Abstracts from the 88th Annual Technical Meeting of the Oklahoma Academy of Science Oklahoma City University November 13, 1999

The following nine abstracts of papers presented at the 88th meeting of the Academy were scheduled to be published in Volume 80 of the Proceedings. They were inadvertently omitted, and therefore are published here. The editors apologize profusely to the authors and readers for this omission.

THE BUBBLE STRIP METHOD FOR MEASURING DISSOLVED HYDROGEN CONCENTRATION IN WELL WATER: A COMPARISON OF TWO TYPES OF BUBBLE STRIPPING CELLS. **D.M. McInnes,[1] D. Kampbell.[2]** [1]Man Tech Environmental Technology, Inc., 919 Kerr Research Drive, Ada, OK 74820, Department of Chemistry, East Central University, Ada, OK 74820. 2U.S. EPA, Office of Research and Development, National Risk Management Laboratory, Subsurface Protection and Remediation Division, 919 Kerr Research Drive, Ada, OK 74820.

The Bubble Strip Method was developed for determining concentrations of dissolved H_2 in ground water. This information can aid in assessing the viability of employing the strategy of monitored natural attenuation (MNA) to restore sites contaminated with chlorinated hydrocarbons. The method was applied by pumping well water into a sample cell, and then injecting 20 mL of air to produce a head space. Well water was then pumped through the cell, causing dissolved H_2 to partition between the two phases. The head space was then analyzed by gas chromatography, and $[H_2]$ in solution was calculated using Henry's Law. Two sample cell designs were compared, the Microseeps Cell and the Chapelle Cell. Kinetics of equilibration studies were conducted with each cell, employing various solution flow rates at both 4°C and 21°C. The Microseeps Cell compared favorably in this regard, with the added benefit of costing significantly less. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

PRODUCTION OF NATURAL CAFFEINE FROM MIXED PLANT CELL CULTURES: *COFFEA ARABICA* (COFFEE) AND *PAULLINIA CUPANA* (GUARANA). **Gianna N. Bell-Eunice and George R. Waller.** Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078.

Caffeine (1,3,7-trimethylxanthine) is a natural occurring compound found in a variety of plants as: *Coffea* (coffee), *Paullinia* (guaraná), *Ilex* (maté), *Camellia* (tea), Cola, and Theobroma (cacao). Caffeine has been long known for its major role in the popularity of coffee, tea, and soft drinks. Studies on caffeine from *Coffea arabica* and *Camellia sinesis* provide an almost complete understanding of the compounds involved in the caffeine metabolic pathway, showing theobromine as the immediate precursor of caffeine and theophylline being the first product formed in the biodegradation pathway. The qualitative analysis of guaran· tissue culture showed predominately the production of theobromine, while the analysis of coffee showed the production of caffeine. A mixed plant cell culture from *Coffea arabica* and *Paullinia cupana* was established, with caffeine and theobromine being analyzed by GC using a DB-35MS column. A marked increase in the caffeine as well as a decrease in the theobromine was found in the mixed cell culture when compared to the coffee and guaran· controls. The physical characteristics of the mixed cell culture show brittleness and differences in growth rates that can be assigned to the effect that each type of cell has on the other. From the results we concluded that the theobromine was partially synthesized by the *Paullinia cupana* and *Coffea arabica* cells and was enzymatically converted to natural caffeine in the mixed culture. This provides a new method of increasing the production of this alkaloid by cell and tissue culture.

THE UNSEEN DANGER: *NAEGLERIA FOWLERI* IN THE WATERS OF TAYLOR FERRY.
Marsha J. Howard, Eric G. Harp, R. David Lacey, Bonnie L. Riportella and David T. John. Basic Sciences, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107.

Naegleria fowleri is a pathogenic free-living ameba that causes a rapidly fatal central nervous system infection. The disease has been reported worldwide but never in Oklahoma before August 1998, when a 3-year-old girl became infected while playing in the water at Taylor Ferry, and subsequently died. This study was to sample water at the site during the public use season, to determine the levels of *N. fowleri*, and to identify any conditions that coincided with the levels. 18 collections were made May-Sept. 1999 (~4/month). Ten 50 mL water samples were taken at each visit (181 total). Samples were returned to the lab, centrifuged, decanted, and the pellets transferred to *E. coli* coated agar plates. Plates were incubated at 42°C and observed for ameba plaques. Each plaque was considered a separate isolate and amebae were subcultured to broth medium. 970 isolates were obtained from the 181 samples collected. As ameba numbers reached 10^4-10^5/mL, mice were inoculated intranasaly to determine pathogenicity. 160 isolates have been inoculated into mice to date, of which 12 caused disease. Of the 12, 4 were May samples, 7 June, and 1 September. These months had lower water temperatures (av 27 vs. 31°C), lower pH (av 7.7 vs. 8.5), and higher rainfall (av 7.2" vs. 0.3") than July and August.

