Thirty-Sixth Annual AOA Research Conference Abstracts, 1992: Part 2

Part 2 contains abstracts of the Poster Session to be presented at the Thirty-Sixth Annual AOA Research Conference. For the convenience of attendees, abstracts appear in their scheduled sequence, and are numbered for easy reference. Part 1, abstracts in the Burroughs Wellcome Fellows, Biomedical Sciences, and Clinical Sciences categories, appeared in the September issue.

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Poster Session

P01

EFFECT OF A SPINAL IMPLANT ON FELINE DETRUSOR RESPONSES. B. Degenhardt, D.O. and R.J. Theobald, Jr., Ph.D. Kirksville College of Osteopathic Medicine, Departments of Osteopathic-Theory and Manipulation and Pharmacology, Kirksville, MO 63501.

This project was designed to provide pilot data on the effects of an induced somatic lesion at the level of L_1 and L_2 on the urinary bladder activity of the cat. In naive, female cats a 2 to 4 mm length of stainless steel, 20 gauge, was carefully sutured into the paraspinal muscles at the L_1 - L_2 level. The cats were allowed to recover for 2 weeks. During the recovery period, the cats were gently shaken for 30 minutes to induce an increase in autonomic activity. After recovery, bladder function was determined in an acute experiment where detrusor pressure was measured in response to nerve stimulation and drug administration.

The results show that implantation of the metal rod altered detrusor responsiveness to nerve stimulation and drug administration. Generally, the spinal implant decreased detrusor response to stimulation and increased detrusor response to inhibition regardless of the nature of the stimulation or inhibition. Bladder contractions induced by pelvic nerve stimulation or the administration of acetylcholine or ATP were of less magnitude in cats with the spinal implant than they were in control cats. Similarly, inhibition of pelvic nerve evoked bladder contractions by hypogastric nerve stimulation or the administration of norepinephrine, adenosine or ATP was greater in spinal implant cats than the control cats. The results of this pilot study suggests that a somatovisceral interaction exists in the L_1 - L_2 region of the cat that influences detrusor responsiveness.

P02

LOW-BACK PAIN (LBP) TREATMENT BY HIGH VELOCITY LOW AMPLITUDE (HVLA) OSTEOPATHIC MANIPULATIVE THERAPY (OMT) AND EFFECTIVENESS MEASURED BY ELECTROMYOGRAPHY (EMG), PLASMA CATECHOLAMINES AND BETA ENDORPHIN.
M.F. Krpan, B.A., L.A. Harrington, B.S., R.J. Beckman, B.S., D.R. Boesler, D.O., D.A. Hoganson, Ph.D., E.P. Finnerty, Ph.D., and M.A. Kilmore, Ph.D., Depts of Physiology/Pharmacology and OMM, UOMHS and Dept of Biology, Drake University, Des Moines, Iowa.

Previous work demonstrated that HVLA OMT decreased the EMG activity in the lumbar paraspinal musculature concomitant with a subjective sensation of diminished pain and movement restriction (JAOA, 1988,88:991-997). More recent studies have shown that there is an increase in the skin microcirculation that correlates statistically with the decreased EMG activity. This study measures plasma norepinephrine and epinephrine before and after HVLA, OMT to attempt to quantitate the reduction of LBP sensation and to correlate these markers with EMG and microcirculatory changes. Twenty-six subjects have been recruited to date. Twelve subjects experienced LBP and another twelve were pain free. Each group of twelve were divided into HVLA OMT and nontreatment subgroups. Two additional subjects had LBP concomitant with dysmenorrhea. HVLA OMT, decreased EMG activity during movement and relieved LBP and muscle spasm. Preliminary analysis shows that norepinephrine does not appear to change with HVLA OMT. However, epinephrine appears to decrease with diminished pain. Further data is being obtained from more subjects and the plasma analyzed for beta endorphin.
(Supported in-part by a Quad Cities Osteopathic Foundation grant.)

P03

EFFECT OF CRANIAL BONE MOBILITY ON CRANIAL COMPLIANCE. S. R. Heisey, Sc.D. & T. Adams, Ph.D., Michigan State University, Dept. of Physiology, East Lansing, MI 48824.

Using a customized device for measuring cranial bone movement, we have determined that not only are cranial sutures mobile, but also this flexibility contributes to cranial compliance. This instrument was firmly attached to the surgically exposed parietal bones of anesthetized adult cats (Na pentobarbital, 36 mg/kg ip) so that it spanned the sagittal suture to measure sagittal suture movement (SSM). Cannulas were placed in the brain's lateral ventricles for injecting graded volumes of cerebrospinal fluid (CSF) and for recording intracranial pressure (ICP). Cranial compliance (CC) is defined as the ratio of a change in intracranial volume (ICV) to a change in ICP. Sagittal suture compliance is the ratio of SSM to a change in ICP. Experiments were conducted with the head held firmly in a stereotaxic frame and also with the head free of restraint. How much ICP increases with an increase in ICV conventionally is thought to depend on the displacement of CSF into the spinal subarachnoid space, its absorption into the venous circulation as well as by displacement of blood volume from the cranium. Our data indicate that cranial bone movements at their suture junctions also contribute to CC and its reciprocal, cranial elastance. Our data also show that any restriction of SSM increases elastance. For example, cranial elastance of the unrestrained cat head was 4.5 torr/μm. For animals whose cranial bone movements were restricted, it was 19.7 torr/μm. This is a >400% difference. The more distensible and flexible are the sutures, the greater the ability of the cranium to buffer ICP increases. We provide a hydraulic model to account for the dynamic and steady state relationships between intracranial fluid volumes and pressure as affected by cranial suture mobility. We propose that any calculations of cranial compliance, elastance, cranial bone motion and other related measures consider any form of head restraint as an important test variable. (*Supported by A.O.A. grants # 90-05-294 & 91-05-294*).

P04

THERAPEUTIC ALTERATIONS IN THE TIME COURSE OF STRENGTH RECOVERY FOLLOWING EXERCISE-INDUCED MUSCLE INJURY. L.M. McCullough, B.S., R.R. Conatser, M.S., G. Chleboun, M.S., and J.N. Howell, Ph.D. Ohio Univ. Coll. of Osteopathic Med., Somatic Dysfunction Res. Inst., Athens, OH 45701

In order to assess the effects of various therapeutic interventions on the time course of recovery from muscle injury, we have examined the time course of the recovery of strength following exercise-induced injury. Injury was induced in the elbow flexors with 3 bouts of eccentric contractions carried to failure. Loads equalled 90% of the subjects' isometric maximum force, measured at an elbow angle of 90°. Mean strength as measured immediately after the exercise fell to 60% of pre-exercise control. Following the exercise, subjects were placed randomly into treatment groups. Group I (N=20): subjects performed (nearly) isometric contractions of the elbow flexors with loads equal to 35% of their isometric max. twice daily (20 reps of 5 sec duration separated by 5 sec rests). Group II (21) - subjects performed an unloaded movement of the arm involving the full range of elbow motion, also 20 reps twice daily. Group III (14): over the 5 measurement days subjects were given 2 or 3 osteopathic manipulative treatments by experienced physicians. Physicians were given no instructions as to how to treat the subjects, but they did record the procedures they used. Group IV (17): subjects were given flurbiprofen, 100 mg b.i.d. Group V (15): subjects were given a placebo. Group VI (19): untreated controls. In all groups strength continued to fall for 24 hours after the injury, but thereafter recovery was quite linear for 5 days, after which time the recovery rate slowed. Recovery was significantly faster in Groups I and II than in the control group. Recovery was also faster in both the drug and placebo groups (IV and V) than in the nontreated controls, but recovery rate was not significantly faster in the drug group than in the placebo group. (Drug and placebo supplied by Upjohn; supported by AOA Grant #259)

P05

TIME COURSE OF MUSCLE CELL DEATH FOLLOWING ECCENTRIC EXERCISE. J.N. Howell, Ph.D., G. Chleboun, M.S., D. Karapondo, M.S. and R.R. Conatser, M.S. Ohio Univ. Coll. of Osteopathic Med., Somatic Dysfunction Research Inst., Athens, OH 45701

