trauma, tumors, ischemia, Alzheimer's disease, Parkinson's disease, Down's syndrome, multiple sclerosis and AIDS (Mayer AMS., *MEDICINA* 58:377, 1998). We have recently characterized inflammatory mediator generation by *Escherichia coli* lipopolysaccharide (LPS)-activated BMΦ, our *in vitro* model to investigate anti-inflammatory marine natural products (Mayer, AMS et al., *SHOCK* 11:180, 1999). The marine anti-inflammatory Pseudopterosins (PS) isolated from the marine gorgonian *Pseudoterogorgia elisabethae*, appear to inhibit inflammatory cells eicosanoid release production by a yet undetermined mechanism (Mayer, AMS et al., *Life Sciences* 62:401,1998). The purpose of this investigation was to study the effect of 4 PS analogs, namely PSA, PSE, PSA methyl-ether (PSAM) and PSC on phorbol ester (PMA) and opsonized zymosan (OPZ)-stimulated thromboxane B_2 (TXB_2) and superoxide anion (O_2^-) generation from *E. coli* LPS-activated rat microglia (BMΦ). O_2^- was determined by superoxide dismutase-inhibitable reduction of ferricytochrome C and TXB_2 using commercially available immunoassays. PSA, PSAM, PSC and PSE strongly inhibited PMA- stimulated release of O_2^- (IC_{50}=2-2.5 μM) and TXB_2 (IC_{50}=0.8-1.5 μM) but only weakly affected OPZ-stimulated production of O_2^- (IC_{50} > 10 μM) and TXB_2 (IC_{50}= 3.8 or > 10 μM, respectively). In conclusion, concomitant inhibition of BMΦ O_2^- and TXB_2 appears to suggest PS inhibit eicosanoid generation by a cyclooxygenase-independent mechanism, possibly distal to arachidonic acid (AA) release, because BMΦ O_2^- generation is unaffected by cyclooxygenase inhibitors (Mayer, AMS et al., *Soc. Neurosci. Abstr.* 23:1223, 1997). We hypothesize that a higher specificity of PS toward BMΦ PKC isozymes activated by PMA rather than OPZ might explain the observed weak effect of these compounds on OPZ-stimulated O_2^- and TXB_2 generation. Supported by NOAA, U.S. Dept. Commerce Grant NA66RG0477 (Project R/MP-73) and Midwestern University.

B12

CADMIUM-INDUCED LUNG INJURY: EVIDENCE FOR A SPECIFIC INCREASE IN THE PERMEABILITY OF E-CADHERIN AND VE-CADHERIN DEPENDENT CELL- CELL JUNCTIONS. CA Pearson, PC Lamar, RJ Niewenhuis and WC Prozialeck. Pharmacology Department, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL. 60515

Although it is well-known that respiratory exposure to Cd^{2+} results in pulmonary edema, the specific mechanisms underlying this effect have yet to be elucidated. Studies from our laboratory have shown that Cd^{2+} can disrupt the tight junctions between epithelial cells in culture by interfering with the normal function of the Ca^{2+}-dependent cell adhesion molecule E-cadherin. The objective of the present study was to determine whether the early pneumotoxic effects of Cd^{2+} might result from specific changes in the permeability of E- and VE-cadherin-dependent cell-cell junctions in the lung. Male CF1 mice were given either saline or $CdCl_2$ (65 nmoles) via intratracheal instillation. After 24 hours, the animals were euthanized, and the lungs were removed and either subjected to bronchoalveolar lavage or analyzed for histopathologic changes. The results showed that Cd^{2+} caused an increase in lung weight and in the protein content of the lavage fluid. The microscopic analyses showed evidence of edema and capillary congestion but little evidence of injury to the alveolar epithelial cells or the capillary endothelial cells. In addition, the samples from the Cd^{2+} -treated mice showed a decrease in the amount of E-cadherin in the epithelial cells of the alveoli and small bronchioles and of VE-cadherin in vascular endothelial cells. These findings suggest that Cd^{2+} -induced pulmonary edema may be a consequence of relatively specific increases in the permeability of the cadherin-dependent cell-cell junctions in the alveolar epithelium and the vascular endothelium. (Supported by Grant #R01 ES06478 from the NIEHS).

B13

CADMIUM DISRUPTS CADHERIN-DEPENDENT CELL-CELL JUNCTIONS IN ROS 17/28 CELLS. WC Prozialeck, PC Lamar and CA Pearson. Pharmacology Department, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL. 60515

While the osteotoxic effects of Cd^{2+} have long been recognized, the mechanisms by which Cd^{2+} affects bone are not well-understood. Recent studies from our laboratory have shown that Cd^{2+} can selectively damage the E-cadherin-dependent junctions between many types of epithelial cells in culture. At the same time, studies from other laboratories have shown that E-cadherin and related cadherins are present in osteoblast-like cells and play a key role in the differentiation of the cells into their epithelial phenotype. The objective of the present study was to determine if Cd^{2+} could disrupt cadherin-dependent cell-cell junctions in the rat osteoblast-like cell line, ROS 17/28. The cells were grown to confluence on glass coverslips in DMEM and then exposed to 0-20 μM Cd^{2+} in a physiological saline solution. The integrity of cell-cell junctions was assessed morphologically, and cadherin-like cell adhesion molecules were visualized by immunofluorescence using a pan-cadherin primary antibody. Exposure to Cd^{2+} for 2-4 hours caused the cells to separate and retract from each other without detaching from the growing surface. This effect coincided with the loss of pan-cadherin immunoreactive material from the cell borders and a reorganization of the actin cytoskeleton, but occurred well before the cells showed evidence of serious injury or the loss of membrane integrity. These results indicate that Cd^{2+} can disrupt the cadherin-dependent cell-cell junctions in ROS 17/28 cells, raising the possibility that some of the osteotoxic effects of Cd^{2+} might result from the disruption of cadherin dependent cell-cell junctions in bone. (Supported by Grant RO1 ES06478 from the NIEHS).

B14*

EXPRESSION OF VASOPRESSIN-ACTIVATED CALCIUM MOBILIZING RECEPTOR (VACM-1/CULLIN-5) mRNA IN HUMAN BREAST EPITHELIAL CELLS AND BREAST CANCER CELL LINES. Amyna Husain, Alfred Hicks, and Michael J. Fay, Department of Pharmacology, Midwestern University, Chicago College of Osteopathic Medicine, 555 31st Street, Downers Grove, IL 60515.

