Abstracts from the 82nd Technical Meeting Ada, Oklahoma 1993 November 12

This is a new Section for the *Proceedings*. As the title indicates, it contains abstracts from the preceding OAS Technical Meeting. However, it should be noted that *not every* paper presented at the Meeting is represented by an abstract. Only abstracts that were prepared and submitted on a special form, and for which a special processing fee was paid, are reproduced here; they are reproduced exactly as the authors submitted them.

This first experience with reproducing abstracts prepared on a special form revealed one major problem. The forms supplied in the OAS Newsletter were produced xerographically; the forms actually used sometimes were copies of copies; photocopying machines sometimes distort the image. As a result, some of the abstracts were received on forms that are appreciably different in size from those originally provided. In this issue it was easy to accommodate the irregular sizes (two per page rather than the planned four), but only by happenstance. For future meetings, *printed forms* (rather than photocopied forms) will be provided to those who need them; abstracts will be accepted only if they are prepared on the special, printed form.

If you plan to submit an abstract for publication, *please request a printed abstract form from the Editor.* The editors shall be grateful.

EFFECTS OF ANTIESTROGENS ON INSULIN LIKE GROWTH FACTOR-I (IGF-I)-STIMULATED GROWTH OF MDA-MB-231 BREAST CANCER CELLS.

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The objective of the present study was to compare the activities of antiestrogens Analog II, tamoxifen (TAM), and ICI-182,786 (ICI), on IGF-I stimulated growth of MDA-MB-231 estrogen receptor (ER) negative human breast cancer cells. Analog II has been shown to inhibit the growth of both ER positive and ER negative breast cancer cells, which indicates that this compound may not act exclusively at the ER. Cells grown in RPMI media containing 5% CPSR, a serum replacement with low mitogenic activity, were exposed to IGF-I (10^{-12M} to 10^{-18}M), Analog II (10^{-6}M), TAM (10^{-6}M), ICI (10^{-6}M), or a combination of growth factor and antiestrogen for 3, 5 and 7 days. Cell proliferation was assayed using the Neutral Red Assay. Analog II did not inhibit IGF-I stimulated cell growth. TAM produced a 30-90% inhibition of IGF-I-stimulated cell growth and ICI inhibited stimulated cells 10-80% at all IGF-I concentrations at 3, 5 and 7 days ($p<0.05$). Our results indicate that TAM and ICI are effective in reducing IGF-I-mediated growth. Further studies on the expression of the IGF-I receptor gene and quantitation of the gene product will be conducted to elucidate the mechanism by which antiestrogens modulate breast cancer cell growth. (This study was supported by OCAST grant HR2-009).

FLAGELLATES OF *NAEGLERIA FOWLERI.*

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Naegleria fowleri is a free-living ameboflagellate and the cause of a fatal human disease known as primary amebic meningoencephalitis. Amebae are able to transform into nonfeeding, nondividing, transient flagellates. The purpose of this study was to define the conditions for maximum transformation, or enflagellation, in *N. fowleri.* Amebae were grown axenically, washed with Page's ameba saline, suspended in 4°C ameba saline, and agitated at 100 rpm in a gyrotory shaker. Samples were taken hourly to 8h, fixed and stained with D'Antoni's iodine, and examined by light and phase-contrast microscopy. Within 90 min of suspension in buffer, amebae began to round-up and form 2 short, blunt flagella. As the cells elongated into flagellates, the flagella likewise lengthened. Maximum enflagellation occurred between 4-5h after suspension. After a time as flagellates, the cells reverted to amebae, regained ameboid movement, and eventually resorbed their flagella. Maximum enflagellation varied greatly (0-55% flagellates) among the 13 isolates tested. A 37°C transformation temperature (23, 30, 37 42°C tested) yielded the most flagellates. The phase of growth affected enflagellation with early stationary phase amebae producing the most flagellates. Amebae grown without agitation yielded more flagellates than amebae grown with agitation. However, agitation of the ameba/buffer suspension produced more flagellates. The number of flagella per flagellate ranged from 1-5, although 92% of the cells had 2 flagella. (Supported in part by EPA grant R-818106)

DEVELOPMENT OF TITLE III SUPPORTED SCIENCE LABORATORIES

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Title III is a federally funded program to assist institutions with minimal resources and a high number of PELL Grant recipients. These funds are used to develop programs to strengthen the institution. In this case, course laboratory exercises were developed to give students a "hands-on" experience (now required by industry) and an enhanced scientific literacy. The laboratory exercises were added to existing or new courses in environmental science, computer networking, molecular biology, and statistics. Computer analysis allows students a "working" knowledge of topological environmental analysis of water, soil, and air samples; management protocol, shared files and diagnostic support; isolation purification, and assessment of mitochondrial DNA and protein; and categorical data analysis utilizing software packages such as GLIM, SAS, S-PLUS, STAT-XACT.

THE INFLUENCE OF SELECTED SURFACTANTS AND WATER QUALITY PARAMETERS ON SURFACTANT-ENHANCED SOIL FLUSHING OF HYDROCARBONS FROM CONTAMINANTED SOIL.