Following eccentric exercise of the human elbow flexors to failure under heavy load (90% of isometric max.; elbow angle = 90°), the integrated EMG amplitude during submaximal, isometric contractions (up to 20% of isometric max.) increased 2 to 3 fold (N=37). Associated with failure was a decrease in mean power frequency (MPF) of the EMG from about 60 Hz to less than 50 Hz. This decrease was less than the decrease in MPF associated with concentric exercise carried to failure, suggesting lesser metabolic fatigue. The increased EMG amplitude required to produce a given force after exercise indicates that at least some fibers, capable of generating action potentials, could generate little force, either because of fatigue or cellular injury. Maximum isometric force output of the eccentrically exercised muscles decreased by almost 50%. Recovery was slow, taking weeks in many subjects (50% recovery in 2 weeks; N=39) After concentric exercise, the EMG amplitude required to produce submaximal contractions recovered in three days. It is likely that injured fibers, able to produce action potentials but little force after eccentric exercise, subsequently lose their ability to produce action potentials and become inexcitable. The time course of recovery of the normal EMG/force ratio may indicate the time course of death of these cells, which must ultimately be replaced by the regeneration of new cells. The motor units involved must include those recruited during low level isometric contractions, <20% of isometric max. (Supported by AOA Grant #259)

P06

MEASURABLE CORRELATES OF CERVICAL DYSFUNCTION. W.L. Johnston, D.O., J. Vorro, Ph.D., R.P. Hubbard, Ph.D., M.C. Beal, D.O. Departments of Family Medicine, Anatomy, and Biomechanics, Michigan State University, College of Osteopathic Medicine, East Lansing, MI 48824.

Cervical region disability, pain and dysfunction represent major clinical problems that have often resisted objective description. As a result, there is continuing need for instrumental correlates of palpable motion asymmetries that accompany such motor dysfunctions. Once established, instrumental correlates can confirm/deny physiologic changes in musculoskeletal response to manipulative and other forms of treatment. Four of our studies have addressed this need. Each combined instrumental measures with palpatory findings: the first was a kinematic study; the next two were myoelectric; the fourth was clinical, involving manipulative treatment. During subject selection for the first three studies, individual response to a test for cervical sidebending directed division of 17 asymptomatic subjects into two groups, 6 symmetric (controls), and 11 asymmetric (six were more limited in sidebending right and five left). Kinematic and myoelectric measurements were made during six primary rotary movements of the head/neck, performed actively and passively. Kinematic and myoelectric data supported the palpable findings of group differences in cervical function: in the asymmetric group there was reduced total range in all six primary rotations and in secondary deviations; muscles in the asymmetric group were slower to initiate action and reduced in time and strength of contraction. During the study of manipulative treatment, four patients with motion impairment and chronic cervical pain after cervical injury had periodic myoelectric measurements. Following manipulative management (4 to 8 months), data revealed parallel improvement in subjects' complaints, physician's findings and in the instrumental record of electrical activity of the cervical musculature. (Supported by AOA Grant #79-05-79)

P07

QUANTIFICATION OF THE CRANIAL RHYTHMIC IMPULSE IN HUMAN SUBJECTS. J.M. Norton, Ph.D., G. Sibley, B.S., and R.E. Broder-Oldach, B.S.. University of New England College of Osteopathic Medicine, Biddeford, ME 04005.

The characteristics of the cranial rhythmic impulse (CRI) were measured in twenty (20) healthy human subjects by a single experienced examiner using the standard vault hold. The flexion phase of the CRI was indicated by a switch activated by examiner knee pressure and connected to a chart recorder. Average duration of flexion and extension, cycle length, and CRI frequency were subsequently obtained for 6-10 cycles for each subject; mean values for these parameters for 20 subjects were 7.43 ± 1.26 sec, 8.98 ± 2.23 sec, 16.41 ± 3.34 sec, and 3.89 ± 0.72 cycles/sec, respectively. Flexion occupied 45.3% of the typical CRI cycle. Amplitude was determined by the examiner using an arbitrary five-point scale; average amplitude for 20 subjects was 3.03 ± 0.72, and amplitude and frequency were only weakly correlated. The durations of flexion and extension were found to be significantly correlated with one another using nonlinear regression, and both were inversely correlated with CRI frequency. Nonlinear regression analysis indicated limiting values for flexion duration of 9.9 and 5.1 sec at the lowest and highest CRI frequencies, respectively. The duration of extension also approached a lower limit of 5.1 sec at high frequencies but showed no upper limit at low frequencies, suggesting that longer cycle lengths and therefore lower frequencies are achieved by lengthening the extension (or "non-flexion") phase of the CRI cycle. (This work was supported by AOA Grant #91-14-345.)

P08

FAILURE OF A TISSUE PRESSURE MODEL TO PREDICT CRANIAL RHYTHMIC IMPULSE FREQUENCY. J. M. Norton, Ph.D. University of New England College of Osteopathic Medicine, Biddeford, ME 04005.

A recently developed simple tissue pressure model for the cranial rhythmic impulse, or CRI, (JAOA 91:975-994) was based on the assumption that the CRI may arise in soft tissues as the result of a complex interaction of cardiovascular and respiratory rhythms from both the subject and the examiner. To investigate this hypothesis, CRI frequency was measured in twenty (20) healthy human subjects by a single experienced examiner using a standard vault hold. The flexion phase of the CRI cycle was indicated on a chart recorder using a switch activated by examiner knee pressure; average duration of flexion and extension, cycle length, and CRI frequency were subsequently obtained for 6-10 cycles for each subject. During the period of CRI monitoring, heart rate (HR) and respiratory frequency (RF) for both subject (s) and examiner (e) were measured and recorded using body surface electrodes and a pneumograph. The values for HR(e), RF(e), HR(s), and RF(s) obtained during the period of CRI monitoring were entered into the tissue pressure model and yielded calculated values for the frequency of variations in net tissue pressure (Pnet). These calculated Pnet frequencies were then compared to the measured CRI frequencies using standard regression formulae; no significant correlation was found. More complex versions of the published tissue pressure model are being developed and will be applied to the data generated in this study in a continuing search for the physiological basis for the CRI. (This work was supported by AOA Grant #91-14-345.)

P09

INTEREXAMINER AGREEMENT IN THE CHARACTERIZATION OF THE CRANIAL RHYTHMIC IMPULSE. G. Sibley, B.S., R. E. Broder-Oldach, B.S., and J. M. Norton, Ph.D.. University of New England, Biddeford, ME 04005.

In order to determine interexaminer agreement in the characterization of the cranial rhythmic impulse (CRI), ten different examiners were asked to monitor the CRI of the same subject within a three hr period using a standard vault hold. The flexion phase of the subject's CRI was indicated by a switch activated by examiner knee pressure and connected to a chart recorder. The average duration of flexion and extension, cycle length, and CRI frequency were subsequently determined for each examiner; mean values for all examiners were 7.14 ± 1.94 sec, 11.76 ± 6.44 sec, 18.90 ± 7.78 sec, and 3.71 ± 1.46 cycles/sec, respectively. When the examiners were sorted by years of experience in CRI determination, inter-examiner agreement was found to greatest among examiners with >10 yr of experience, and least among examiners with <5 yr of experience. Examiners were also asked to make a subjective assessment of the subject's CRI amplitude (using an arbitrary five-point scale) and vitality, and to identify the region of greatest restriction in the vault of the subject. There was less agreement among the examiners in these determinations. Amplitudes ranged from 2-4 and the vitality was reported as normal to fatigued, with no noticable agreement even when the examiners were sorted by experience. All of the examiners with >5 yr of experience did agree that the area of greatest restriction was anterior, while those with <5 years of experience indicated restriction in varying locations. (This study was supported by AOA Grant #91-14-345.)

P10

CALCIUM L-CHANNEL BLOCKERS AND DANTROLENE SODIUM REDUCE PERSISTENT HINDLIMB FLEXION IN SPINALIZED RATS. M.F. Anderson, DO & I.T. Earnhardt, PhD St.Elizabeth's Hospital of Boston, Psychiatry Dept., Boston, MA 02135 & University of New England, Pharmacology Dept., Biddeford, ME 04005.

Stimulation (2mA, 7ms, 100Hz, 60min) across the musculature of the upper hindlimb in the spinalized rat produces persistent hindlimb flexion. Previously, we have shown that NMDA receptor antagonists interfere with the induction of this flexion. Given NMDA receptor stimulation indirectly may activate voltage sensitive calcium L-channels, the dose response of pretreatment with nimodipine, nifedipine and diltiazem on the induction of persistent hindlimb flexion was explored.