A novel vasopressin binding protein was recently cloned from a rabbit renal medullary cDNA library. This novel protein, named the vasopressin-activated calcium mobilizing receptor (VACM-1), is unique in that it does not share sequence homology to the known vasopressin receptors. It is now evident that VACM-1 shares sequence homology to an emerging family of proteins that have been named cullins. The major function attributed to cullins is the targeting of short-lived cell cycle regulatory proteins (e.g. G1 cyclins and cyclin-dependent kinase inhibitors) for degradation by the ubiquitin-mediated proteasome pathway. Since the region of the chromosome (11q 22-23) where *VACM-1/cullin-5* is located is associated with loss of heterozygosity (LOH) at a frequency of 38% in primary breast cancer and 84% in local recurrent breast cancer after surgery, the VACM-1/Cullin-5 gene product is a putative tumor suppressor. Previously, we demonstrated that both MCF-7 estrogen receptor α positive and MDA-MB-231 estrogen receptor α negative human breast cancer cells express VACM-1/Cullin-5 mRNA and protein using the techniques of RT-PCR and Western blotting, respectively. We have expanded these initial studies to examine VACM-1/Cullin-5 mRNA expression using the techniques of Northern blot analysis and RT-PCR in primary human breast epithelial cells, immortalized but nontumorigenic human breast epithelial cells, and the MCF-7 and MDA-MB-231 human breast cancer cell lines. All of the cells examined expressed VACM-1/Cullin-5 mRNA as indicated by RT-PCR and Northern blot analysis. Experiments are planned to examine VACM-1/Cullin-5 function in these cells with the use of antisense technology.

*Student presentation competition.

B15*

RENAL SYMPATHETIC NERVE ACTIVITY DURING EXERCISE IN RABBIT PREGNANCY. M. Augustus, J. Alberts and K. O'Hagan. Department of Physiology, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL 60515

Little is known of how neural control of the circulation during exercise is affected by normal pregnancy. The renal sympathetic (renal SNA) and hemodynamic responses to 3 graded intensities of treadmill exercise was measured in 3 term pregnant and 5 nonpregnant NEW female rabbits. Three bouts of 5 minutes of exercise (7 m/min @ 0% grade; 10 m/min @ 6% grade, 10 m/min @ 17% grade) were performed, and bouts were separated by 45 min of rest. Renal SNA was monitored via electrode recording electrodes. Resting mean arterial pressure (MAP) was lower (~11 mmHg) in the pregnant animals. Dynamic exercise increased MAP, HR and renal SNA in both groups of animals. While the absolute exercise MAP achieved by the pregnant rabbits was lower at each intensity, the relative MAP response to the exercise bouts was unaffected by pregnancy. Neither the absolute nor the relative HR or renal SNA responses to exercise were affected by pregnancy. These preliminary data suggest that the sympathetic drive to visceral circulatory beds during exercise is not affected at term pregnancy. This study was supported by NHLBI and a DCOM Summer Fellowship to M. Augustus.

B16*

A FLUORESCENT GLYCATION PRODUCT DERIVED FROM CARNOSINE J.A. Nagib, B.S., S.S. Shakry, B.S. and N.W. Seidler, Ph.D. University of Health Sciences, College of Osteopathic Medicine, Department of Biochemistry, 1750 Independence Avenue, Kansas City, Missouri 64106

Protein glycation as indicated by advanced glycation endproducts (or AGEs) occurs in various diseases such as Alzheimer's disease (AD). The senile plaques in AD, which contain polymerized β-amyloid peptide, are composed of AGEs. L-Carnosine (β-alanyl-L-histidine) prevents nucleation and aggregation of beta-amyloid peptide. The mechanism of this process is not known. Coincident with L-carnosine's antiglycation effects is a chemical reaction with glycating agents. In our study, we used glyceraldehyde 3-phosphate (Glyc3P) as the glycating agent. We observed the appearance of a fluorescent product (excitation 365nm; emission 440nm) following reaction between L-carnosine (100mM) and Glyc3P (5 and 10mM) for 90min at 40°C which resulted in 562 and 1240rfu, respectively. The following heterocyclic ring structures can be found following glycation reactions: pyrroles, pyridones, pyrazines, imidazolones and pentosidines. While imidazolones and pentosidines exhibit fluorescence, they are derived from arginine residues. The L-carnosine-Glyc3P adduct may represent a previously unidentified glycation product. Fluorescent product formation was time, temperature and concentration dependent. We also observed that the amino group content of L-carnosine decreased upon incubation with Glyc3P, suggesting that the free β-amino group of β-alanine participates in the reaction. These findings will lead us to enhancing our understanding of the anti-glycation properties of L-carnosine.

Supported in part by a grant from the UHS Division of Research.

*Student presentation competition.

B17

ASPARTATE AMINOTRANSFERASE INTERACTIONS WITH CARNOSINE G.S. Yeargans, B.S. and N.W. Seidler, Ph.D. University of Health Sciences, College of Osteopathic Medicine, Department of Biochemistry, Kansas City, MO 64106

Carnosine (β-alanyl-L-histidine) exhibits therapeutic potential for a wide variety of pathologic conditions. While carnosine shows tissue specificity, carnosine mechanism of action appears to involve a global scavenging of free radicals, such as superoxide anion radicals, and other reactive oxygen species, such as sugar-derived and lipid-derived aldehydes. The nature of the interactions between carnosine and the proteins that it protects is poorly understood. This study examined the specific interactions between carnosine and the enzyme aspartate aminotransferase (AAT) that is known to be very susceptible to glycation and that is thought to play a protective role in ischemic myocardium. Carnosine binds tightly to AAT (K_D = 25.7μM) with an estimated two binding sites as determined by Scatchard analysis. Carnosine's binding to AAT alters AAT's Tm (temperature at which half of the enzymes are denatured). In the presence and absence of carnosine, AAT (Tm) was 71.5°C and 73.5°C, respectively. With native AAT, carnosine effectively prevents glyceraldehyde (Glyc)-induced protein glycation as measured by nitroblue tetrazolium (NBT)-reactivity and 365nm absorbance. In the presence of Glyc, carnosine lowered the NBT-reactivity and 365nm absorbance by 40 and 50%, respectively. With denatured AAT, carnosine had no effect on the Glyc-induced increase in NBT-reactivity, but prevented the Glyc-induced increase in 365nm absorbance by 24%. In conclusion, we observed that carnosine binds AAT with high affinity and that the binding may contribute to its effectiveness as an anti-glycation agent.

Supported by a Grant from the UHS Division of Research.

B18

POTENTIAL OF SULPHONAMIDES FOR CANCER THERAPY. [1]W. Richard Chegwidden, PhD, FRSC and [2]Ian M Spencer, PhD
[1]Lake Erie College of Osteopathic Medicine, Erie, PA 16509 and [2]Sheffield Hallam University, Sheffield S1 1WB, UK.

