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BETA-GALACTOSIDASE ACTIVITY AND ISOZYMES OF RIPENING CHILE.

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Pungent peppers (*Capsicum annuum* cv. 'Joe Parker', New Mexico Red and Green) are harvested at the mature green or dehydrated red stage development. Mature green chile is used as fresh fruit or canned, while dehydrated red chiles are made into chile powder (e.g. paprika or red pepper). Experiments were designed to investigate enzymes that may play a role in ripening (color change from green to red). When xylosidase, glucosidase, and galactosidase activity were compared, β-galactosidase (BG) was most prominent in all stages of ripening (green, turning and red) and increased as the fruit ripened. Different pepper types were compared for BG activity. All pepper types tested (NM type, Hungarian yellow, Bell, and Jalapeno) showed an increase in BG activity upon ripening. HPLC cation exchange chromatography of green chile protein extracts showed one large acidic and one smaller basic BG peak, whereas, red chile protein extracts showed a very small acidic BG peak and a large basic BG peak. In addition, IEF-PAGE indicated that green chile contained a prominent acidic BG, whereas, red chile contained a prominent basic BG.

ANALGESIC POTENCY OF ALPHA ADRENERGIC AGONISTS IN AMPHIBIANS.

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We compared the analgesic effects produced by subcutaneous (s.c.) or intraspinal (i.s.) injection of four alpha-adrenergic agonists in an amphibian pain model. The nociceptive threshold (NT) was determined by the acetic acid test using serial dilutions of acetic acid applied stepwise until a hindlimb wiping reflex occurred. Dexmedetomidine (DM), clonidine (CL), epinephrine (EP) and norepinephrine (NE) produced a dose-dependent analgesic effect lasting at least four hours. Dose response curves were parallel and the relative potencies compared to EP=1 were 4.8 (DM), 0.02 (NE) and 0.01 (CL). after s.c. and 3.6 (DM), 2.1 (NE) and 0.09 (CL) after i.s. Pretreatment with selective alpha-2 antagonists, yohimbine and atipamezole, significantly decreased the analgesic effects of alpha agonists, whereas the alpha-1 antagonist, prazosin, had no significant effect. We conclude that descending noradrenergic fibers from the brainstem to the spinal cord may modulate ascending pain pathways via activation of alpha-2 receptors.

FUNGAL CELL WALL DEGRADING ENZYME ACTIVITY OF VINE-DECLINE PATHOGENS ON CANTALOUPE TISSUE.

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Vine declines of cucurbits are caused by several pathogens; *Macrophomina phaseolina* (Charcoal Rot), *Monosporascus cannonballus* (Vine Decline), *Didymellabryoniae* (Gummy Stem Blight), *Diaporthe melonis* (Purple Stem), and recently, *Acremonium* sp. (Acremonium Collapse). Experiments were designed to determine if these pathogens produced cell wall degrading enzymes on cantaloupe tissue. Enzymes assayed were beta-galactosidase (BG), alpha-mannosidase (AM), polygalacturonase (PG), and cellulase (CE). Fungal pathogens were grown in a minimal salt broth with either commercial pectin, Cm-cellulose, cantaloupe root, hypocotyl or stem tissue as the substrate. Aliquots were removed from the shake cultures after 0,3,6, and 9 days of growth. Bioassays indicated that AM was not produced by any of the fungi on the substrates described. BG, CE, and PG were produced by *M. phaseolina*, *D. bryoniae*, and *D. melonis*. *Acremonium* sp. produced CE and PG. *M. cannonballus* produced CE only.

PEROXIDASE ISOZYMES OF FROZEN SWEETCORN. T. W. Cluck, K. Collings, J. K. Collins, C. Biles, E. V. Wann and P. Perkins-Veazie. East Central Univ., Ada, OK 74820 and USDA-ARS, Lane, OK 74555.

Peroxidase is a plant enzyme associated with off-flavors in fruits and vegetables. Peroxidase activity is commonly used by the food industry to predict off-flavor development in frozen food. Experiments were designed to analyze peroxidase activity in three corn cultivars ['Florida Staysweet' (sh2), 'Merit' (su1) and 'Bodacious' (su1/se)] before and after unblanched frozen storage. Corn proteins were extracted from acetone powders of kernels and separated by isoelectric focusing-polyacylamide gel electrophoresis and Native-PAGE. Banding patterns differed according to genotype and storage duration. All genotypes contained a peroxidase isozyme having a molecular weight of 80 kD and pI of 4.5. The su1/se and sh2 genotypes produced an additional peroxidase band of 13.8 kD An additional peroxidase isozyme (pI 5.0) was resolved from the su1 genotype after 12 months of storage. Although variation in banding patterns was observed, total peroxidase activity did not parallel flavor changes in frozen unblanched supersweet (sh2) or sugary enhanced (su1/se)] sweet corn genotypes Changes in total peroxidase activity may not predict flavor changes in all genotypes. However, the presence or absence of certain peroxidase isozymes may be useful in predicting off-flavor development in frozen corn.