TAURINE MODULATION OF SYNAPTOSOMAL [^{3}H] DOPAMINE UPTAKE IN RAT STRIATUM.
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Male Sprague-Dawley rats (4-months old; N=3) were sacrificed and both the left and right striata were removed on ice. Synaptosomes were obtained following homogenization in Tris-HCl-buffered 0.32M sucrose (pH=7.4) using a glass-teflon homogenizer. The synaptosomal fraction was resuspended in oxygenated (95% O_2/5% CO_2) buffer containing (mM); NaCl (126), KCl (4.8), $CaCl_2$ (1.3), $MgSO_4$ (1.4), sodium phosphate (16), glucose (6) modified to pH = 7.4. Pargyline (100µM) prevented oxidation by monoamine oxidase. Nonspecific uptake was determined by 1 µM mazindol. Synaptosomes were incubated for 3 minutes at 30°C in the presence of 10nM [^{3}H] dopamine, and either 0 (control), 1, 10, or 20mM taurine. Filtration and a 15-second wash with ice-cold 25mM Tris-HCl terminated the reaction. To examine the effect of taurine on the Km and Vmax of the DAT, synaptosomes were incubated in the presence of either 0 (control), 1, 10, or 20mM taurine and 8 concentrations of [3H] dopamine (10-500nM). Taurine inhibited the uptake of dopamine at physiological concentrations (10mM) and kinetic analysis revealed that this effect was due to an increase in the Km of the transporter for [^{3}H] dopamine. These results suggest that taurine, at phsyiological concentrations, is a modulator of the dopamine transporter. In addition to other modulatory functions, taurine can also influence dopaminergic function through changes in receptor number and transporter function. (Supported by OSU-COM intramural funds.)

EFFECTS OF LPS PRIMING ON HUMAN NEUTROPHIL'S RESPONSES TO BACTERIAL CHEMOTACTANT.
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Chemotaxis is the direct movement of phagocytic cells toward the site of invading organisms in response to chemotactic factors. The most important chemotactic factors are the products of the complement cascade, but selected host cell products and bacterial cell components may also function as chemotactic factors. The purpose of this study was to exam-

ine the chemotactic response of human neutrophils to the bacterial chemotactant f-MLP after exposure to lipopolysaccharides (LPS). The LPS used in this study were extracted from batches of *Pseudomonas aeruginosa* PA01 grown with different carbon sources. Fresh samples of neutrophils were incubated with various LPS and then placed into wells on agarose plates. These plates were incubated at 37°C at 5% CO_2 for 90 minutes, and then visualized with a Wright stain. The neutrophil chemotactic response was determined by measuring the area of neutrophil migration as compared to the control. Our results indicated that LPS inhibited chemotaxis from 40-67%, depending on the carbon source of the LPS. In summary, LPS may be a significant virulence factor that enables *P. aeruginosa* to inhibit the immune response, and thereby facilitates recurrent infections such as those associated with cystic fibrosis.

BIOCHEMICAL ALTERATIONS IN A METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ADAPTED TO VANCOMYCIN RESISTANCE. **Marvita D. McGuire and Robert S. Conrad.** Department of Biochemistry and Microbiology, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107.

Methicillin resistant *Staphylococcus aureus* (MRSA) is emerging as a devastating hospital acquired infection. Vancomycin, a glycopeptide cell wall inhibitor, is the most effective antibiotic available against this organism. Recent reports of Vancomycin Intermediate *Staphylococcus aureus* (VISA) clinical isolates have renewed concerns of the continuing efficacy of vancomycin. A clinical isolate of MRSA was cultured in cation supplemented Mueller-Hinton broth (CSMH) in the presence of vancomycin. The clinical isolate was step-wise adapted to grow in the presence of 30μg/mL of vancomycin as determined by the minimum inhibitory concentration (MIC). The vancomycin resistant *S. aureus* (VRSA) subsequently lost resistance to β-lactam antimicrobials as well as the ability to secrete β-lactamase. Additionally, the VRSA lost the ability to secrete coagulase, but not catalase. VRSA was no longer capable of fermenting lactose and trehalose as compared to the original isolate, however continued to ferment glucose. Membrane proteins were extracted and compared to the MRSA parent by SDS-PAGE. Those studies have indicated an overexpression of proteins presumed to be Penicillin Binding Proteins. VRSA was found to be highly resistant to lysostaphin, and grows at a slower rate than the original MRSA isolate.