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A technology for enhanced oil recovery was transferred to *in situ* hazardous waste remediation of soil containing hydrocarbons using laboratory shaker methods. Radiolabeled hexadecane, o-cresol and phenanthrene were used as the target compounds (TCs). Sandy soil was contaminated with TC levels of 4.62, 9.24 and 13.86 mg/gram of soil. The soil was washed with three surfactants (Sandoxylate SX-408, NAXCHEM K, and INPROVE) in 0.5 %, 1.0 % and 2.0% solutions and water. Ground water parameters used to alter the flushing solution were pH levels of 5.5, 7.0 and 8.5 and brine levels of 5,000, 10,000 and 20,000 mg/1. The amount of TC desorbed and flushed from the soil was measured with a scintillation counter. Significant TC desorption parameters were TC type and concentration and surfactant type and concentration. Brine and pH levels were found not to be significant in TC desorption. The surfactants flushed over 3 times as much hexadecane and 9 times more phenanthrene from the soil as the water flushes. For o-cresol, the amounts flushed were not significantly different for the two solution flushes.

ANIONIC SURFACTANT MOBILITY IN A FINE-GRAINED UNSATURATED SOIL.

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Surfactants are presently being considered for use in flushing organic contaminants from soils. The effectiveness of using surfactants for this purpose will depend on their mobility with respect to unsaturated flow conditions. Consequently, our study tested the mobility of two anionic surfactants in an unsaturated fine-grained soil using procedures and boundary conditions described by Brown and Allred (1992) and Bruce and Klute (1956). Two factors having a possible impact on the mobility of the anionic surfactants were investigated. These factors include resident soil cations and soil organic matter. Enhancement of anionic surfactant mobility was then tested using soil treatment and surfactant solution additives. Both surfactants exhibited a varying degree of retardation with respect to the wetting front and affected a reduction in diffusivity for the soil to which they were exposed. The mobility of one of the surfactants, an alkylether sulfate, did not appear to be influenced by the resident soil cations or organic matter. Methods of mobility enhancement for this surfactant were not effective. Soil divalent cations and organic matter did have a subtle influence on the mobility of the second surfactant, a linear alkylbenzene suffonate. Also, the mobility of this surfactant was enhanced by both a soil treatment preflush with sodium chloride and the addition of alkalis to the injected surfactant solution.

ESTROGEN INDUCTION OF PROGESTERONE RECEPTORS IN ADIPOSE TISSUE OF SHEEP.

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Previous work in this laboratory had shown the presence of estrogen receptor (ER) in gluteal and perirenal adipose tissues with similar physical characteristics to the ER in uterine tissue. In contrast we failed to detect ER in omental adipose tissue. Studies were undertaken to determine if estrogen treatment (ER implant) of sex steroid depicted (ovariectomized/adrenalectomized) ewes would result in an upregulation of progesterone receptor in adipose tissues in a fashion similar to that seen in reproductive tissues such as uterus which was used as our internal control. Western blot analysis of sheep treated for 7 days indicated that PR was induced in uterus>gluteal>perirenal tissues consistent which the concentration of ER contained in these tissues. Ligand binding techniques (Scatchard analysis and sucrose gradient analysis) indicated that specific binding for radiolabeled R5020 was induced in E_2 treated animals with gluteal having more specific binding than perirenal and omental tissue (gluteal = 29.2. perirenal 8.1. omental 4.2 f moles/mg Protein) Sucrose gradient profiles showed specific binding in both the 5S and 9S regions of the gradient with only the 9S region showing sensitivity to PR antibody treatment. The nature of the specific R5020 binding in the 5S region of the gradients has yet to be determined but accounted for 100% of the specific binding in omental tissue and approximately 66% of the specific binding in perirenal and gluteal adipose tissues. These data suggest that the low levels of ER present in some adipose tissues may be physiologically significant in that E_2 treatment appears to induce PR in these tissues as well as reproductive tissues. These data suggest a direct role for sex steroids in adipose accretion and metabolism (supported in part by OCAST grant #HR9-031)

CRYPTOCOCCUS YEASTS IN SOIL ARE INDIGENOUS.

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The indigenicity of yeasts found in soils has been considered questionable on the grounds that yeasts found on or near soil surfaces were probably deposited there and that yeasts do not survive long in soil. Knowing that Ross Desert (Antarctica) Cryptococcus spp. are both unique and adapted to that environment and that other yeasts can become seasonally dominant in the peat soils of South Georgia I. (subAntarctic), we investigated yeast survival and growth in other soils. Cryptococcus spp can both grow and survive in other than cold desert soils. Soils from the Atacama Desert (Chile) yielded habitat-adapted Cryptococcus spp. after 10 years of refrigerated storage. A Cr. laurentii isolate similarly held in sterilized OK soil has survived well for over 4 years. Cr. albidus grew ± added nutrient in an OK soil. The large population (10^7 cfu/g) resulting from nutrient enhanced growth has remained essentially stable over the more than 74 days since, though held at the stressing temperature of 25 °C. (Supported in part by Oklahoma State University.)