CUTANEOUS MYIASIS IN A TULSAN WHO VACATIONED IN CENTRAL AMERICA. David T. John and David A. Henderson, Departments of Biochemistry & Microbiology and Research, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107.

Myiasis, Gr. *myia* fly, is the infestation of tissues with fly larvae, or maggots. Myiasis is common in domestic and wild mammals all over the world and is a relatively frequent occurrence in rural areas where people are in close contact with domestic animals. This is a report of specific myiasis caused by a larva of the human botfly, *Dermatobia hominis.* A mature larva of *D. hominis* emerged from a boil-like lesion on the scalp of an adult female approximately 10 weeks after she had returned from a 2-week vacation to Costa Rica. Prior to the larva's emergence the woman had been to 3 physicians for treatment, all of whom had prescribed antibiotics for a presumed bacterial infection. The living larva was observed and photographed, fixed in 70% ethanol at 60°C, cooled to room temperature, and after 24h processed for scanning electron microscopy. The live larva was 15mm long by 8mm wide and white in appearance. The anterior end possessed 2 anemone-like spiracles and a pair of well-developed oral hooks, used for movement and invading the skin. The abdominal segments contained rows of short spines or hooklets that anchored the larva in the tissue. The posterior end of the larva had a pair of spiracular plates located in a small deep cleft. The traveler recovered without incident from her case of cutaneous myiasis.

BREAST CANCER CELL MIGRATION: INFLUENCE OF ANTIESTROGENS. A.C. Mathew, J.T. Pento, J.J. Dmytryk, T.T. Rajah, A.S.M. Abidi and G.M. Hurt, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

An important characteristic of tumor malignancy and the major cause of patient mortality is the metastatic nature of malignant cancer. In this study, the antimetastatic activity of antiestrogens [tamoxifen (TAM): ICI-182,780 (ICI); and Analog II (AII)] on highly invasive, estrogen receptor (ER)-negative MDA-MB-231 (MDA) and non-invasive, ER-positive MCF-7 (MCF) human breast cancer cell lines was investigated using an *in vitro* wound technique. Cell cultures were treated with antiestrogen or vehicle in the control group for 4 days in a 60mm petri dish. The cell cultures were then wounded mechanically, by pressing a razor blade into a confluent cell culture and removing one half of the monolayer. Thereafter, cell movement was evaluated by counting the number of cells 1) that migrated into the wounded area on the surface of the dish and 2) that "floated" into the wounded area and reattached. The results with MDA cells indicate that ICI produced a 30% decrease in cell migration, AII did not alter MDA migration, while TAM caused a 100% increase in cell migration. Further, ICI and AII treatment produced a 40-50% decreased in the number of "floating" MDA cells while TAM had no effect. With MCF cultures few "floating" cells were observed. ICI and AII treatment produced a 20-40% decrease in MCF migration while TAM increased migration by approximately 15%. In conclusion, this study demonstrates that ICI and AII inhibit breast cancer cell migration and that TAM enhances cell migration in this *in vitro* wound model. (Supported in part by OCAST grant HR2-009 and NIH grant CA 62117.)

COMPARATIVE CHEMICAL ANALYSES OF LIPOPOLYSACCHARIDES EXTRACTED FROM PSEUDOMONAS AERUGINOSA STRAINS WITH VARYING ANTIBIOTIC SENSITIVITIES. M.J. Howard, S.L. Stewart, and R.S. Conrad. Department of Biochemistry/Microbiology, Oklahoma State University College of Osteopathic Medicine Tulsa, OK 74107.

The correlation between antimicrobial susceptibility and bacterial cell composition was studied in *Pseudomonas aeruginosa* by comparative chemical analyses of lipopolysaccharides (LPS) extracted from strains with varying sensitivities. All comparisons were relative to strain PAO1 wild type. The degree of susceptibility among these strains was established by determining the minimal inhibitory concentrations for selected antimicrobials. The levels of neutral sugars were similar except that supersusceptible strain Z61 was deficient in rhamnose. Major differences were noted in the fatty acid content whereby Z61 had elevated levels of 2-hydroxydodecanoic acid which was almost totally absent in the polyinyxin resistant isolate A strain. Strain Z61 had a greatly reduced level of dodecanoic acid which was somewhat elevated in isolate A. No major differences were noted in amino compounds, amino sugars and phosphates. The various LPS were examined for their ability to bind polymyxin B and its deacylated nonapeptide derivative. Although some differences in binding affinity were noted, these differences were not proportional to observed differences in antimicrobial sensitivies.

