Abstracts of Scientific Presentations
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Platform Sessions

PS1 Non-melanoma Skin Cancer and Pathological Evaluation of SKH-1 Mouse Treated with Oral Inositol Hexaphosphate (IP6)
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Approximately 1 million new cases of non-melanoma skin carcinoma (NMSC) occur in the US every year due to exposure to UV radiation; thus, the development of novel therapeutical or protective agents is imperative to combat NMSC. Inositol hexaphosphate (IP6) is a naturally occurring polyphosphorylated carbohydrate, abundantly present in many plants and in certain high-fiber diets such as cereals and legumes. Naturally occurring IP6 has a striking broad-spectrum anticancer activity in various in-vitro and animal models. IP6 affects major pathways in malignancy such as proliferation, cell cycle progression, metastasis, invasion, and angiogenesis, and induces apoptosis. We investigated the protective effects of IP6 in drinking water on the incidence of UVB induced-skin cancer in the SKH-1 (Crl:SKH1-hr) murine model. Mice were divided into 2 groups (15 mice/group). The treatment group received 2% IP6 in drinking water and UVB exposure. The control group received UVB exposure. All mice were fed with a special IP6-deficient diet (AIN 76A). The treatment group started receiving 2% IP6 in the drinking water 3 d prior to radiation. The mice were irradiated 3 times/wk starting at 1.5 kJ/m2 dose with weekly increments of 1.5 kJ/m2 to a final dose of 9.0 kJ/m2; tumor formation was monitored until the 31st wk. At the 31st wk, the tumor incidence was 5-fold lower in the mice treated with 2% IP6 in drinking water (P < 0.01) and tumor multiplicity was approximately 4 times lower in the mice treated with 2% IP6 in drinking water (P < 0.01). Histopathological evaluations showed squamous papilloma (67%) and epidermal hyperplasia (33%) in the IP6-treated mice versus squamous papilloma (14%), epidermal hyperplasia (72%), cornifying epithelioma (7%), and undifferentiated mesenchymal neoplasm (7%) in control mice. Our results indicate that 2% IP6 in the drinking water significantly decreased the percentage of mice with tumors as well as the average number of tumors per mouse. These results imply a protective effect of oral IP6 against UVB radiation-induced skin tumor formation.

PS2 Tissue Vaccines for Prevention of Prostate Cancer
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Prostate cancer is a significant clinical condition of men, with nearly 250,000 new cases diagnosed and 30,000 deaths each year. Due to the paucity of effective treatments for the refractory disease, focus has been given to prevention, primarily related to dietary manipulations. While some data for dietary prevention is compelling, widespread adoption of broad dietary changes is unlikely. In contrast, vaccination offers the opportunity to prevent disease in large numbers of individuals without requiring sustained behavioral changes. To determine if vaccination is a potential strategy for prevention of prostate cancer, we evaluated the utility of a tissue vaccine derived from glutaraldehyde-fixed tumor (GFT) tissue. Subcutaneous tumors generated in Lobund-Wistar (LW) rats with the syngeneic PAIII prostate cancer line were harvested, dissociated, and treated with 3% glutaraldehyde. Groups of 30 LW rats were treated with intravenous methylnitrosourea (30 mg/kg) to induce autochthonous prostate tumors and were then vaccinated subcutaneously (SC) monthly from 2 to 10 mo of age. At 10 mo of age, gross and histological examination of prostates showed a 90% reduction in the incidence of prostate cancer compared to media-vaccinated controls (included to demonstrate lack of treatment effect by media). To determine if this vaccine could be used as a xenogeneic preparation, immunocompetent Ncr-Foxn1™ mice were vaccinated SC with the GFT vaccine; their splenocytes harvested 7 d after the last boost and co-incubated with human PC346 prostate cancer cells (group 1); and orthotopically transplanted into syngeneic BALB/c nu/nu mice. Groups of 20 nu/nu mice were treated this way or with PC346 cells co-incubated with splenocytes from media-vaccinated mice (group 2); or transplanted with untreated PC346 cells (group 3). Ten weeks later, the mice were euthanized and prostates evaluated histologically for tumor growth. The incidence of prostate cancer was reduced by 70% in group 1 mice compared to those in groups 2 and 3, indicating that this vaccine has xenogeneic efficacy. In summary, tissue vaccines are effective at preventing prostate cancer in both syngeneic and xenogeneic models.
PS3 Diagnostic Power of Circulating Tumor Proteins and Physiological Parameters Tested in an Immunodeficient Rat Orthotopic Human Lung Cancer Model