CHARACTERIZATION OF THE AMEBA ISOLATED FROM THE FIRST CONFIRMED CASE OF PRIMARY AMEBIC MENINGOENCEPHALITIS IN OKLAHOMA. **Eric G. Harp, Marsha J. Howard and David T. John.** Department of Biochemistry and Microbiology, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107.

Primary amebic meningoencephalitis (PAM) is a fatal brain disease caused by the free-living ameboflagellate, *Naegleria fowleri*. The first confirmed case of PAM in Oklahoma occurred in August of 1998. The victim was a 3-year-old girl who became infected while playing in the water at Taylor Ferry on Fort Gibson Lake. A diagnosis was made by histologic identification of amebae in brain tissue and by cultivation of amebae in axenic medium. The isolate was designated HBT-1. Amebae were elongate or ëlimaxí in shape, from the Latin word meaning slug, and moved in a directional manner by eruptive, blunt pseudopodia called lobopodia. When amebae were suspended in distilled water, they transformed into temporary biflagellate forms. Cysts were spherical with a smooth surface. Growth was evaluated in 3 axenic media, Balamuth's, Nelson's and Mix, at 37°C, with Mix medium producing maximum growth of 1.1×10^6 amebae/mL at 72 hr. The LD_{50} dose was calculated as 132 amebae for an inoculum of 10μl instilled intranasally in 21-day-old male CD-1 mice. The identity of the HBT-1 isolate was confirmed as *N. fowleri* by indirect immunofluorescence and by isoenzyme analysis using acid phosphatase and propionyl esterase.

SYSTEMIC BUT NOT INTRATHECAL MORPHINE ANALGESIA SUPPRESSES ANTIGEN-SPECIFIC AND MITOGEN-STIMULATED LYMPHOCYTE PROLIFERATION. **Jena G. Hamra[1] and Tony L. Yaksh.[2]** [1]Department of Pharmacology and Physiology, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107; [2]Department of Anesthesiology, University of California, San Diego, LaJolla, CA.

Considerable evidence suggests that opioids interact with the immune system. However, the influence of spinal opioid systems on immune competency has not been examined. Therefore we examined the effect of a 5 hour period of analgesia following either subcutaneous or intrathecal injections of morphine on rat lymphocyte proliferation and the expression of cell surface receptors. Morphine was administered twice (time 0 and 2.5 hours) either subcutaneously (10mg/kg) or intrathecally (30µg) to naïve rats or rats immunized with keyhole limpet hemocyanin (KLH). Rats were sacrificed five hours after the first dose of morphine, splenic lymphocytes isolated and incubated with KLH or PHA for proliferation assays or labeled with monoclonal antibodies directed at cell surface receptors (T and B cell, CD4, CD8 and CD25). Systemic morphine suppressed both PHA and KLH stimulated lymphocyte proliferative responses, increased the number of cells expressing the CD4 receptor, but did not alter the expression of the interleukin-2 (CD25) receptor. Naltrexone blocked morphineís effects on lymphocyte proliferation. Intrathecal administration of morphine did not significantly alter either lymphocyte proliferative responses or cell surface receptors. These results show that equianalgesic doses of systemic but not intrathecal morphine suppress both mitogen and antigen stimulated lymphocyte proliferation and this action is not the result of alterations in IL-2 receptor expression.

AN ELECTROPHYSIOLOGICAL STUDY OF THE LIGHT-EVOKED POTENTIALS IN THE OPTIC NERVE OF THE FROG, *RANA PIPIENS*. **J.H. Hunt, W.E. Finn, and P.G. LoPresti.** Department of Pharmacology and Physiology, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107; Department of Electrical Engineering, University of Tulsa, Tulsa, OK 74104.

Age-related macular degeneration and retinitis pigmentosa are two major eye diseases producing blindness. Our research focuses on developing an artificial vision system to restore sight in these patients. The initial objective is to understand the flow of visual information from the retina to the brain via the optic nerve. In this study, the optic nerve in the frog, *Rana pipiens*, was prepared for nerve recording in two steps. First, the frog was anesthetized with MS-222 and the optic nerve was surgically exposed via the roof of the mouth. The following day, the frog was treated with tubocurarine and the optic nerve transected and drawn-up into a suction recording electrode. The eye was stimulated with 3 second pulses of light focused on the retina from various color light emitting diodes while recording the evoked compound action potentials from the optic nerve. Unique patterns of nerve response were observed for each selected wavelength band. Both "on" and "off" evoked signals were recorded and analyzed off-line with a computer.