SEM OF A MATURE LARVA OF THE LATIN AMERICAN BOTFLY, *DERMATOBIA HOMINIS*.

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A live larva, 36 h after emergence from the scalp of an Oklahoman who had vacationed in Costa Rica, was fixed and gently scraped clean of its waxy surface coat, then processed for SEM. The larva measures 15 mm long and 8 mm wide at center, tapering to a 3.5 mm diameter head and a 1 mm diameter posterior spiracle. Its body is ventrally curved, flattened ventrodorsally, and in ventral view, consists of nine 1 mm thick rounded, disk-like segments demarcated by grooves. The anterior rims of segments 1-6 bear posteriorly pointed, thorn-like spines. Striking features of the head are a pair of ventral hooks and a pair of anterior spiracles. The 0.5 mm long, ventroposteriorly directed tusk-like hooks are each embedded in an ovoid "turret" and were observed in life to protract and retract. The anterior spiracles are cylindrical extensions of the head that protrude laterally, dorsally and anteriorly at 45º angles from the central axis of the larva and have their lumina obscured by thick fringes of villi that are mostly cylindrical. Some villi were fractured in a transverse plane revealing a core of contiguous polyhedral cells with 3-5 arching columnar apical processes forming intersecting arcades. Some of these cells were covered apically by a smooth, tentlike "membrane".

THE EFFECTS OF MUTANT *PSEUDOMONAS* EXOTOXIN A MOLECULES ON SPLENOCYTES FROM NUDE MICE.

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Exotoxin A (PE) produced by *Pseudomonas aeruginosa* is a toxin that inhibits protein synthesis via the ADP-ribosylation of elongation factor 2 (EF-2). Studies have demonstrated that exotoxin A induces a GM-CSF dependent proliferation of immature T lymphocytes from athymic nude mice. Various mutants of exotoxin A were examined for their ability to induce the proliferation of splenocytes from nude mice. PE43, which lacks amino acids 5-224, was able to induce splenocyte proliferation but at higher doses than native PE. PEgly276, a mutant that does not undergo proper intracellular cleavage, was able to induce proliferation but at higher doses than native PE. CRM66, which has an amino acid substitution that makes it unable to bind to EF-2 was able to induce proliferation. Two ADP ribosylating mutants, PE589 and PE553D, were unable to induce splenocyte proliferation. These same mutants were tested for their ability to induce GM-CSF production in splenocytes. The toxins that induced proliferation also induced the production of GM-CSF. These results suggest that ADP-ribosylation may have a role in the induction of proliferation and/or the production of GM-CSF in splenocytes from nude mice.

DEVELOPMENT OF AN INDIRECT IMMUNOFLUORESCENT ASSAY FOR THE IDENTIFICATION OF PATHOGENIC FREE-LIVING AMEBAE.

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Pathogenic free-living amebae cause serious human disease including infection of the eye and the central nervous system. During a previous environmental survey in the Tulsa area we obtained 34 pathogenic ameba isolates from water and swab samples and identified them on the basis of morphology, pathogenicity to mice and reactivity with concanavalin A. The purpose of this study was to develop an indirect immunofluorescent assay to confirm the identity of the 34 environmental isolates. The amebae (antigen) used in the assay were *Acanthamoeba castellanii*, *Naegleria australiensis* and *Naegleria fowleri*. Antiserum was produced in rabbits which were inoculated with the above amebae. Test amebae were fixed to serological slides with acetone or 2% formalin in methyl alcohol. Serial 2-fold dilutions of antiserum were applied to the amebae and the slides were incubated at 37°C, rinsed, exposed to fluorescein-labeled antirabbit IgG and counterstained with Evans' blue. The overall results of immunofluorescence with homologous antiserum was 1:128 for *A. castellanii*, 1:512 for *N. australiensis* and 1:512 for *N. fowleri*. Formalin-methanol was a better fixative than acetone. (Supported by Oklahoma Partners for Biological Sciences Summer Research Award to DAA)

SPATIAL ABILITIES AND THEIR CORRELATIONS WITH MICROBIOLOGY ACHIEVEMENT.