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Clinically relevant animal models of human cancer that allow tumor growth and metastasis to closely mimic that of human disease are necessary for the evaluation of putative therapeutics. Presently, there are no animal models for human lung cancer that mimic human disease and show clinically relevant protein biomarker activity. It is hypothesized that circulating levels of lung cancer-associated proteins may correlate with physiological measurements from an orthotopic H460 human non-small cell lung carcinoma (NSCLC) model in immunodeficient rats. An orthotopic lung cancer model was developed in the nude rat (HSD:R-H-FOXN1RNU) using non-invasive intratracheal instillation of 1 x 10^7 H460 cells in 0.1 ml phosphate buffered saline (PBS). Human H460 cells from American Type Culture Collection were screened for mycoplasma and bacteria and banked at Centocor. Pilot studies showed that animals developed solid lung tumors by 23 d after tumor cell implantation. In this study, body weights and clinical observations, blood gas, and blood analytes from naïve and tumor-bearing animals were recorded twice weekly. Serum samples were collected to quantitate circulating tumor-secreted human IL-8, p53, VEGF, and MMP-9, which were correlated with all parameters to track disease progression. Individual animals were euthanized via CO2 asphyxiation when moribund or at 62 d after tumor cell implant, whichever came first. MMP-9 and p53 were not significantly detectable when moribund or at 62 d after tumor cell implant, whichever came first. MMP-9 and p53 were not significantly detectable in the serum. Circulating human VEGF was detected at high levels on the day of death in some of the tumor-bearing animals. Human IL-8 was detectable in all tumor-bearing animals and correlated with markers of respiratory acidosis (pH, P = 0.012; TCO2, P = 0.024; P = 0.007; and HCO3, P = 0.029), and with surface body temperature (P = 0.001). IL-8 significantly correlated with survival (P < 0.001), indicating an association with tumor burden, since the tumor could be the only possible source of this cytokine. Circulating human IL-8 may be a useful clinically relevant tumor protein marker, due to its correlation with survival as well as multiple physiological parameters associated with disease progression.

PS5 Infection with Enterohepatic Helicobacter spp. Increases Bile Flow in Male C57/LJ Mice: Implications for Drug Research and Metabolism

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Enterohepatic Helicobacter spp. are enzootic in academic rodent colonies. Little is known regarding the ability of these organisms to alter biliary homeostasis. The purpose of the current study was to determine if helicobacters alter the flow or concentration of bile and its lipid constituents. Helicobacter free male C57/LJ mice were purchased and divided into two experimental groups. Group 1 was infected with both H. hepaticus and H. rodentium at 4 to 5 wk of age; group 2 remained uninfected (n = 20/group). After 10 wk of infection, mice were anesthetized and hepatic bile cannulation was performed. After collection, bile was volumetrically analyzed to determine flow rate. Cholesterol concentration was quantified by enzymatic assay, bile salt concentration was calculated by high-performance liquid chromatography (HPLC), and phospholipids were calculated via Bartlett’s assay. There were no significant differences in the concentrations of cholesterol, phospholipid, or bile salts, nor were there significant differences in cholesterol or phospholipid flow rate. In contrast, bile flow rate (0.5145 ± 0.03 compared with 0.6313 ± 0.04 ml/h/100 g; P < 0.05) and bile salt flow rate (16.50 ± 2.2 compared with 25.07 ± 3.0 ul/h/100 g; P < 0.05) were both significantly elevated in infected animals. Regression analysis revealed that differences in bile flow were independent of bile salt flow. Increased bile salt independent flow arises from excesses in one of two biliary solutes (glutathione or bicarbonate). Helicobacter spp. infection may alter these constituents by inducing hepatic inflammation, which increases glutathione, or by converting biliary urea to carbon dioxide, which in normal alkaline bile would rapidly be converted to bicarbonate. In conclusion we demonstrate that infection with enterohepatic Helicobacter spp. alters bile salt flow and bile flow. These results contrast with well-described models of acute infection which
demonstrate bile stasis. Since hydrophobic drugs and metabolites require biliary excretion, these data imply that Helicobacter spp. may markedly alter pharmacology and toxicity studies due to increased bile flow.

PS6 Co-infection with Helicobacter bilis in C57BL/6 Mice Attenuates Gastric Pathology and Alters the Systemic Immune Response Associated with H. pylori Infection