Following spinalization of adult Long Evans rats under halothane anesthesia, animals were pretreated with one dose of a particular test agent (nimodipine: 0.03, 0.1, 0.3, and 1.0mg/kg, i.p., nifedipine: 0.1, 0.3, 1.0, and 3.0mg/kg, i.p., diltiazem: 0.03, 0.1, 0.3, and 1.0mg/kg, i.p.) and wound clips were applied to the musculature of the right upper hindlimb. After a 30min waiting period, current (2mA, 7ms, 100 Hz, 1 hr) was delivered across the clips. In all groups, poststimulation flexion was reduced in a dose dependent manner. In an additional group of urethane anesthetized, nimodipine pretreated rats, mean arterial pressure (MAP) was monitored via a carotid artery pressure transducer. While a dose dependent effect was observed on MAP, the induced flexion was not appreciably affected by MAP (MAP<100) nor different from that of the above, dose matched, nimodipine pretreatment groups.

Since 1) the systemic administration of calcium L-channel blockers may promote skeletal muscle relaxation and 2) NMDA receptor activation may effect release of intraneuronal calcium ion stores, the dose response of pretreatment with dantrolene sodium was studied. Dantrolene sodium theoretically inhibits the release of both skeletal muscle and neuronal calcium ion stores, but does not influence MAP. Following the above protocol, dantrolene sodium (0.1, 0.3, 1.0, and 3.0mg/kg, i.p.) pretreatment reduced poststimulation flexion in a dose dependent manner, but was without effect on MAP. These data suggest that induction of a persistent hindlimb flexion in the spinalized rat depends on both adequate 1) neuronal and skeletal muscle intracellular calcium ion levels and 2) activation of skeletal muscle afferents. As such, the calcium L-channel antagonists and dantrolene sodium may prophylax against chronic pain syndromes including somatic dysfunction when administered in the setting of acute neuromuscular trauma. (Support: Mead Johnson Research Fellowship Grant & Department of Psychiatry, St. Elizabeth's Hospital of Boston)

P11

CEREBRAL EVOKED POTENTIALS IN SOMATIC DYSFUNCTION
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Cerebral somatosensory evoked potentials (SEPs) were elicited by magnetic stimulation of the paraspinal muscles at L2 and L5 levels in twenty normal subjects and sixteen patients with lumbosacral somatic dysfunction. A focal point coil with a mean diameter of 4.7 cm (Cadwell MES-10) was placed tangentially to the skin. The stimulus strength was sufficient to induce a visible muscle twitch without producing muscle contraction in the legs. The potentials recorded over the scalp (Cz-Fpz) consisted of several component P30, N40, P50, N70 and P90. Experiments performed during vibratory and electrical stimulation and voluntary muscle contraction suggest that muscle spindle receptors provide the afferent input responsible for the early components of SEPs. In patients with somatic dysfunction (i.e., lumbosacral torsion), the amplitudes of P30-N40 and N40-P50 SEPs were significantly decreased at the level of the dysfunction (L5) on the side of increased paraspinal muscle tone as compared to the SEPs from the opposite side at L5 and bilaterally at L2, each of which displayed normal resting muscle tone SEPs. After OMT, the somatic dysfunction resolved and the amplitude of the SEPs normalized. Utilization of this technique has potential for quantitative evaluation of somatic dysfunction and osteopathic manipulation.

Partially funded by a grant from the 41st Fund at the University of California, Irvine.

P12

COCAINE DISTRIBUTION IN TESTIS, EPIDIDYMIS AND SPERM OF MICE.
JJ Orris, BA, HW Hitner, PhD, MP Mahalik, PhD* and SA Cosmi, MS. Phila College of Osteopathic Med, Phys/Pharm Dept Phila, PA 19131-1696 and Ohio Northern Univ School of Pharmacy, *Ada, OH 45810..

Evidence is accumulating to suggest that male-mediated effects may contribute to adverse reproductive outcome. Recent in vitro reports suggest that cocaine-exposed spermatozoa may influence abnormal fetal development. The present investigation measured in vivo cocaine and benzoylecgonine (BE) concentrations in plasma, testis and epididymis over time following sc administration of 4 mg/kg cocaine HCl to CF-1 mice. Gas chromatography/mass spectrometry was used for determination of cocaine and BE levels. Cocaine and BE were present in all tissues examined. Plasma cocaine levels decreased from a mean of 525.2 ng/ml at 30 min. to 54.2 ng/ml at 180 min. Levels of cocaine in testis and epididymis decreased slowly over time with concentrations in the epididymis (4515.0 ng/gm tissue at 180 min) higher than concentrations in the testis (1092.0 ng/gm at 180 min) at each time interval measured. BE levels persisted over the time periods measured and reflected the longer half-life of this metabolite. Autoradiographic analysis of spermatozoa obtained from male mice treated with tritiated cocaine revealed strong binding affinity. Scanning electron microscopic analysis revealed concentrated binding to the spermatozoal acrosomal head and flagellated tail. The presence of substantial amounts of cocaine in the epididymis over an extended period of time, as well as evidence of cocaine binding to spermatozoa, suggest potential for preimplantation embryotoxicity.

P13

PERSISTENT HINDLIMB FLEXION IS ASSOCIATED WITH AN EXPERIMENTAL PERIPHERAL MONONEUROPATHY.
B.J. Winterson, S. Hanning, & D.J. Mokler Depts. Physiol. & Pharmacol., Univ. New Eng.Coll.Ost.Med., Biddeford, ME 04005.

Loose ligation of the common sciatic nerve produces an experimental mononeuropathy (EMN), a syndrome of hyperalgesia, trophic changes and "guarding" of the hindlimb. We have been studying the persistent hindlimb flexion induced by prolonged percutaneous electrical stimulation. In the present study, we examined rats with EMN to determine whether they also developed persistent hindhimb flexion.

EMN was produced in Long-Evans rats by the mephod of Bennett and Xie (Pain, 1988) (LIGATE). On days 3,7,10, and 14 rats were anesthetized with pentobarbital and persistent flexion was measured by applying known weights until leg lengths were equal. LIGATEd rats routinely showed flexion on the ligated side (3d: X=6.2g; 7d: X=4.8g; 10d: X=6.4g; 14d: X=7.1g). Sham surgical controls showed little flexion (overall X= 1.1g). To test whether the flexion was due to the hypersensitivity of the foot, the foot was anesthetized with lidocaine using an ankle block and an injection into the foot pad. There was no reduction in flexion (X= 7.1 g). Rats were spinalized at T7 and the flexion remained (X= 6.0 g). The 5-HT_2 agonist DOI reduced flexion in spinalized animals (X= 1.6 g). Finally, in 2 animals dorsal rhizotomy did not produce a decrease in persistant flexion (X= 8 g). The results suggest that the persistent flexion of EMN is a segmentally maintained process that does not depend upon sahsory input from the foot. These features are similar to persistent hindlimb flexion induced by electrical stimulation. Thus, in two chronic pain models, there are similarities suggesting a central origin for, at least, the "guarding" response (postural asymmetry). (Supported by AOA grant 90-14-290.)

P14

VALIDATION OF THE MINI-MENTAL STATE EXAMINATION--A PILOT STUDY.
A. W. Houtz, Ph.D., J. R. Hall, Ph.D., H. G. Micklin, D.O., D. P. Colvin, D.O., P. A. McBride, M.S. Texas College of Osteopathic Medicine, Department of Psychiatry and Human Behavior, Fort Worth, Texas, 76107.

The Mini-Mental State Examination (MMSE) is one of the most widely used instruments for detecting cognitive dysfunction. The MMSE has been used in clinical and research settings and has also been utilized diagnostically although the validity of this screening test remains questionable. The purpose of the present study was to examine the ability of the MMSE to identify cognitive dysfunction and to predict level of performance with a comprehensive neuropsychological battery, the Halstead Reitan Neuropsychological Test Battery (HRNTB).

The MMSE and HRNTB were administered to 36 consecutive, diagnostically mixed, outpatient referrals for neuropsychological evaluation. Correlations between the MMSE and the General Neuropsychological Deficit Score (GNDS) were statistically significant, but modest. Comparison of the MMSE classification of cognitive impairment with the GNDS classification of neuropsychological dysfunction revealed that the MMSE misclassified 94.4 percent of the sample. Correlations between the MMSE severity ratings and the GNDS diagnostic categories were found to be nonsignificant.