LEAFHOPPER TRANSMISSIBILITY OF THE PHYTOPATHOGEN SPIROPLASMA CITRI AFTER REPEATED SUBCULTURE IN ARTIFICIAL MEDIUM.

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Spiroplasma citri, a wall-less prokaryote which causes citrus stubborn and horseradish brittleroot diseases, is transmitted in nature by the leafhopper Circulifer tenellus. After repeated passage of S. citri in artificial medium, insect transmissibility may be lost. S. citri line BR3-P, passed over 130 times, has lost its transmissibility. S. citri line BR3-M, passed 43 times after its isolation from horseradish, was tested for transmissibility by C. tenellus. S. citri line BR3-T, isolated from a line of infected turnips in which it had been maintained for 10 years, and previously shown to be transmissible, was used as a positive control treatment. Liquid medium was used as a negative control treatment. Suspensions of each S. citri line were injected into the body cavity of young adult C. tenellus using heat-drawn needles. The leafhoppers of each treatment were caged separately with sugarbeet plants for a 2 week latent period, then transferred in groups of 3 to cages with turnip plants for a 3 day inoculation access period. The leafhoppers were then frozen and the turnip plants placed in a greenhouse for 4 weeks prior to being assessed for S. citri infection by enzyme linked immunosorbent assay (ELISA). Results of 4 replications of each treatment showed that S. citri line BR3-M was transmissible, with an overall transmission rate of approximately 40%. This data verified that S. citri line BR3-M retains transmissibility following as many as 43 passages through artificial medium.

PATHOGENIC FREE-LIVING AMEBAE IN TULSA AREA WATERS.

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Pathogenic free-living amebae are responsible for serious human disease including infection of the eye and the central nervous system. The purpose of this study was to conduct a year-round sampling program to isolate and identify pathogenic free-living amebae in Tulsa area waters. Water and cotton-swab samples were collected from a variety of sites in the Tulsa area. Water was centrifuged and the sediment placed on non-nutrient agar with living *Escherichia coli*. Swab samples were placed on *E. coli*-coated agar and all plates were incubated at 42 °C. Amebae obtained from the agar surface were subcultured in liquid medium and incubated at 37 °C. Pathogenicity was determined by intranasal inoculation of 21-day-old male CD-1 mice using 10^4-10^5 amebae per mouse. Amebae were cultivated from brain tissue of dead or dying mice. A total of 34 pathogenic isolates were obtained from 2,016 processed water and swab samples. Pathogenic amebae were isolated during every month of the year and were identified as *Naegleria australiensis* (38%), *Acanthamoeba* species (35%), *Naegleria fowleri* (18%), and leptomyxid amebae (9%). Pathogenic leptomyxids, although a cause of fatal disease, have not previously been reported from the environment. The greatest percent recovery of pathogens occurred during the spring and autumn. The prevalence of pathogenic free-living amebae in the sampled waters was 1 pathogen per 3.4 liters of water. (Supported by EPA grant R-818106)

EFFECT OF ANTIESTROGENS ON GENOTOXICITY.

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Our previous work indicates that cyclopropyl antiestrogens (Analog II) are potent inhibitors of breast tumor cell growth and have the potential to inhibit the growth and metastatic spread of breast cancer. The aim of the present study was to investigate the genotoxic potential of Analog II. Genotoxic effects of Analog II and tamoxifen were studied at the hypoxanthine phosphoribosyl transferase (HGPRT) locus in V79 cells. This assay identifies and selects mutants by taking advantage of the biochemical pathways by which a cell synthesizes DNA. The doses of the compounds tested for genotoxicity ranged from 10^{-7} to 10^{-6}M. The mutation frequency /10^6 cells treated with tamoxifen at both concentrations was three times over control (5.81 vs 1.81) This report is in agreement with other studies which reveal that tamoxifen is a potential genotoxic carcinogen. The mutation frequency/10^6 cells treated with Analog II was approximately twice the control value at 10^{-6}M. Analog II did not produce any genotoxicity at 10^{-7}M (2.98 vs 1.81). However further studies on genotoxicity involving 1) in vivo studies and 2) metabolic activation of the antiestrogens are necessary before definite conclusions can be drawn. Additional genotoxicity studies are being undertaken in our laboratory. (Supported by OCAST grant HR2-009).

NEONATAL LUNG DAMAGE MONITORED BY EXHALED VOLATILES

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Ventilation of premature newborns with high levels of oxygen can result in oxidant-related lung damage. The lung damage can be monitored non-invasively by analyzing the newborn’s breath for specific compounds. Pentane and ethane are two compounds of particular importance. These compounds are only derived from free-radical induced lipid peroxidation of linolenic and linoleic fatty acids precursors. The derived alkanes can be observed directly using relatively simple techniques in which large exhalation volumes are loaded directly onto a packed column for ambient temperature chromatography with FID detection. This method of analysis simplifies earlier studies in which water and carbon dioxide were removed prior to cryotrapping and separation using capillary columns. These developments should allow for more wide-spread utilization of breath analyses to monitor hypoxic, hyperoxic and many other ischemic and inflammation-related processes.