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Spatial ability includes the subabilities spatial visualization, spatial orientation, and flexibility of closure. A science course which has not been examined for correlations between spatial ability and achievement is undergraduate microbiology. 158 microbiology students were pretested with the Paper Folding Test of Spatial Visualization, the Cube Comparison Test of Spatial Orientation, and the Hidden Figures Test of Flexibility of Closure. Spatial Orientation correlated with laboratory grade ($r=.18$; $p<.02$); spatial visualization correlated with final examination grade ($r=.17$; $p<.03$) and laboratory grade ($r=.19$; $p<.02$). Noting gender differences, spatial orientation correlated with laboratory grade for females ($n=113$; $r=.21$; $p<.03$) while flexibility of closure correlated with laboratory grade for males ($n=45$; $r=.37$; $p<.02$). Equitable teaching of microbiology could include incorporating spatially enhancing teaching techniques, especially into the laboratory component of the course, to improve the chances for success of entering students with lower spatial abilities.

SEX RATIO STUDY OF *MYOTIS VELIFER* IN WESTERN OKLAHOMA.

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Myotis velifer, the cave myotis bat, is a permanent resident of Oklahoma found in hibernacula caves and maternity roosts. More males than females have been reported in hibernating populations in Kansas and Texas. However, the sex ratio in Oklahoma has not been documented. In several visits to hibernacula, it appeared females outnumber males. The sex ratio of hibernating bats in a major hibernaculum was monitord for an entire season. There was a constant female-bias throughout the sampling period (64% females). The sex ratio of bats in peripheral caves was examined to determine if the sexes were segregating among caves. There was also a female-bias in regional caves (76% females overall). The sex ratio of young *M. velifer* at a maternity roost was examined to determine if an equal number of male and female bats were born. There was an equal sex ratio of young flying pups, so the low percentage of males during hibernation is not due to a differential sex ratio at birth. It is still unclear why the sex ratio is unbalanced during hibernation.

HYDROLOGY OF A CREATED WETLANDS.

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Forty-five acres of mitigation wetlands were created along Polecat Creek near Jenks, OK to replace a site that was filled when the Creek Turnpike was built. Because three of five mitigation sites do not hold water as designed, hydrologic studies were conducted to establish flow regimes. Three groups of water are defined by levels of dissolved solid (low, moderate, and high) and by similarities in saturation index for calcite, dolomite, and gypsum. These associations show the influence of flood waters on one pond and indicate that a second may be leaking to an adjacent pool dammed up in a local hollow. A third pond is indicated to be saturated with respect to calcite and may therefore be in communication with more highly concentrated ground water. Monitoring of ground water is recommended at several sites to document the hydrologic relationships and to determine whether clay liners under the ponds have failed.

A SIX-LEGGED *RATTUS* IN OKLAHOMA

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A six-legged black rat (*Rattus rattus*) was collected by Roger D. Cowan on January 18, 1993, in Lawton, Comanche County, Oklahoma, and brought to the Cameron University Department of Biology. The specimen was photographed, subjected to an x-ray exam, and afterwards deposited in the Cameron University Museum of Zoology (CUMZ 1209). The specimen was an immature male measuring 232 mm in length and weighing 40 grams, and appeared normal except for two extra hind limbs. One of the additional legs was located just right of the penis, and dissection revealed the leg muscles were atrophied and the head of the femur was joined to the posterior edge of the right pubis by connective tissue. The femur was shorter than the femur of the normally developed left hind leg, although the tibia and fibula were of normal size for the specimen. The other extra leg originated as a single right femur but split antero-posteriorly 9 mm from the head, terminating into two lower legs each having a tibia, fibula, well formed foot and fully developed muscles such as the gracilis and gastrocnemius. The legs were probably nonfunctional due to their sharing of a single femur. Accessory appendages have been reported in small mammals such as the family Leporidae (Murie, *J. Mamm*. 15:162 1934), but information regard this phenomenom in rodent famlies is scant.

ENVIRONMENTAL SOIL EXERCISES FOR GENERAL EDUCATION STUDENTS.

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As a result of a four year Title III grant, a number of laboratory exercises were developed in environmental studies. The laboratory apparatus and equipment, purchased through the Title III laboratory equipment project, are now available to develop other laboratory exercises. This project was to develop laboratory procedures to allow general education students, enrolled in a chemistry class with no scheduled laboratory, the experience of a laboratory through some water and soil analysis activities. The students measured the pH of water and soil. The collection of a representative sample was emphasized. In addition, phosphorous in soil was determined using water and water with a little acetic acid added. The phosphorous was enhanced in the acetic acid sample as expected. The students were asked to add hydrochloric acid to some of the soil sample to determine whether the soil was basic or acidic. They were also asked to correlate the soil, pH, phosphorous and acidity tests. The students used funnels for separation, pH meters, colormeters, balances, and grinders for soil particle size. They learned basic instrumental techniques as well as laboratory activities and safety.