The results of this study indicate that the MMSE seriously underestimates the level of cognitive impairment in samples of heterogenous cerebral dysfunction. The results also suggest that the MMSE is unable to detect neuropsychological impairment in patients with moderate cerebral dysfunction. Thus, relying on the MMSE as an indicator of cognitive dysfunction is extremely tenuous and may result in frequent misidentification of organicity.

P15

SEDATIVE-INDUCED ANALGESIA IN A NON-MAMMALIAN VERTEBRATE PAIN MODEL; M.A. Mitchell, Ph.D., C.W. Stevens, Ph.D. and A.J. Klopp, M.S.; Oklahoma State University, College of Osteopathic Medicine, Department of Pharmacology and Physiology, Tulsa, OK 74107

A non-mammalian model for assessment of analgesic efficacy using amphibians has been shown to be sensitive to opioid, alpha-2 adrenergic and non-steroidal anti-inflammatory analgesics. In the present studies, the ability of the sedative drugs, ethanol and the chlorpromazine, to induce analgesia was studied in the common grass frog, *Rana pipiens*. Animals were assessed for changes in the pain threshold following subcutaneous administration of ethanol (1000 to 17300 nmol/g) or chlorpromazine (100 to 1000 nmol/g). Pain thresholds were measured using the acetic acid test in which dilute concentrations of acetic acid were applied dropwise to the dorsum of the hindlimb until a wiping response was observed. To prevent tissue damage, a cut-off time of 5 sec was imposed if no response was observed. Both ethanol and chlorpromazine produced a dose-dependent increase in pain thresholds giving ED_{50} values of 3,416 nmol/g and 214 nmol/g, respectively. While no sedation/motor dysfunction was noted for ethanol treated animals except at the highest dose, chlorpromazine caused frank sedation and loss of righting reflex at all doses. These data are similar to studies in rodents using thermal algesiometric tests (hot plate, tail flick) which showed parallel increases in analgesia and depressed motor response following treatment with ethanol or chlorpromazine. Furthermore, these results provide additional support for the development of an alternative, non-mammalian vertebrate model for analgesic efficacy and pain research. Supported in part by OSU-COM Intramural funds and the Whitehall Foundation (CWS).

P16

A RECEPTOR FOR LAMININ IN THE BRAINS OF POSTNATAL MICE L. Luckenbill-Edds,Ph.D., C. A. Kaiser,B.A., and D. D. Powell,B.S. Ohio University College of Osteopathic Medicine, Department of Biological Sciences, Athens, OH 45701

Laminin, a large glycoprotein of the basement membrane that lines epithelia, muscle fibers, and nerves, promotes the growth of nerve cell processes in vitro. Laminin has also been detected in the brains of developing embryos in situ where it is postulated to promote or guide nerve cell outgrowth. Our research addresses the following questions: Is a **receptor** present in the brain with which laminin might interact during developmental stages? If present, what is the pattern of development of the receptor? To localize the receptor, an antibody to the receptor was raised in rabbits using affinity-purified receptor (M_R = 110kD) from mouse brains (courtesy of Dr. Hynda Kleinman, NIH). This receptor is known to bind a specific sequence of the laminin molecule identified as a neuron growth-promoting sequence. Mouse brains from embryonic and postnatal stages were processed using standard immunocytochemical procedures (Vector immunoperoxidase ABC Kit).

Results show that the receptor is present at postnatal (P) stages (P1,7,8,25,30) in fiber bundles within the brain such as the internal capsule. No staining was detected in embryonic brains. Our results point to a possible role for the 110kD receptor in the growth of fiber bundles postnatally, and raise interesting questions about its role in the regulation of growth and regeneration in adult and aging brains. Supported by AOA Grant #89-08-296.

P17

MUSTARD OIL APPLICATION INCREASES PERSISTING HIND LIMB FLEXION (SPINAL FIXATION) IN RATS. M.M. Patterson, Ph.D., M.J. Bartelt, M.A., T.Jackson, B.S., & E.S. Johnson, B.A. Dept. of Psychology and College of Osteopathic Med., Ohio University, Athens, OH 45701.

Persisting hind limb flexion may be induced by applying approximately 40 mins of (2-4 mA) electrical stimulation to spinalized rats' hind limbs, thus inducing a *spinal fixation* of the reflex alterations (e.g., Steinmetz, Lupica, Secard & Patterson, *Physiol. and Behav, 29*, 1039-1044, 1982). Additionally, Woofe and Wall (*J. Neuroscience, 6*,1433-1442, 1986) reported that increased c-fiber input could increase spinal cord responsiveness for up to 3 hours. The present study examined the effects of mustard oil induced c-fiber activation on the induction of spinal fixation.

We surgically anesthetized (50 mg/kg ip) 20 rats and randomly assigned them to mustard oil or saline groups. We then transected the rats' spinal cords at T7 and placed them in the stimulation apparatus. Twenty min prior to stimulation, we applied the mustard oil to the experimental rats right feet. Immediately following 30 min of 1.5 mA (100pps) stimulation to the right hind limb, we assessed the spinal fixation by measuring the weight needed to remove the right hind limb flexion.

The mustard oil application significantly ($p<.0005$) increased the fixation, from 5 g in the control group to 19 g in the mustard oil group, thus indicating that c-fiber activation enhances fixation. We suggest that the enhancement of fixation by c-fiber activation is mechanistically similar to the sensory and motor changes which result from tissue injury: tissue injury activates c-fibers which in turn enhances the spinal processes which store and maintain excitability alterations. These spinal processes may at least partially modulate the persistence of pain and could be implicated in chronic pain, phantom limb pain and other chronic syndromes. In addition, the rapid induction of somatic dysfunction seen in some cases of strain or sprain may be enhanced by the increased c-fiber inputs. Supported by American Osteopathic Association grants 90-08-319 and 91-08-319.

P18

ENVIRONMENTAL STRESSORS INCREASE PERSISTING HIND LEG FLEXION (SPINAL FIXATION) IN RATS. M.M. Patterson, Ph.D., M.J.Bartelt, M.S., E.S. Johnson, B.A., T. Jackson, B.S. Dept. of Psychology and College of Osteopathic Med., Ohio University, Athens, OH 45701

In 1981 we demonstrated that stimulating a spinalized rat's leg for approximately 40 min induces a flexion which persists for at least 3 days (Steinmetz, Cervenka, Robinson, Romano, & Patterson, *JCPP, 95*, 548-555, 1981). Because this leg flexion fixates in the spinal cord it has been termed *spinal fixation*. The development of fixation following exposure to the stress conditions, forced swimming and noxious noise, was the focus of the present studies.

In the first study, we placed the experimental rats into a water bath and forced them to swim for 8 min. In the second study we exposed the experimental rats to noxious noise (approx 100 db) for 12 hours. Following exposure to either the control or the stress condition, we surgically anesthetized (Nembutal 50 mg/kg ip), and performed spinal transections. Following the 30 min of right hind leg stimulation (3-4 mA, 100pps), we assessed the fixation by measuring the weight required to remove the right leg flexion.

In both studies the environmental stressors greatly increased the fixation ($p<.0005$). The swimming stress group's (n=9) fixation was 36 g compared to their control group's (n=9) of 28 g, while the noise stress group's (n=16) fixation was 38 g compared to their control group's (n=16) of 32 g.

These findings confirm that generalized organismic stressors enhance the induction or severity of spinal fixation. Exposure to stressors increases responsiveness to pain and may also increase long-term spinal excitability. We suggest that these excitability alterations may be induced by somatic and visceral malfunctions and may in turn contribute to the development of chronic pain and other syndromes such as phantom limb pain. In addition, the exposure of an individual to environmental stressors after a somatic dysfunction with its associated facilitated segment has been induced may exacerbate the detrimental effects of the somatic dysfunction and would suggest that following treatment, the patient should avoid stressors for as long as possible to avoid reintroduction of somatic dysfunction symptoms. Supported by American Osteopathic Association grants 90-08-319 and 91-08-319.

P19

EMBRYONIC IMPLANTATION AND D.C. ELECTRIC FIELD STIMULATION AS A METHOD FOR RECONSTRUCTING THE DAMAGED MAMMALIAN VISUAL SYSTEM. M. F. Zanakis,Ph.D., R.M. Cebelenski,B.S., A. Bosak,B.S., P. Jacovina, and B. H. Hallas,Ph.D., New York College of Osteopathic Medicine, Old Westbury, N.Y..