The sulphonamides acetazolamide (DIAMOX) and methazolamide (NEPTAZANE), which have previously found widespread use in the systemic treatment of glaucoma and altitude sickness, are highly specific inhibitors of carbonic anhydrase (CA). This enzyme catalyses the hydration of CO_2 to bicarbonate, which is an essential substrate for a range of metabolic pathways, including that of *de novo* pyrimidine synthesis. Whilst a low flux through these pathways may be accommodated by the uncatalysed rate of bicarbonate production, the additional activity of CA may be required for the higher rates associated with enhanced cell growth in diseases such as cancer and psoriasis. We examined this hypothesis and investigated the role of CA in this process. The effects of CA inhibitors, acetazolamide, methazolamide and ethoxzolamide, on the growth in culture of U937 cells (established from human histiocytic lymphoma) and Raji cells (from Burkitt's lymphoma) were examined. The relative effectiveness of these three sulphonamides in inhibiting cell growth correlated with their effectiveness as inhibitors of CA activity. Addition of nucleotide precursors to the growth medium appeared to abolish the inhibition of growth, by acetazolamide and methazolamide, of both cell lines

Our data indicate that carbonic anhydrase activity is necessary for the enhanced growth rate of cancer cells in culture, and that carbonic anhydrase may play a role in providing bicarbonate for nucleotide synthesis. This suggests that specific sulphonamide inhibitors of carbonic anhydrase, such as those previously used in the treatment of glaucoma, have potential in cancer therapy.

B19*

ALZHEIMER'S DISEASE INCREASES THE SUSCEPTIBILITY OF PROTEINS TO OXIDATIVE DAMAGE. P.L. Marshall, B.S., C. C. Conrad, Ph.D., and R. W. Gracy, Ph.D. University of North Texas Health Science Center, Molecular Aging Unit, Dept. of Biochemistry and Molecular Biology, Ft. Worth, TX 76107.

Reactive oxygen species (ROS) are generated by a variety of sources from the environment and normal cellular functions. ROS include free radicals such as superoxide and hydroxyl radicals, nonradical oxygen species, and reactive lipids and carbohydrates. The accumulation of oxidized proteins appears to play a causative role in many age-related diseases including Alzheimer's disease (AD). The purpose of this study is to identify the proteins that are most susceptible to ROS damage and to use these as potential biomarkers for the early diagnosis of AD.

Levels of oxidatively modified plasma proteins were examined from AD patients, non-AD controls and AD-relatives. Oxidative modification was measured by reacting the protein carbonyls with 2,4 dinitrophenyl hydrazine (DNPH). Specific protein oxidation was assessed from Western blots of electrophoretic gels using antibody to the DNP-derivatives. Statistically significant elevations ($p<0.05$) of total oxidized proteins were observed in both AD subjects and AD relatives when compared with non-AD controls. In addition, a specific protein (MW=73kD) was uniquely oxidized in the plasma of AD subjects. Furthermore, this protein from AD subjects was more susceptible to *in vitro* oxidation via the Fenton reaction.

In addition, fibroblasts from AD individuals were more susceptible to oxidative damage by hydrogen peroxide and Fenton Reaction than age-matched controls and with increased oxidative stress, there was an increase in AD fibroblast death. This data suggests a potential of identifying the sensitive oxidized protein biomarkers both in cell cultures and in the plasma of AD patients.

This research is supported by grants from the Robert A. Welch Foundation (BK0502) and the Alzheimer's Association (IIRG-98-037).

B20*

EFFECTS OF OPPOSITE FORELIMB STIMULATION ON HINDLIMB SPINAL FIXATION IN RATS. S. Thompson, B.S., M.M. Patterson, Ph.D. and A. Ullman, B.S., University of Health Sciences College of Osteopathic Medicine, Departments of Osteopathic Principles and Practice, and Physiology, 1750 Independence Avenue, Kansas City, MO 64106-1453

Spinal fixation is a long-term alteration in the excitability of spinal reflex circuits.The present study investigated the effects of stimulating the left forelimb on the amount of fixation in the right hindlimb. Stimulation of the forelimb produces a reflex flexion in that limb, and a crossed flexion pattern in the opposite hindlimb. It was hypothesized that simultaneous stimulation of the forelimb should increase the amount of fixation produced by stimulation of the hindlimb.

Twelve Sprague-Dawley rats were anesthetized with Nembutal (sodium pentobarbital 50 mg/kg) and simultaneously stimulated on their left forelimb and right hindlimb (3mA; 45 min.) and compared to controls (12 animals, unilaterally stimulated on the right hindlimb). Measurements of fixation were taken immediately following the stimulation.

The results showed no statistically significant differences in fixation between the two groups. The data suggest one of two possibilities; (i) that the contralateral opposite limb stimulation produced little or no actual excitatory drive on the interneurons of the hindlimb being stimulated and/or (ii) the fact that the animals had an intact neuraxis lessened the effect of the crossed-flexor drive from the forelimb stimulation. In previous studies (e.g., Steinmetz, Beggs, Molea and Patterson, 1985, Brain Research, *327*: 312-315), we have shown that fixation produced in the intact animal is decreased somewhat from that produced by the same stimulus parameters in the spinalized animal. This suggests that the higher nervous system has an inhibitory effect on the fixation process. The possible role of the opposite limb crossed flexor reflex in the production of fixation will be explored further in spinalized animals, if such a preparation is feasible.

Supported by AOA Bureau of Research Grant 95-03-319

B21*

EFFECTS OF CROSSED EXTENSOR ACTIVATION ON SPINAL FIXATION IN RATS. L. Dally, B.S. and M.M. Patterson, Ph.D., University of Health Sciences College of Osteopathic Medicine, Departments of Osteopathic Principles and Practice, and Physiology, 1750 Independence Avenue, Kansas City, MO 64106-1453

Spinal fixation is a long-term alteration in the excitability of spinal reflex circuits. This study assessed the effect of crossed extensor pathways on the fixation of heightened excitability of flexor reflex circuits. Twelve Sprague-Dawley rats were anesthetized with Nembutal (sodium pentobarbital 50 mg/kg) spinalized, bilaterally stimulated on their hindlimbs (3mA; 45 min.) and compared to controls (12 spinalized, unilaterally stimulated). A statistically significant decrease in post stimulation flexion ($p=.016$) and force ($p=.0467$) was found in the experimental group. This demonstrated (i) that the spinal cord does play a role in limb fixation that is secondary to heavy nociceptive input; (ii) that sensory inputs responsible for fixation do activate the motoneurons responsible for generation of the crossed extensor reflex; and (iii) that activation of the crossed extensor reflex by concomitant left hindlimb stimulation is presumed to decrease flexion fixation of the right hindlimb by inhibiting the contralateral flexion. A series of 12 intact (nonspinalized) rats bilaterally stimulated produced no statistical difference in fixated limb flexion when compared to the unilaterally stimulated intact cohorts ($p=.082$ distance; $p=.551$ force). Previous studies by Patterson and his colleagues (e.g., Steinmetz, Beggs, Molea and Patterson, 1985, Brain Research, *327*: 312-312) have demonstrated that fixation in intact animals is inhibited relative to spinalized groups. The present data are consistent with the previous findings — an intact neuraxis will negate at least partially the crossed extensor activation.These data begin to suggest a possible means of reversing via manipulation the facilitated segment often discussed in the osteopathic literature.