This laboratory have demonstrated that various age embryonic eyes implanted into the host anterior chamber, are capable of growth through a tibial nerve bridge into the host superior colliculus. This "reconstructed visual system" is capable of establishing synapses betwen the implanted ganglion cells and the brain. In other experiments to facilitate growth and regeneration D.C. electric field stimulation of the Central Nervous System, has demonstrated beneficial effects in the restoration of damaged axons. The present study reports reconstruction of the damaged mammalian visual system by combining embryonic implantation and D.C. stimulation. In all experiments embryonic rodent eyes were removed and attached to a 3 cm segment of tibial nerve. The eye with attached nerve was inserted into the anterior eye chamber of an adult rat host while the free nerve end was inserted through a burr hole in the cranium into the contralateral superior colliculus. A 1.5μA constant current D.C. stimulator (Traxon) was applied to the nerve, so that the cathode was oriented distal to the lesion. Control animals received sham (no current) stimulators. After several weeks, animals were intraocularly injected with HRP, or HRP was applied to a cut portion of the tibial bridge and the animal was sacrificed 48 hours later. TMB processing was performed on sections of the eye, nerve bridge and brain followed by histological analysis of the visual system. Preliminary studies show an increased number of HRP labeled axons in the host brain of those animals receiving concurrent DC stimulation in addition to embryonic implantation. These studies indicate that D.C. treatment, coupled with embryonic implantation might be a viable method of reconstructing the damaged mammalian visual system.

P20

D.C. ELECTRIC FIELDS AS A METHOD FOR RESTORING THE DAMAGED RODENT OPTIC NERVE. A. Bosak,B.S., B.H. Hallas,Ph.D., R. M. Cebelenski,B.S., P. Jacovina, M.F. Zanakis,Ph.D., New York College of Osteopathic Medicine, Old Westbury, N.Y. and American BioInterface Corp., New York, N.Y.

Previous studies, performed on the Peripheral (PNS) and Central Nervous System (CNS), have demonstrated that chronic D.C. stimulation of damaged neuronal tissue results in what appears to be either regenerated axons or a "rescuing" of these fibers from degeneration. Which ever mechanism may be operating, the present study was aimed at further characterizing the extent and degree of viability of these axons in the damaged rodent optic nerve model. Adult rat optic nerves were crushed and a 1.5μA constant current D.C. stimulator (Traxon) was applied so that the cathode was oriented distal to the lesion. Control animals received sham (no current) stimulators. After 5 weeks, animals were intraocularly injected with a 20% solution of HRP and sacrificed 48 hours later. TMB processing of the injected eye, optic nerve and brain followed by histological analysis of the visual system revealed that HRP was axonally transported to the brain in "active" stimulator animals, but not in "inactive" (control) stimulator animals. HRP transport could be demonstrated in virtually all animals that were treated, and in none of the animals that were not. These studies lend further support to D.C. treatment of damaged CNS tissues to achieve regeneration or to spare fibers from degeneration. Additional studies are in progress which are aimed at analyzing the underlying mechanisms.

P21

ONTOGENY OF POSITIONAL BEHAVIOR IN MACACA mullata: CHANGES IN BODY SEGMENT MASS DISTRIBUTION WITH AGE. J. P. Wells, Ph.D., WVSOM, Div. of Structural Biology, Lewisburg, WV 24901; J. E. Tournquist, Ph.D., Dept. of Anatomy, Univ. of PR, San Juan, PR 00396.

Changes in body segment proportions i.e. location of segment centers of mass effect the location of the total body center of mass relative to the supporting substrate. Therefore, it is logical to assume that the development of locomotor skills and the selection, by the animals, of appropriate branch angles, diameters, and vegetation types will be inextricably bound to morphology. This study presents data collected from animals from the free ranging population on the island of Cayo Santiago, part of the Carribean Primate Center. Precise ages are known for all specimens. These morphological data will then be used, along with a positional behavioral study currently in process, to determine the changes in locomotion and posture of developing young animals.

Data in this study have shown that both limbs increase relatively less in length than the trunk+head, but more in mass. This finding is most predominant in the hindlimb. Change in length is not the most dramatic finding but rather it is the overall percentage of body weight. This observed weight increase is further linked to a proximal shift in the center of mass. The most likely explanation for this shift relates to increase in muscle mass in the proximal hindlimb.

Thus, within the first eighteen months of life the rhesus monkey body proportions change from having a balance in the fore and hindlimbs to a clearly hindlimb dominant animal. The behavioral study will focus primarily on animals between birth and 18 months of age.

P22

INCORPORATION OF A DISSECTION TABLE VENTILATION SYSTEM INTO THE GROSS ANATOMY LABORATORY. W. D. Martin, Ph.D., J. W. Nemitz, Ph.D., R. M. Fisk, Ph.D., J. P. Wells, Ph.D. WVSOM, Department of Anatomy, Lewisburg, West Virginia, 24901.

A table ventilation system which draws air across the cadaver and away from the table top has been designed to fit the standard Shandon-Lipshaw AN-52 dissection table. The system removes fumes and odors from the cadaver or fluid collected in the dissection table. The system is designed to fit the dissection table with no modification of the table needed. Each "U" shaped unit consists of a pair of hollow collection arms attaching to a collecting manifold at one end. Each arm has an adjustable slot along its length in which the opening can be varied from 1/4 inch to virtually closed to regulate the air flow along the length of the table. The arms and manifold can be raised from the table surface to a height of 1.5 and 3 inches to accommodate larger cadavers. During dissection the manifold is coupled to a separate central ventilation system through a flexible duct which is uncoupled when the laboratory is not in session to allow the table top to be closed. The air is exhausted directly; however, if recirculation of air is necessary a filtering system can be employed to remove chemicals and odors. Furthermore, an individual fan unit and filtering system can be utilized if a central ventilation system cannot be used. Eight hour exposure data for formaldehyde concentrations sampled by detectors positioned 5" and 20" above the table top containing a known volume of 4% formaldehyde under varying air flow velocities will be presented.

Funded by capital improvement funds provided to WVSOM by the West Virginia Board of Trustees.

P23

THE EFFECT OF HIGH LEVELS OF VITAMIN E ON THE PROGRESSION OF ATHEROSCLEROSIS IN THE WATANABE HERITABLE HYPERLIPIDEMIC RABBIT. A.K. Willingham, Ph.D., C. Bolanos, E. Bohannan, and R.J. Cenedella, Ph.D. Department of Biochemistry, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

It has been demonstrated that probucol, a hypocholesterolemic drug, additionally acts as an antioxidant to prevent the progression of atherosclerosis in the Watanabe rabbit. Since vitamin E is a naturally occurring antioxidant, we tested its effect on the progression of atherosclerosis in these rabbits. Watanabe rabbits (5-6 mos of age) were divided into two groups (7 each). One group was fed standard Purina Rabbit Chow and the second was fed the same diet supplemented with Vitamin E at 2000 mg/kg diet. (Purina Rabbit Chow contains about 30 mg Vitamin E/kg diet). After 9 mos. the rabbits were sacrificed and plasma lipids were determined and the extent of plaque formation in the aortas was measured.

The extent of plaque formation (% of the total surface covered with fatty streaks) was not significantly different in the two groups. Aortic arch, 70%; thoracic aorta, 30%; abdominal aorta; 30%. However, there were significant differences observed in plasma lipids. Total cholesterol, LDL cholesterol and triglycerides were lower in the Vitamin E fed group. The mean values (mg/dl) $\pm$ SEM were: total cholesterol, control = 328 $\pm$ 14, vitamin E = 267 $\pm$ 30; LDL cholesterol, control = 223 $\pm$ 15, vitamin E = 167 $\pm$ 23; and triglycerides, control = 564 $\pm$ 111, vitamin E = 380 $\pm$ 47. These results indicate that high levels of Vitamin E may at least have a beneficial effect on lowering plasma lipids observed in hyperlipidemia. Supported by F. Herbert Fields Grant and KCOM Warner Fund.

P24

COMPARISON OF GENOMIC DNA HYBRIDIZATION ASSAYS FOR THE IDENTIFICATION OF *ACINETOBACTER SP.* IN CLINICAL ISOLATES. J. B. Oliver, B.S., J.D. Jollick, Ph.D., M.C. Modrzakowski, Ph.D., Ohio University C. O. M. Athens, Ohio 45701.