Supported by AOA Bureau of Research Grant 95-03-319

B22

Salivary Cortisol Variation in Institutionalized Elderly. GJ Harper, Ph.D., Ohio University, Department of Social Medicine, College of Osteopathic Medicine, Athens, OH 45701.

Salivary cortisol is increasingly being used as a stress marker in several populations. Some studies indicate that with age, people become hypercortisolemic. However, few studies have examined normal population variation in the elderly. Cortisol levels typically follow a strong circadian rhythm with the highest values in the morning and a steady decline throughout the day. This project examined cortisol patterns in older adults living 2 institutional settings. It was hypothesized that cortisol levels would be significantly higher in nursing home (NH) residents than independent-living (IL) residents.

Participants included 27 NH residents and 29 IL residents in Ohio, age 87+/- 8 and 80+/- 6, respectively ($p<0.05$). Daily saliva samples were collected from each participant at 8am, 12pm, 4pm, 8pm over 3 consecutive days. Salivary cortisol levels were determined by chemiluminescence. After outliers were removed, mean cortisol levels among NH were 13.6, 9.4, 9.8 and 10.23 nmol/l, respectively and 8.7, 7.7, 7.4, 7.3 nmol/l among IL residents . ANOVA and repeat measures ANOVA results indicated that individual cortisol levels and cortisol patterns did not significantly differ between groups($p>0.05$).

Although levels and circadian patterns do not differ between groups, average levels were significantly higher and more variable than reported for younger populations. Circadian patterns were distinctly different from younger populations. High levels may be due to pathology, age or repeated exposure to stressors. Altered circadian rhythms were likely due to inactivity and sleep disruption common to institutional life. Thus, cortisol may be an inappropriate stress marker for institutionalized elderly.

*Student presentation competition.

B23*

ABSORBANCE PROPERTIES OF A CARNOSINE-CONTAINING GLYCATION PRODUCT

S.E. Shinkey, B.S. and N.W. Spidle, Ph.D.
University of Health Sciences, College of Osteopathic Medicine, Department of Biochemistry, Kansas City, MO 64106

Protein glycation (or non-enzymatic glycosylation) contributes to the pathogenesis of various diseases such as Alzheimer's disease (AD). AD involves formation of intracellular protein glycation adducts evidenced by the fact that the neurofibrillar tangles are comprised of advanced glycation products (AGEs). Anti-glycation compounds such as L-carnosine prevent the glycation-induced aggregation of neuronal proteins and Tenilsetam, another AGE-inhibitor, was shown to provide clinical relief of AD symptoms. We are interested in L-carnosine's antiglycation properties. We hypothesized that L-carnosine forms glycation adducts. We examined the glycation adducts between L-carnosine and glyceraldehyde 3-phosphate (Glyc3P). We determined the absorbance spectral changes over time during incubation of 10mM L-carnosine with 5 mM Glyc3P at 37°C. There was a shift in the absorbance maximum from 316nm to 288nm indicating a rearrangement of the L-carnosine structure. Additionally, a time dependent increase in absorbance at 288 nm occurred suggesting that this putative adduct increases in concentration over time. We examined the product's biological effects. We used a target enzyme, aspartate aminotransferase (AAT), which is susceptible to glycation. We assessed the inhibitory effects of the adduct. After a 4 hr incubation of AAT with 5mM Glyc3P at 37°C no activity remained (control: 126 μmoles/min per mg prot). In the presence of the adduct, 49% of control activity (62 μmoles/min per mg prot) remained. The adduct inhibited AAT to a less degree than did the glycating agent Glyc3P, supporting the hypothesis that L-carnosine is a biologically relevant anti-glycation agent.

Supported in part by a grant from the UHS Division of Research.

B24

CYTOKINE LEVELS IN CLUSTER HEADACHE PATIENTS

L. Mueller, D.O., A. K. Gupta, MS IV, T. P. Stein, Ph.D.
UMDNJ-SOM
Department of Family Medicine
Stratford, NJ 08084

The pathophysiology of cluster headache while much debated, is still largely unknown. Most studies have focused on vascular or neural mechanisms. Exciting new research is directed at examining possible neuroimmunologic mechanisms. Urinary levels of proinflammatory cytokines Interleukin-1 beta (IL-1B) interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFa) were studied in 11 male episodic cluster headache patients during and outside of active headache cycles and compared with healthy nonheadache male control subjects. Comparative means revealed lower levels of all cytokines tested in cluster subjects versus controls, but did not achieve statistical significance. No differences were found between cluster headache groups during and outside of headache cycles. Lower levels of Interleukin IL-1B, IL-6 and TNFa in cluster headache patients may signify a disordered bidirectional neuroimmune network that may cause, contribute to, occur independent of, or be an associated epiphenomenon of cluster headache pathophysiology. Further research to expand proposed neuroimmunologic mechanisms of cluster headache may contribute to diagnostic markers of disease or future therapeutic modalities such as recombinant cytokines, cytokine antagonists, or medications to modulate cytokine production.

B25

MYOSIN ISOFORM COMPOSITION AS A MEASURE OF BENEFICIAL EFFECTS OF MANIPULATIVE THERAPY ON MUSCLE HEALTH IN A RAT MODEL OF ARTERITIS

B.H. Hollar, Ph.D., M.A. Andrews, Ph.D., J. Conley, B.S., M. Wells, Ph.D., and J. Sozan, Ph.D. New York College of Osteopathic Medicine, Departments of Neuroscience and Physiology, Old Westbury, New York 11568

In our previous studies, we have established a reliable method to produce rheumatoid arthritis in Sprague-Dawley rats and to study the effects of OMT, anti-inflammatory agents and exercise have on joint disorder, leg and ankle extension and gait (stride length) using computerized motion analysis (please see accompanying abstract and poster for details). In an attempt to determine individual muscle fiber changes produced by OMT, anti-inflammatory agents and exercise, arthritis was induced in three groups of Sprague-Hawley rats as previously described (JAOA 97(4) 207-214 1997). Each group of animals received only one form of treatment over the course of twelve weeks. At the end of the twelve weeks, the gastrocnemius (medial head) and the tibialis anterior muscles from both legs were dissected out and analyzed. The muscle was homogenized by ultrasonication and proteins were separated overnight at constant voltage by modified SDS gel electrophoresis at 10°C. The two principal isoforms of skeletal muscle myosin could be resolved and quantified using digital densitometry to assess changes in isoform distribution. Preliminary results indicate that in untreated animals, there was a trend in some muscles toward increasing the content of the slow myosin isoform in the arthritic leg relative to the untreated leg, indicating a change in the use of this leg. This trend was not found in animals treated with manipulation. Supported by ADA Grant #99-07-403

B26*

BRONCHIAL GLANDS IN ASTHMA : A MORPHOMETRIC ASSESSMENT.