DNA homology is a useful discriminating test for the identification of *Acinetobacter sp.* (glucose nonfermenting, gram negative bacteria). Four genomic DNA probes were tested for the identification of the following DNA homology groups: 1) the *Acinetobacter calcoaceticus - Acinetobacter baumannii* complex, 2) *Acinetobacter haemolyticus*, 3) *Acinetobacter lwoffii*, and 4) *Acinetobacter johnsonii*.

Two methods of preparing clinical bacterial isolates as DNA targets for the hybridization assay were compared. Bacterial strains putatively identified as *Acinetobacter sp.* as well as a variety of positive and negative controls were used to prepare targets quantitatively and qualitatively.

Either method was useful for the detection of *Acinetobacter sp.* from among negative controls (100% sensitivity, 87.5% specificity). Only the assays using quantitatively prepared DNA targets were able to show significant differences between the four DNA homology groups. The qualitative method showed significant differences only in the *Acinetobacter calcoaceticus-Acinetobacter baumanii* complex group.

Genomic hybridization assays are useful for the detection of *Acinetobacter sp.* in clinical isolates. Identification of *Acinetobacter* DNA homology groups requires quantitatively prepared targets. Development of more specific DNA probes should allow the use of qualitatively prepared targets in a clinical setting.

P25

POLY(ADP-RIBOSE) POLYMERASE ACTIVITY- ASSOCIATION WITH RENAL INVOLVEMENT IN SLE. R.M. Pertusi, D.O., H.Y. Chen, M.D., M.S., B.R. Rubin, D.O., E.L. Jacobson, Ph.D. Texas College of Osteopathic Medicine, University of North Texas, Ft. Worth, TX 76107.

Poly (ADP-ribose) polymerase is a nuclear enzyme which is activated by DNA strand breaks and is believed to function in the maintenance of chromatin structure during DNA repair. Assays of poly (ADP-ribose) polymerase activity in lymphocytes from SLE patients showed a 70% decrease in activity (Sibley, et al. Arthritis and Rheumatism 32, 1045-1049, 1989 and Pertusi, et al. Arthritis and Rheumatism 34 (5):R5, 1991). We have initiated studies to determine an association between decreased poly (ADP-ribose) polymerase activity in peripheral blood lymphocytes of patients with SLE and altered renal function. We studied 13 patients fulfilling 4 or more ARA (1982) revised criteria for SLE. SLE patients were considered to have altered renal function if they fulfilled criteria #7 of the ARA (1982) revised criteria for SLE (persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not performed OR cellular casts--may be red cell, hemoglobin, granular, tubular, or mixed). Polymerase activities ranged from 10-220 units. 83% (5/6) of patients with activities less than or equal to 75 units had renal involvement. 14% (1/7) of patients with with activities greater than or equal to 76 units had renal involvement. This data suggests a trend toward lower polymerase activities in SLE patients with renal disease.

P26

QUANTITATIVE ASSESSMENT OF BOWEL VIABILITY
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Intraoperative assessment of ischemic canine small bowel using Doppler US,fluoroscein index and Electronic Contractility Meter(ECM) generated EMG & Threshold Stimulus Level(TSL)(mA) is being investigated at RWJ University Hospital.This abstract addresses the utility of the ECM,a novel strain gauge probe applied to the serosa of intestine,which can measure surface EMG & impedance signals and deliver a TSL needed to produce a bowel contraction.

Thirty-three mongrel dogs underwent operative laparotomy where a 40 cm. length of distal ileum was marked and its mesenteric blood supply ligated. An 8 cm. central area of this segment was futher devascularized by Marginal artery ligation. Relaparotomy is performed in 24 hours.TSL data is used to determine cut points for resection & reanastomosis of ischemic but theoretically viable bowel margins.Dogs surviving 12 days=survivors,less than 12 days=deaths.There were 23 survivors and 6 deaths 4 dogs were eliminated from the study.Mean histologic grading of multiple sections of resection margins was performed assigning values of grades A(normal histology),B(ischemic changes extend to lamina propria) both viable,and grades C(only serosa intact),D(transmural infarct) both nonviable. Chi-Square analysis was performed comparing viable and nonviable margins in survivors & deaths, $X^2=10.3$, $p < .005$,statistically significant,suggesting that the ECM TSL data can successfully predict viable cut points in ischemic canine small intestine.

Sponsors:University of Medicine and Dentistry of New Jersey Student Summer Research Fellowship and R.E.Brolin,M.D.,Dept.Surgery,RWJ University Hospital

P27

INDUCTION OF LOW T_3 SYNDROME IN EXERCISING WOMEN DOES NOT ELEVATE BASAL CORTISOL LEVELS. T.D. Law, B.S., A.B. Loucks, Ph.D., E.M. Heath, Ph.D., J.R. Watts, B.S., & H.L. Brient, B.S. College of Osteopathic Medicine, Department of Biological Sciences, Ohio Univ., Athens, OH 45701.

Adrenal activation during intense exercise has been hypothesized to disrupt reproductive function in women, and both Low-T_3 Syndrome and mild hypercortisolism have been observed repeatedly in amenorrheic athletes. Therefore, we performed a randomized prospective cohort experiment to determine the effect of severe energy deficiency on basal cortisol levels in intensely exercising women. Twenty-nine untrained, regularly menstruating women all performed 5.4 MJ/day of supervised ergometer exercise at 70% of aerobic capacity for four days in the early follicular phase of the menstrual cycle. A clinical dietary product (Ensure™, Ross Labs) was used to control energy availability (dietary energy intake minus exercise energy expenditure). Fourteen subjects received less than the threshold of energy required to prevent Low T_3 Syndrome. For nine days including three days before treatments, blood was sampled once daily at rest at 8am. Initially, T_3, fT_3, and cortisol were in the normal range for all subjects. Repeated measures two-way ANOVA (Time X Energy) revealed that energy deficiency in the intensely exercising women suppressed levels of T_3 (by 17%, $p<0.01$) and fT_3 (by 8%, $p<0.05$) in the women receiving the sub-threshold diets, but did not elevate their cortisol levels ($p=0.11$, one-sided) either absolutely or in comparison to the women receiving super-threshold diets. This experiment had statistical power for more than 99% likelihood of detecting a shift of two standard deviations in cortisol levels beyond the normal range, if such a shift had occurred. Thus, to the extent that our experimental protocol is a sufficient test, neither the so-called "stress" nor the energy cost of exercise appears to elevate resting 8am cortisol levels. The hypercortisolism observed in amenorrheic athletes may not be related to their dietary and exercise regimen. (Supported by AOA, OU, and Ross Labs.)

P28

DETECTION OF HELA CELL RECEPTORS FOR COXSACKIEVIRUS B3 BY A MODIFIED WESTERN BLOT ANALYSIS

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Coxsackieviruses are established causitive agents of acute and persistent myo/pericarditis in man. These and other viruses are reported as responsible for myopathies necessitating up to 8% of heart transplants. Coxsackievirus B3 (CB3), the primary cause of viral myo/pericarditis, is amenable to study at the organismal level due to the availability of murine model systems, biological infectivity assays, and genomic cDNA and probe assays. However, insufficient knowledge has been obtained regarding pathogenesis at the cellular level of attachment and penetration to be able to understand early infection events and viral tropism.

The purpose of this study continues to be to establish methods and to identify CB3 cellular receptors. The approach taken herein was to iodinate purified CB3 surface proteins and to permit interaction of labeled CB3 with SDS-PAGE-resolved HeLa S3 membranes and other cell fractions in a modified western blot system. CB3, propagated in BGM cells, was collected by polyethylene glycol precipitation and purified by differential centrifugation and two cycles of cesium chloride isopycnic banding. Purified CB3 was iodinated using Pierce IODO-GEN to a specific activity of 1.1×10^5 cpm/ug protein. Samples (67 ug) were incubated in phosphate buffered saline with SDS-PAGE-resolved preparations of whole cell, nuclei and membrane proteins derived from cells by an osmolytic method and blotted onto Sure-blot nylon membranes. Following washings, autoradiograms of the modified western blots revealed virus adsorption bands in both membrane and nuclei fractions.

The M_r of these bands were approximately 300,000, 105,000, and 21,500 daltons and the latter two bands appeared in both nuclei and membrane fractions. No banding was observed in disrupted whole cell fractions. American Heart Assn. Ia-G15

P29

CYTOTOXIC EFFECTS OF CADMIUM AND CADMIUM-METALLOTHIONEIN IN LLC-PK_1CELLS. Dawn R. Wellington, Peter L. Lamar and Walter C. Prozialeck., Chicago College of Osteopathic Medicine, Downers Grove, IL 60515.