D.J. Sommer, B.S.[1]; J.E. Sheppard, B.S.[1]; B.A. Simon, B.S.[1]; A.T. Morrissey, Ph.D.[2]
NOVA SOUTHEASTERN UNIVERSITY/COLLEGE OF OSTEOPATHIC MEDICINE[1] and NSU/COLLEGE OF MEDICAL SCIENCES[2], Ft. Lauderdale, FL. 33328

The abnormalities of mucus production and clearance are important components of the asthma pathogenesis. We hypothesize that the airway submucosal glands in severe asthmatics produce secretion that is both quantitatively as well as qualitatively different from that of the non-asthmatics. In this abstract we present the quantitative assessment of submucosal gland abundance in asthmatics as compared to non-asthmatic controls.

To measure the submucosal gland profiles in the airway walls, we used the Image-Pro® Plus a computerized morphometry program on digitized images of paraffin embedded H&E and PAS stained tissues from bronchi. The uniformly stratified samples were taken at autopsy from 8 male and 9 female patients who died in status asthmaticus and from 6 control subjects (accidental death). The airway wall areas were determined by tracing the luminal and ab-luminal surfaces of the bronchial cross sections. The measured glands were related to the wall area and to the luminal perimeter, which have been shown to reliably correlate with airway constriction.

In comparison to controls the asthmatics had a larger ($p<0.05$), submucosal gland profile area, characterized by submucosal gland hypertrophy. Preliminary data indicate nearly two fold larger gland area in asthmatics 12.6 ± 5.9 % (±SEM) v.s. controls 6.8 ± 2.9 % (±SEM) of the bronchial wall. The larger secretory organ may impart a significant contribution to the quantity of mucus secretion. We are currently investigating the qualitative modifications of the airway secretions and their participation in the asthma pathogenesis. Support: NSU/CMS Faculty Research Grant, and NSU/COM Student Grant.

*Student presentation competition.

B27*

Low density lipoprotein oxidation is inhibited by water extracts of the medicinal herb Achyrocline satureioides. *H Wang-Flores OMSII, T Menini, MS, MD, and A. Gugliucci, MD, PhD* Touro University College of Osteopathic Medicine, Division of Basic Sciences, Vallejo, CA 94592.

Hypothesis. A considerable amount of work is devoted at present to the role of different synthetic and natural antioxidants as inhibitors of LDL oxidation and their putative effect to counteract atherogenesis. Achyrocline satureioides is a medicinal herb used in many countries for its choleretic, antispasmodic and hepatoprotective properties. The presence of the flavonoid quercetin and its derivatives, and of different phenolic acids in the aerial parts of this plant has led us to study the antioxidant activity of its extracts vis-a-vis human LDL.

Methods. Human low density lipoprotein (LDL) was isolated by sequential ultracentrifugation. Copper and hydrogen peroxide-induced autoxidation of LDL was monitored in the presence or in the absence of different concentrations of Achyrocline satureioides water extracts. Lipid peroxidation was monitored by diene conjugates and thiobarbituric acid-reactive substances analysis.

Results. Water extracts of Achyrocline satureioides are capable of inhibiting the initiation and the propagation of LDL oxidation in vitro. They inhibit lipid peroxidation at all concentrations in a dose-dependent manner. Significant differences in lag-time, slope and maximum rate of oxidation could be detected by both assays employed. In terms of mass, the antioxidant potency of water extracts of Achyrocline satureioides is in the same order of magnitude than that of ascorbic acid.

Conclusions. The results obtained suggest that the extracts of A. satureioides possess significant free radical scavenging and antioxidant activity in vitro, a fact that should encourage future in vivo studies. *Sponsored by TUCOM*

B28

SYLVIAN FISSURE MORPHOLOGY IN A POPULATION OF TONAL AND NON-TONAL LANGUAGE SPEAKERS. J.T.Martin, Dr.rer.nat., D.H.Nguyen, B.S., and R.A.Sugerman, PhD Western University of Health Sciences, College of Osteopathic Medicine of the Pacific, Pomona, CA 91766

Language processing occurs largely in the perisylvian areas of the left hemisphere. The planum temporale (PT), an area located on the dorsolateral surface of the temporal lobe, is thought to be involved in the first stages of language processing. Tonal language speakers, e.g., Vietnamese, use tonal variation of a single sound segment to represent different meanings, whereas in non-tonal languages, e.g., English, shifting the intonation of a word does not affect its meaning. In order to attend to pitch contours, tonal language speakers may require more neural circuitry. Therefore, we hypothesized that the planum temporale would be larger in tonal language speakers. To study this question, we measured the perimeter length of various segments of the Sylvian Fissure in 35 Vietnamese cadaver brains and 50 Caucasian brains. Brains were divided into three subtypes according to the work of (Witelson & Kigar, J.Comp.Neurol., 1992). Within the most frequent subtype the segment encompassing PT was relatively larger in the left hemisphere in Vietnamese brains. This is probably a reflection of language processing differences because the two populations were not significantly different in this measure on the right side. These data raise questions of whether cell assemblies needed for processing tonal features expand during childhood language learning, or alternatively, whether certain anatomical features may predispose a population to develop one type of language over another.

B29

TECHNIQUES FOR ENSHEATHING CELL IMPLANTATION INTO THE RAT OPTIC NERVE (B.H. Hallas, Ph.D., G. Salam, D.O., W.D. Maxwell, Ph.D., M.R. Wells, Ph.D.) New York College of Osteopathic Medicine, Department of Neuroscience, Old Westbury, New York 11568.

Hypothesis. To develop a method for the implantation of cultured cell grafts of glial cells into the mammalian optic nerve to promote axonal regeneration. **Methods**. Rat optic nerves were exposed through a lateral approach with a gentle retraction of the eye. A lesion of local glial cells was made by an injection through a micropipette of 2µl of 0.1% lysolecithin in saline with a small amount of methylene blue added. After 20 minutes, olfactory ensheathing cells labelled with Dil were introduced through a micropipette. Animals were allowed to survive for 1 to 30 days when the optic nerves were examined for the presence of demyelinated areas and the presence of labelled cells. **Results**. Injections of lysolecithin created local areas of demyelination within the optic nerve. Labelled cells could be identified in these areas for up to two weeks after injection, with some suggestion of migration. The ability of grafts to myelinate optic nerve axons is presently being examined. **Conclusions**. A method has been developed for the implantation of ensheathing and other cultured cells into the rat optic nerve. The techniques may be consistent with the use of autologous cells and may be used in studies to support the regeneration of axons in the optic nerve. Supported by the New York College of Osteopathic Medicine of the New York Institute of Technology.

B30

INITIAL RESULTS OF THE TREATMENT OF NERVE COMPRESSION SYNDROMES WITH MANIPULATIVE THERAPY. M.A.W. Andrews, Ph.D.*, B.H. Hallas, Ph.D.#, J.R. Contray, B.S.#, and A.M. Petrizzo, D.O.+, *LECOM, Erie, PA; #NYCOM of NYIT, Old Westbury, NY; +Community Gen. Osteopathic Hosp., Harrisburg, PA.

Continuing our investigation of the effects of nerve compression syndromes on muscle function, we have investigated the effects of twice weekly manipulative therapy on rat hindlimb muscle (with a unilaterally induced piriformis syndrome) using the techniques of Hallas, et al. (1997 JAOA 97:207-214). Again, as in previous studies, we have assessed the sensitivity of fast-twitch (extensor digitorum longus; EDL) muscle fibers to Pi, the primary fatigue metabolite in muscle, as a marker of muscle functional change. Unilateral compression of the rat sciatic nerve was produced by suture ligation at either 100 or 35 mm Hg, at the level of the obturator tendon. Animals were euthanized at one, two and four weeks post-ligation, the EDL removed and chemically demembranated (0.5% Triton X-100 in control solution), and single fibers dissected out. All experiments were run at 22° C, pH 7, with solutions formulated by solving the set of simultaneous equations describing the multiple equilibria of ions in solution. Solutions contained (mM): 5 EGTA, 20 imidazole, 2 Mg^{2+}, 5 MgATP, 15 phosphocreatine and 100 u/ml creatine kinase (to maintain a constant [ATP]). Appropriate Ca^{2+} was added to assure attainment of maximal force (F_{max}) under all conditions, and KH_2PO_4 (Pi) was added as required. All solutions had a total ionic strength of 200 mM. It was found that the sensitivity of EDL fibers to Pi was increased (less force generated) in direct proportion to duration of ligation and to the compression force. Furthermore, under all conditions (but not in all cases), there was a tendency for amelioration of this sensitizing effect if hindlimb manipulation therapy was used. These results provide initial evidence that deleterious effects of nerve compression syndromes may be treated by manipulation therapy, however, additional research is needed because, due to the low number of animals used (n = 5 in each case) and the fact that amelioration was not seen in all cases, results were not found to be statistically significant (Support: AOA Bureau of Research).

*Student presentation competition.

Medical Education

M01

Assessment of Medicaid Purchased Health Care in Rural Appalachia

C. Simpson, DO, A. Pixley, Ph.D.
Ohio University, College of Osteopathic Medicine
Department of Family Medicine
Athens, OH 45701

The work mandated of several components designed to maximize the impacts of managed care that could be applied to the region to maximize access and improve medical care while attempting to bring these costs to the State's Medicaid budget.

Methods

The Project Team mapped clusters of contiguous counties that were formed around the region's geography, economic, and health system medical areas. Once preliminary "model" clusters were identified, Medicaid utilization data was analyzed on a county-wide and cluster-wide basis.

Results

Initial cluster modeling identified six county groupings and one sole county for a total of seven preliminary model service areas. In-cluster hospitalization use for the clusters ranged from 59% to 77%, while in-cluster ambulatory services ranged from 82% to 93%; in-cluster dental services ranged from 69% to 91%. Per-user clusters, utilization across the instruments (inpatient, ambulatory, dental) was consistent; high in-cluster hospital use extended with higher in-cluster ambulatory use. Based on our findings, a cluster of counties was recommended as a pilot managed care site.

Conclusions

This study accomplishes the natural variation of health care utilization and access to care across part of the rural SE Ohio. Although designed to identify a site where a Medicaid managed care program can be implemented, it also speaks to the limitations that this and other pilot programs may have in generalizing health policy decisions even within a regionally focused rural area. How initiatives derived from models implemented in other communities may face significant, unexpected barriers that will impede successful attainment of programmatic objectives.

The authors gratefully acknowledge the support of the Ohio Board of Regents and the Ohio Department of Human Services for their support.

M02

An Examination of the AOA Data Base

C. Simpson, DO, A. Pixley, Ph.D.
Ohio University, College of Osteopathic Medicine
Department of Family Medicine
Athens, OH 45701

Problem Statement

The address maintained by the AOA is the preferred mailing address that the physician provides to the AOA. The assumption that mailing address and practice location are synonymous may be in error, since there is no published information showing the relationship of these variables.

Methods

DO FPs that recently completed residency training were identified from the AOA master file. A survey was sent to the AOA identified preferred mailing address. Each physician was asked if the mailing address used was the physician's primary practice location.

Results

Ninety-six of the 235 responding physicians, answered that the mailing address sent by the AOA was their primary practice location. One hundred and thirty nine of the 235 responding physicians indicated that the mailing address was not their primary practice location. Overall, (59%) report that the mailing address and practice address either are the same, or are located in the same county.

Conclusions

For recently trained DO FPs, preferred mailing address as maintained by the AOA is not synonymous with primary practice location. Practically speaking, 94% of respondents report that their mailing address and practice address are located in the same county. Eleven percent of respondents practiced in one county and receive their mail in a different county.

As this author knows to define rural location more precisely than the county level, this problem may become more acute. We would encourage the AOA to include primary practice location in its national database.

The authors gratefully acknowledge the support of the AOA, and the Ohio University, College of Osteopathic Medicine.

M03

Complementary and Alternative Medicine Research Curriculum Development With Emphasis On Osteopathic Manipulative Medicine: A Proposal

A. T. Still, D.O., Ph.D., J.C. Licciardone, D.O., M.S., M.S.A., J. Su, B.A.S. The University of North Texas Health Science Center, The Physical Medicine Institute, Department of Manipulative Medicine, Ft. Worth, TX. 76107

The Physical Medicine Institute in conjunction with the Department of Manipulative Medicine developed a Predoctoral Research Fellowship in CAM at the University of North Texas Health Science Center in 1998 as an extension of the Predoctoral Teaching Fellowship in CAM. This program was designed to develop successful researchers in complementary & alternative medicine (CAM), specifically OMM. It collaborates with the School of Public Health, Graduate School of Biomedical Sciences, & various departments of Clinical Medicine; and is coordinating efforts to offer predoctoral fellows the combined D.O./M.P.H. degree through enrollment in a variety of research & CAM related courses. We propose to enhance the administrative, curricular, mentoring, and funding structures of the current research program.

Development is targeted at: (1) curriculum expansion with courses on biostatistics; epidemiology; clinical trial design; research methods; responsible conduct; ethical & regulatory issues in research, (2) development of new CAM-focused courses; (3) program extension to attract a wider audience including faculty, pre- and post- doctoral fellows, & allied health professionals; (4) establishment of annual CAM research conferences; (5) development of Continuing Medical Education courses in CAM; (6) formalization of various degree tracks; and (7) use of computer technologies for curricular advancement.

Research fellows will acquire the skills necessary to successfully develop basic science & clinical research projects, obtain funding, implement studies & publish quality research in CAM with the opportunity to advance toward various degrees in combination with or addition to their Doctor of Osteopathy degree. Fellows, at present, are competitively selected from a diverse multicultural pool of osteopathic medical students from the UNTHSC. This evolving predoctoral fellowship program has a successful track record of graduating accomplished clinical researchers, educators & administrators in CAM. This expanded & improved program will continue to develop future leaders and researchers capable of successful and competitive clinical & basic science research in CAM.