Although cadmium is known to cause severe damage to the epithelium of the proximal tubule, the mechanisms underlying this effect have yet to be elucidated. Recently, Prozialeck, et al. showed that ionic cadmium (Cd^{2+}) selectively damages the junctions between cells of the porcine renal epithelial cell line, LLC-PK_1 (*Toxicol. Appl. Pharmacol. 107*:81-97, 1991). The objective of the present studies was to determine whether or not cadmium that is bound to metallothionein (a low molecular weight metal binding protein) might cause similar damage to the junctions between LLC-PK_1 cells. This is an important issue because much of the circulating cadmium *in vivo* is bound to metallothionein.

Confluent LLC-PK_1 cells on Falcon Cell Culture Inserts were exposed to Cd^{2+} or a Cd-metallothionein complex (Cd-Mt) from either the apical or the basolateral compartments for up to 48 hours. The integrity of cell junctions was assessed by monitoring the transepithelial electrical resistance (TER), and the viability of the cells was evaluated by monitoring the release of lactate dehydrogenase into the medium. The results showed that exposure to Cd^{2+} from the basolateral compartment for 2-4 hours caused a pronounced decrease in TER, without affecting cell viability. By contrast, exposure to Cd^{2+} from the apical compartment, or Cd-Mt from either compartment, had little effect on TER, until the cells began to die, which usually occurred after 8-24 hours of exposure. These results indicate that Cd^{2+} selectively damages the junctions between LLC-PK_1 cells by acting at a site on the basolateral cell surface. By contrast, Cd-Mt has no specific effects on the junctions between LLC-PK_1 cells. (Supported by NIH Grant ES05656).

P30

EFFECTS OF L-ORNITHINE, PUTRESCINE AND SPERMIDINE ON DEVELOPMENT OF MOUSE EGGS OBTAINED AFTER INDUCTION OF OVULATION OR AFTER NATURAL MATING. E.M. Brandon, B.A. and L.J. Van Winkle, Ph.D., Chicago College of Osteopathic Medicine, Department of Biochemistry, Downers Grove, IL 60615.

Polyamines are needed for cell proliferation (Heby, 1981) and it is probably through this mechanism that early development is influenced. Inhibitors of polyamine synthesis have detrimental effects on preimplantation mouse embryos *in vitro* (Alexander, 1978, 1979) and recently reported is the positive effect of diamine putrescine on *in vitro* mouse egg development (Sawicki et al., 1991). We reinvestigated the developmental effects of putrescine, as well as the effects of its precursor L-ornithine and its product spermidine.

Eggs from ICR and CF1 mouse strains were tested, each strain subdivided into one group that had been naturally mated, and one in which gonadotropins were used to induce ovulation and mating. These four groups were treated with 1 μM-1000 μM solutions of L-ornithine, putrescine, and spermidine.

Results showed that eggs from ICR but not CF1 mice developed more frequently into 4-cell conceptuses in culture when the eggs had been obtained from mice that had mated naturally vs. those that had been gonadotropin-treated. One-100 μM putrescine, L-ornithine, or spermidine did not improve development of eggs, and 1000 μM of each of these substances reduced the frequency of development of eggs to blastocysts.

We concluded that gonadotropin-treatment can be detrimental to *in vitro* development in some stains of mice, and that 1000 μM concentrations of the polyamines are inhibitory to development also. We also concluded that 1-100 μM putrescine is not necessarily beneficial to development for all strains of mice under all conditions of culture.

P31

PRELIMINARY RESULTS OF INTERVENTION VS. USUAL CARE FOR SMOKING CESSATION OF INPATIENTS

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ABSTRACT

Cigarette smoking is the most important preventable cause of illness and death in the United States, responsible for 390,000 or one sixth of all deaths. 90% of smokers want to quit but are unable to do so.

The objectives of this study were two-fold:

- Are brief interventions by a physician effective?
- Can the positive effect be sustained for six months?

We randomly divided all smoking patients admitted to the inpatient service during a three month period into an intervention group (n=25) or usual care group (n=24). The intervention group was given an average of 10.4 minutes of counseling by a family physician during their admission. All patients were then followed at three days, one month, and six months after discharge to evaluate the effectiveness of the intervention.

The intervention group was noticeably more successful in smoking cessation than the usual care group at all follow-up visits.

In conclusion, severity of the patient's medical condition increases their desire to quit smoking. However, without a workable plan, motivation alone is ineffective. Spending a minimal amount of time in counseling and providing the patient with a plan is highly successful in smoking cessation. Therefore, it only makes sense that each family physician should make it a priority to provide their smoking inpatients with the opportunity to quit.

P32

PLASTINATION OF HUMAN ANATOMICAL SPECIMENS T. N. Kvist, Ph.D., Philadelphia College of Osteopathic Medicine, Department of Anatomy, Philadelphia, Pennsylvania, 19131.

The purpose of this study is to employ a new plastination technique to prepare permanent, dry anatomical specimens and body sections for teaching purposes. Anatomists have long sought such a technique for preserving soft tissues to avoid having to use specimens stored in fixative solutions that must be kept wet and become brittle with time. The plastination procedure uses silicone rubber, epoxy resin and polyester copolymer to preserve previously perishable biological material in a permanent, dry and odorless life-like state. Prosected specimens plastinated with silicone rubber are resilient, flexible, and durable on handling and make excellent teaching/museum specimens. Tissue sections impregnated with polyester copolymer and epoxy resin make high quality thick, opaque body slices and transparent sections respectively because of their hardness and refractive indices.

Embalmed specimens prosected to show topographical anatomical relationships have been dehydrated in a series of cold acetone baths at -25° C (to prevent shrinkage), impregnated with a silicone rubber mixture (patented and available from von Hagens, West Germany) at -25° C under vacuum at a pressure of 2 mm of Hg, warmed to room temperature and gas vapor cured (hardened) in a desiccator. The topographical anatomical relationships of these plastinated specimens will then be correlated with cadaver dissection and with radiographs and angiograms provided by the Radiology Department. In addition, thick and thin plastinated body slices will be used to study and correlate topography with CT scans and MRI images posted in the gross lab as each region of the cadaver is dissected.

Considerable student time is now spent manually dissecting cadavers, that could be used more profitably in learning cross-sectional anatomy, a subject so important in understanding diagnostic CT scans and MRI images. Plastinated tissue sections closely resemble diagnostic CT scans and MRI images and are therefore of significant educational and clinical relevance. Prosected plastinated specimens would then negate the need to dissect. Several dry, plastinated anatomical specimens are on display.

Supported by a FOCUS grant from the American Association of Colleges of Osteopathic Medicine.

P33

REASONS FOR CHOICES OF GRADUATES PRACTICING OUT OF STATE

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The West Virginia School of Osteopathic Medicine has, as one objective, the goal of supplying osteopathic physicians for West Virginia. A recent report shows that the College was the leading provider of primary care physicians practicing in rural Appalachia and West Virginia during the study period. However, faculty and administration remain concerned about the graduates who leave the state.

To determine why some graduates left the state, a survey was done of 406 alumni who completed postdoctoral training and are in practice outside West Virginia. Data from 190 responses (46.8%) are reported here.

Respondents were asked to indicate relevance of 25 items to choice of current practice location, marking all that applied. Most frequently marked were: fiscal promise (46.3%), specialty practice opportunity (43.1%), solo or group practice opportunity (42.1%), good place to raise children (41.6%), sports/recreation opportunities (37.9%), did internship/residency in the area (34%), family live near there (32%), and cultural atmosphere (32%).

Responses regarding incentives which would make graduates return to the state showed a similar mix of practice, family, lifestyle, and economic considerations, including medicare and tort reform. Most of the factors influencing these decisions are beyond the control of the medical school, and must be addressed by society at large.

P34

THE KNOWLEDGE BASE OF MATURE OSTEOPATHIC PHYSICIANS

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The purpose of this study was to explore the pattern of the changes over time in the knowledge base of practicing physicians.