M04

INTEGRATION OF BUSINESS COURSEWORK INTO A MEDICAL SCHOOL CURRICULUM. M.P. Mariotti, Ph.D. and J.W. Trotter, Ph.D., Lake Erie College of Osteopathic Medicine, Erie, PA 16509 and The Pennsylvania State University, Behrend College, Erie, PA 16563

PURPOSE: The goal was to provide LECOM students with the principles of management and practical tools to help them succeed as physician leaders. This study assesses the relevance and student acceptance of this concept. **DESCRIPTION:** In 1997, LECOM introduced a business course titled Healthcare Management I into its first year curriculum. This was a comprehensive course in business principles applied to healthcare, and was designed to help students understand and appreciate the competitive forces of their chosen profession, whether in their own professional practice or in a group. Quality of worklife, as well as profitability, was emphasized. The course focused on the following traditional business principles: Management; Marketing; Accounting; Finance; and Economics. A second course, Healthcare Management II, was added to the second year curriculum in 1999. This course expanded on the foundation presented in Healthcare Management I and offered insight into even more clinically-relevant business aspects. Each course consisted of 30 direct contact hours with 2 exams, and was presented by Ph.D., J.D., C.P.A. and M.B.A. faculty. **ANALYSIS:** Three years of student comments were reviewed. Commentary was segregated into categories indicative of either favorable or unfavorable opinion. **RESULTS:** Students initially resented incorporation of "non-biomedical courses" into the curriculum. They felt it detracted from time they could devote to medical courses and/or National Board preparation. As the program evolved and the initial groups of students entered clinical rotations, feedback in learning sessions became more favorable. The most likely scenario becomes third- and fourth-year students would better appreciate the relevance of healthcare management education. The positive feedback from their peers contributed to the more favorable student perception of this coursework. Student opinion is now positive and supportive. A student club devoted to business has been started on campus, and all successful students are awarded a Graduate Certificate in Healthcare Management from Penn State. Longitudinal studies will examine the opinions of graduates on this topic. **CONCLUSIONS:** This coursework introduces LECOM students to fundamental managerial practices intended to make their careers more profitable and enjoyable. Preliminary information indicates that this is supported positively by students as they progress through their clinical training.

M05

Understanding and Impressions of Osteopathic Medicine: A survey of Truman State University Biology Students. N.K. Sanders, Ph.D. and V. J. Nerini, Truman State University, Division of Science, Kirksville, MO 63501, and B.F. Degenhardt, D.O., Kirksville College of Osteopathic Medicine, OMM Department, Kirksville, MO 63501

Truman State University is a public liberal arts university in rural northeast Missouri. In the same town is the founding osteopathic medical school (Kirksville College of Osteopathic Medicine), and Kirksville is a primarily D.O. community. We hypothesized that close proximity and exposure of Truman students to KCOM, the Still Museum, and a Biology faculty at Truman personally familiar with osteopathic care would lead to a Truman student population with a high degree of awareness of the nature and philosophy of osteopathic medicine. In the current survey, we hypothesized that freshmen pre-med students at Truman would have a greater awareness and appreciation of osteopathic medicine than non pre-med class mates, and that of junior/senior biology pre-med students would be even higher. A survey tool was designed to address this hypothesis, asking students to respond to a series of statements concerning osteopathic and allopathic training and care. Students were also asked in an open- ended question to describe the difference between an osteopathic and an allopathic physician. Preliminary data suggest that junior and senior biology pre-med majors are more knowledgeable about osteopathic medicine than those in freshman biology, and that the freshman non pre-med biology students are the least knowledgeable. Future research plans include a modification of the survey tool to address concerns raised by preliminary data, and expanding the survey to compare responses of Truman students to 1st year KCOM students during orientation week, and 2nd year KCOM students in their last weeks at KCOM.

M06

INDEXING THE WORLD'S OSTEOPATHIC LITERATURE

A Brooks, MLS; MBA; C Elam, MLS; LF Johnson, MIS; D Martin, MSLS;
University of North Texas Health Science Center,
Gibson D. Lewis Health Science Library, Fort Worth, Texas 76107
L Onsager, ML, MA; R Sanders, MA, Med;
Kirksville College of Osteopathic Medicine,
A. T. Still Memorial Library, Kirksville, Missouri 63501

The need for information on osteopathic medicine and osteopathic manipulative treatment has increased greatly in recent years in the United States and worldwide. Currently, access to the 100-year old osteopathic literature is extremely limited, incomplete, and scattered. To solve this problem, the American Osteopathic Association (AOA) and the American Association of Colleges of Osteopathic Medicine (AACOM) are sponsoring the development of the world's first comprehensive index to the osteopathic literature. Similar to MEDLINE, this index will allow the osteopathic profession global access to its own unique literature.

The UNTHSC and KCOM Libraries are now in the fourth year of their five-year contract, with UNTHSC providing project administration, database development, and quality control. UNTHSC is also indexing the current (post-1950) literature, while KCOM, as the founding school for osteopathic medicine, is indexing its unique historical (pre-1950) materials. Using Cuadra STAR database software, the index will soon be accessible via the Web.

The index consists of citations and abstracts of articles, book chapters, audiovisual, and electronic resources from around the world, covering all aspects of osteopathic medicine, osteopathic manipulation, and relevant manual medicine topics. The database can be searched using keywords, National Library of Medicine *Medical Subject Headings (MeSH)*, or specific osteopathic terms. These terms are derived from the osteopathic literature being indexed, the AACOM *Glossary of Osteopathic Terminology*, osteopathic physicians and other resources. A structured thesaurus of osteopathic terms is a major outcome of the project. By helping standardize the osteopathic nomenclature, the thesaurus will facilitate international communication and promote unity within the profession.

(Sponsored by a grant from American Association of Colleges of Osteopathic Medicine (AACOM) and American Osteopathic Association (AOA).)

M07

IPAQ: INTEGRATED PRE-CLINICAL APPLICATIONS AND QUESTION WORKSHOP DESIGNED TO ENHANCE THE COMPREHENSION OF MEDICAL LEARNING OBJECTIVES. R.C. Sexton, Ph.D., W. A Krueger, Ph.D., and M. P. Mahalik, Ph.D. Lake Erie College of Osteopathic Medicine, Erie, PA 16509

PURPOSE: Basic science disciplines were taught as separate units in the first semester curriculum and examined in multidiscipline exams. IPAQ was a 2 hour/week workshop designed to integrate the separate discipline materials in preparation for exams. An assessment of student opinion about the educational value of IPAQ is presented. **DESCRIPTION:** The class of 140 was divided into ten sections. Each section met separately with one student acting as a facilitator who handed out materials, kept records, and managed time. In the first hour of IPAQ, student pairs first prepared and then presented 5 minute oral presentations on specific learning objectives derived from individual discipline lectures presented during the week. During the second hour, students individually answered multiple choice type questions derived from the same learning objectives. The facilitator recorded student answers before assigning an individual student to lead a discussion on a question. Correct answers were provided after a consensus was reached. **ANALYSIS:** After 16 IPAQ sessions, 120 students completed a survey, which used a 5 point Likert scale. **RESULTS**: Students believed that IPAQ: [1] increased their understanding of leaning objectives presented during discipline lectures (86%); [2] helped them integrate learning objectives from multiple disciplines (78%); [3] improved their reading comprehension of multiple choice type questions [71%]; [4] increased their oral communication skills (80%); [5] was more conducive to learning key medical concepts than was the lecture format with a content expert (79%).**CONCLUSION:** IPAQ was valued by students and will be included in future curricula in an expanded version.

M08

Internet Communication with Clinical Students on Rural Rotations

R.W. Foster, D.O., H. H. Baker, Ph.D., MBA, and D. R. Hale
West Virginia School of Osteopathic Medicine
Lewisburg, WV 24901

Purpose. To improve the training of medical students by providing enhanced access to campus-based resources and improving communication between campus-based and off-campus faculty and administrators, primaril for rural family medicine rotations.

Method. In less than three years, in cooperation with the project director an Information Systems Specialist used Allaire ColdFusion® to develop web-based mechanisms for many education/administrative tasks, including a) online student access to rotation objectives and policies, housing information and maps; b) remote submission of case studies, logs, and feedback on sites; c) informing students about open rotations/training opportunities; d) providing information about family practice residency opportunities, with on-line application to affiliated internship/residency programs; e) access to medical journals and text data bases through the institution's medical library; f) secured access by faculty/administration and the involved student to rotation grades and rotation schedule; and g) electronic generation of pictured student identification cards and immunization records with preservation for future access.

Results and Conclusions. While a few students report problems with access from some remote sites, most were very satisfied with internet servic Program objectives are being met.

Support. This project was supported in part by a Grant for Predoctoral Training in Family Medicine, Public Health Service.

M09

One School's Relationship Between Academic Performance and Scores on the June 1999 Level 1 COMLEX-USA

R.W. Foster, D.O., M. H. Baker, Ph.D., MBA, M. K. Cope, Ph.D., J. N. Gorby and R. Fink, Ph.D.
West Virginia School of Osteopathic Medicine
Lewisburg, WV 24901

Purpose. To examine the relationship between academic performance during the first two years at osteopathic medical school and performance on the COMLEX-USA Level 1 examination.

Method. By institutional policy, students at the West Virginia School of Osteopathic Medicine (WVSOM) are required to pass COMLEX-USA Level 1 and Level 2 examinations in order to graduate. For 62 students at WVSOM taking the COMLEX-USA Level 1 examination in June, 1999, simple correlations were calculated between academic performance and Level 1 scores. Correlations were also calculated between Level 1 scores and pre-admissions data.

Results. Correlations with COMLEX-USA Level 1 scores were: Total GPA for the first two years of the curriculum, .55*; GPA for Phase 2 only, .51*; and GPA for Phase 1, .44*. Correlations with pre-admissions data were: MCATs: Biological Sciences, .37*; Physical Sciences, .26; Verbal Reasoning, 0.05; Writing Sample (converted to numerical scale), -0.13; GPAs: Overall undergraduate GPA, .28; Non-science undergraduate GPA, .23; Science undergraduate GPA, .28. (*=sig <.05 after adjustment for 10 correlations.)

Conclusions. Correlations between COMLEX-USA Level 1 scores and measures of academic performance during the first two years of osteopathic medical education were comparable to previously reported correlations of academic performance with licensing examination scores.

M10

Implementation of an Interactive, Web-Based Tool for Learning Anatomical Landmarks

Richard C. Haligren, Ph.D.
Carol L. Monson, D.O.
Michigan State University
College of Osteopathic Medicine
East Lansing, MI 48824

We have developed Internet-based materials designed to support acquisition of knowledge required for evaluation and treatment of somatic dysfunction. We hypothesized that students using these materials would score higher on the anatomical landmark section of both midterm and final exams.

First-year medical students (n = 134) were recruited to participate in the study. Students participating in the study (n = 107) were required to sign an informed consent form. Pre- and post-instruction questionnaires, a pre-instruction test, and the Kolb Learning Style Inventory assessment were administered.

The Internet-based materials were maintained on a dedicated Web site, and were only accessible to students who had activated their computer account. Group 1 (n = 63) included students who enrolled in the study and elected to activate their computer account; Group 2 (n = 44) included students who enrolled in the study and elected not to activate their account; Group 3 (n = 17) included students who elected not to enroll in the study. All students enrolled in the study (Groups 1 & 2) received an introductory packet of materials that included graphical materials showing the location of anatomical landmarks, and instructional materials describing how to activate their computer account. Students who declined to enroll in the study did not receive any materials.

Assessment of student ability to accurately locate anatomical landmarks was measured on a 30-question midterm exam and a 30-question final exam. Students using the Internet-based materials scored significantly higher ($P<.001$) on the anatomical landmark section of both midterm and final exams.

Acknowledgements: This study has been supported in part by Research Grant #98-06-461 from the American Osteopathic Association, Chicago, IL.

M11

INTERNATIONAL ROTATIONAL ELECTIVES IN MEDICINE AND PUBLIC HEALTH

Rose Negrini, D.Sc., and Silvia M. Bonetti, D.O. Office of International Relations, Lake Erie College of Osteopathic Medicine (LECOM), Erie, PA 16509

As we begin the 21st century, the osteopathic medical education and practice faces a global opportunity. LECOM students frequently showed interest in international rotational electives in medicine and public health abroad. As part of our global strategies, we have established a number of international elective sites in countries such as Australia, South Africa, Poland, Romania, Germany, India, and Dominican Republic. LECOM students practically take advantage of these rotations and also are able to meet people from different cultures over the world and have one of the best experiences of their life. Not only do they have the opportunity to experience medicine first hand outside the United States, but they also realize that how much people take human like health care, a house, and a job or education for granted. The following global goals were achieved during the four-year existence of International electives at LECOM: 1) LECOM students were able to educate hospital staff, allopathic doctors, and patients about osteopathic philosophy and practice, 2) students were able to demonstrate OMT, 3) they were able to see clinical cases where they would not have an opportunity to observe them in USA, 4) students learned how more efficiently they can diagnose with minimal clinical laboratory assistance. CONCLUSION: Today, more than ever, society needs osteopathic physicians with global responsibilities to those in need. The LECOM's initiative of "International Electives" encompasses an awareness of global health needs. This unique educational initiative serves as an important model of the efficacy of using osteopathic primary care medicine where resources are scarce.