The measurement instrument used by this study was the National Osteopathic Medical Examination (NOMEX) developed by the National Board of Osteopathic Medical Examiners, Inc. (NBOME) for the American Osteopathic Association (AOA) as a certification examination for the experienced practitioners. The NOMEX was piloted to 145 voluntary osteopathic physicians during the 1990 AOA Annual Meeting. The items were dichotomized as the fact-recalling items (FR) or the clinical application items (CA). Based on the posterior performance, participants were divided into 5 groups according to the number of years of practice: 1-6 years (Group 1), 7-12 years (Group 2), 13-20 years (Group 3), 21-30 years (Group 4), and more than 30 years (Groups 5). The one-parameter Item Response Theory model was used to construct three measurement scales: FR, CA and total exam. Regression models with polytomous categorical variables were established to test the group mean differences.

The results indicated that physicians' performances on all three scales declined monotonously after 12 years of practice. Except the differences between the Group 1 and 2 were not significant, all other pair-wide group comparisons were significant, all favoring the groups with fewer years of practice. The study also found that the time effect had stronger influences on CA performances than on FR performances. (This study does not necessarily reflect NBOME's position.)

P35

IN VITRO EFFICACY OF BETADINE® MICROBICIDES AGAINST CLOSTRIDIUM DIFFICILE(CD) AND ACINETOBACTER CALCOACETICUS (AC). C.S.J. Chelle, M.S., R. P. Grandy, M.S. The Purdue Frederick Company Medical Department, Norwalk, CT 06856

CD and AC have become increasingly common causes of serious nosocomial infections with reports of death in some cases. Because Betadine® has demonstrated effective microbicidal activity against many bacteria and viruses, we measured the critical kill times (CKT) of Betadine® solution (BS) full strength (10% PVP-I), a 1:10 dilution of BS (1% PVP-I) and Betadine® Surgical Scrub (BSS) 50% (use dilution) against CD and AC. The active ingredient in Betadine® Microbicide is polyvinylpyrrolidone (PVP-I). The following CKT were recorded.

In Vitro Killing Time (CKT)

Organism	BS (10%PVP-I)	BS (1% PVP-I)	BSS use dilution)
CD spores[1]	5 min*	< 5 min	< 5 min
CD veg cells[2]	NT	< 30 sec	NT
AC[3]	< 15 sec	< 15 sec	NT

[1] C. difficile, high spore content challenge dose.
[2] C. difficile, high vegetative cell challenge dose.
[3] Acinetobacter calcoaceticus
* A few spores survived to one hour but not longer.
NT = Not tested

We conclude from these in vitro tests that BS full strength and a 1:10 use dilution and BSS at use dilution are effective topical antimicrobial agents against both CD spores and vegetative cells, and AC.

P36

IN VITRO EFFICACY OF BETADINE® SOLUTION AND BETADINE® CREAM AGAINST METHICILLIN RESISTANT STAPH AUREUS (MRSA). C.S.J. Chelle, M.S., R.P. Grandy, M.S. The Purdue Frederick Company Medical Department, Norwalk, CT 06856

MRSA, often found in culture(cx) isolates from wounds, decubiti, and catheter sites, is a frequent cause of nosocomial infections. The rate of kill of Betadine® Solution (BS, a 10 % polyvinylpyrrolidone iodine (PVP-I Solution), Betadine® Cream (BC), a 5 % PVP-I cream, and saline as a control were determined against 5 different hospital isolates of MRSA and one strain of Methicillin Sensitive Staph Aureus (MSSA). Each test agent (BS, BC, saline) was inoculated with 0.5 ml of a 24 hour culture of MRSA or MSSA containing 10^6 - 10^7 organisms and vortexed. Samples (0.1 ml) of the suspension were removed at 30 sec, 1, 2, 3, 5, 10, and 15 minute intervals and transferred to trypticase soy broth containing 0.1% sodium thiosulfate. The following in vitro killing times were recorded.

Organism	Betadine® Cream	Betadine® Sol.	Saline
MSSA	2 min	< 30 sec	no kill
MRSA #1	30 sec	< 30 sec	no kill
MRSA #2	3 min	< 30 sec	no kill
MRSA #3	30 sec	< 30 sec	no kill
MRSA #4	30 sec	< 30 sec	no kill
MRSA #5	30 sec	< 30 sec	no kill

We conclude from these in vitro data that BS and BC are effective topical antimicrobial agents for treatment of MRSA and MSSA. Effective topical treatment with BS or BC of wounds, decubiti, or catheter sites colonized or infected with MRSA or MSSA will help control local infection and many minimize the risk of development of more serious systemic infections.

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THE EFFECT OF BETADINE CREAM VS. SILVADENE CREAM ON RE-EPITHELIALIZATION IN UNINFECTED EXPERIMENTAL WOUNDS,

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Povidone-Iodine, a broad spectrum topical antiseptic, has been used extensively during the past 30 years for many clinical indications including the prevention and treatment of wound infections. The objective of this study was to determine if 5% povidone-iodine cream [Betadine Cream, 5% (BC)] and silver sulfadiazine cream 1% [Silvadene cream, 1% (SC)] influenced the rate of healing, re-epithelialization and irritancy in an uninfected experimental wound model.

Uniform clinical blisters were produced with 50% NH_3OH on the volar aspect of the forearm in 25 normal volunteers and unroofed after 15 minutes. The wound produced is equivalent to a superficial abrasion or a second degree burn. The following treatments were assigned to wound sites: BC, SC, BC vehicle, and no treatment. Wounds were evaluated daily for percent re-epithelialization, percent crusting and presence or absence of infection and irritation.

BC required significantly ($p \leq .01$) fewer days (12.1) for 100% re-epithelialization compared to SC (16.1) and no treatment (15.8). On treatment day 7, the BC sites had a significantly ($p \leq .01$) greater percentage (68%) of wound covered by epithelium compared to no treatment (29%). Results of pairwise comparisons for crusting showed BC had significantly ($p \leq .01$) less crusting compared to no treatment. Irritation was observed on approximately 50% of wound sites for each treatment. There were no reports of infection or other adverse experiences.

Betadine was statistically superior to Silvadene Cream with regard to healing of experimental wounds.

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LOMEFLOXACIN ONCE DAILY THERAPY FOR URINARY TRACT INFECTIONS (UTI) IN THE ELDERLY PATIENTS. D. Ginsberg, D.O.; M.E. Kuss; B. Roniker, M.D. Harleyville Medical Associates, 176 Main Street, Harleyville, Pennsylvania 19438.

Several multicenter clinical studies were undertaken to evaluate the efficacy of lomefloxacin once daily vs norfloxacin, ciprofloxacin or trimethoprim/sulfamethoxazole (TMP/SMX) given twice daily for treatment of complicated or uncomplicated UTI. Therapy for uncomplicated infection lasted up to 10 days and for complicated infection up to 14 days. Approximately 40% of patients treated were over 65 years of age upon entry into these trials. At entry, patients needed to present with signs and symptoms of UTI such as frequency, urgency, dysuria, hematuria, CVA tenderness along with a positive midstream urine culture ($\geq 10^5$ CFU/ml) to be evaluated. Bacteriological and clinical success were evaluated 5-9 days post-treatment. Bacteriological response of lomefloxacin once daily vs. ciprofloxacin for complicated UTI was 95% vs. 94% respectively (n>280); lomefloxacin vs. TMP/SMX was 100% vs. 81%; lomefloxacin vs. norfloxacin was 91% vs. 85%. The added simplicity of once daily lomefloxacin therapy may aid in the patient response to therapy observed.

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EFFICACY OF LOMEFLOXACIN IN TREATING ACUTE BACTERIAL EXACERBATION OF CHRONIC BRONCHITIS (ABECB).

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Several comparative studies were conducted to evaluate once-daily lomefloxacin vs. amoxicillin 500 mg tid or cefaclor 250 mg tid in ABECB; a total of approximately 1,800 patients participated in these studies. Efficacy was evaluated after 10 days' therapy and included amelioration of signs and symptoms of the acute episode as well as eradication of the baseline pathogen from the sputum. Approximately 30% of these patients were over 65 years of age, and 1/3 admitted to being active cigarette smokers at entry. Among the elderly, lomefloxacin eradicated the baseline pathogen in 92% of patients while 79% of the patients treated with either amoxicillin or cefaclor responded. A total of 40% of all patients tested were taking a theophylline product upon entry; lomefloxacin does not interact with theophylline. Quantitative and qualitative assessment of adverse events were similar between drug regimens and age of patients. Once daily therapy of lomefloxacin was more effective than the comparative agents in eradicating the pathogen from the sputum and in aiding in resolving signs and symptoms of ABECB.