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Studies examining experimental Helicobacter pylori (Hp) infection in mice fail to consider the potential effects of enzootic enterohelminic Helicobacter spp., despite the ability of some co-pathogens to alter the pathogenesis of gastric helicobacters. This study investigates the ability of H. bilis (Hp) to modulate the pathogenesis of experimental Hp infection in mice. Four groups of female C57BL/6 mice (n = 30) were orally inoculated with 10⁸ CFU Hp of experimental Hp infection in mice. Four groups of female C57BL/6 mice (n = 30) were orally inoculated with 10⁸ CFU Hp (Hp), Hb (Hb), or Hb followed 2 wk later by Hp (HbHp). Mice were analyzed at 6 and 11 mo post-infection (pi) for gastric pathology. Quantitative PCR for Hp colonization of gastric tissue, ELISA for anti-Hp IgG2a (Th1 isotype) and IgG1 (Th2 isotype), and quantitative PCR for IL-1β, TNFα, IFNγ, IL-10, and IL-13 gastric mRNA were also performed. HbHp mice had significantly less severe gastritis at 6 and 11 mo pi compared to Hp mice (P < 0.05). Atrophy, mucous metaplasia and hyperplasia were also significantly reduced in HpHb mice at 6 mo pi (P < 0.05), while intestinal metaplasia and dysplasia were less severe at 11 mo pi (P < 0.05). Hp mice had significantly less Hp colonization of the gastric tissue compared to HpHb mice (3.32 ± 0.07 compared with 4.58 ± 0.13 log #Hp/μg host DNA, P < 0.001), consistent with greater pathology in Hp mice. Hp mice had significantly higher Th1 associated IgG2a and a trend towards higher Th2-associated IgG1 responses to Hp compared to HpHb mice (P < 0.002, P = 0.10 respectively). Th1-associated cytokines IFNγ and TNFα were also upregulated in gastric tissue of Hp mice compared to HpHb mice at 6 mo pi (P < 0.05), with significant upregulation of IFNγ, TNFα and IL-1β occurring at 11 mo pi (P < 0.05). The Th2-associated cytokine IL-10 was upregulated in HpHb mice compared to HpHb mice at 11 mo pi (P < 0.05). These data suggest that pre-existent enterohelminic helicobacter infection can ameliorate gastric helicobacter pathology and modulate the Hp Th1-associated gastric and systemic immune responses.

PS8 Murine Norovirus, an Intercurrent Variable in a Mouse Model of Bacterial-induced Inflammatory Bowel Disease

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Murine norovirus (MNV) has recently been recognized as a prevalent viral pathogen in mouse colonies and causes disease and mortality in severely immunocompromised mice. Little is known about the potential of MNV to modulate mouse models in biomedical research. We tested the hypothesis that MNV infection alters disease course and immune function in a model of inflammatory bowel disease (IBD). FBV 129PF2-Aecbl1a/−/−/− (mdr1a−/−/− mice (n = 9 to 12 per group) were inoculated by oral gavage with either 1 x 10⁶ PFU MNV-4 or broth (control) 1 wk before gavage with 2 x 10⁷ CFU H. bilis. Mice were monitored for weight loss and clinical signs of colitis, and cohorts were euthanized when severe diarrhea or dehydration consistent with IBD (week 2 to 3 post-infection (pi) was observed. Mouse co-infected with MNV and H. bilis showed increased weight loss (P = 0.0266 and 0.0066, experiments 1 and 2, respectively) by week 2 pi with MNV. Co-infected mice also received higher histologic scores indicative of severe colitis compared with controls (P = 0.0296 and 0.0136, experiments 1 and 2, respectively). To assess whether MNV alters immune responses of dendritic cells (DCs) or T-cells, additional mechanistic experiments were conducted. Purified DCs isolated from MLN of MNV-infected or uninfected mice (n = 5/group) were incubated in vitro with antigen primed T-cells and H. bilis antigen at 2, 7, and 28 d pi, and interferon-γ (IFN-γ) production was measured. T-cells incubated with DCs from MNV-infected mice had increased IFN-γ production at 2 d pi but not at later time points, suggesting DCs prime T-cells more efficiently early after infection. In contrast, recall responses by MLN CD4+ T cells were not affected (data not shown). FACS analysis of MLN T-cells at day 9 pi (n = 5/group) also did not show changes in CD4+ or CD8+ populations or activation markers such as CD44 and CD45RB. Our findings indicate that acute infection with MNV alters antigen presentation by DCs and potentiates bacterial-induced IBD.
PS9 Assessment of Cross-foster Rederivation in the Elimination of Mouse Norovirus and Helicobacter

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Over a 10-mo period, 287 mouse litters were cross-fostered using 1 of 2 paradigms to eliminate murine pathogens including mouse norovirus (MNV) and Helicobacter species (Helicobacter). Paradigm 1 involved cross-fostering litters at less than 48 h of age with no attention to cage bedding material. Paradigm 2 involved cross-fostering litters at less than 24 h of age from cages in which the bedding material was changed within 24 h of cross-fostering. All litters from both paradigms were from strains of mice housed in rooms where MNV and Helicobacter were enzootic. Post-cross-foster rederivation mice were tested for the presence of Helicobacter using 16S ribosome nucleic acid fecal PCR at 4, 8, and 12 wk of age. Mice were tested for MNV using serology at 4 wk and viral nucleic acid fecal PCR at 12 wk. Eighty-six litters were cross-fostered using paradigm 1; 3 tested positive for fecal PCR at MNV at 4 wk. With regard to Helicobacter, 8 litters tested positive at 4 wk and 3 tested positive at 8 wk. No litters tested positive for Helicobacter at 12 wk. Two hundred and one litters were cross-fostered using paradigm 2; 1 tested positive for MNV at 12 wk and 1 tested positive for Helicobacter at 4 wk. These data indicate that cross-foster rederivation can successfully eliminate MNV and Helicobacter from contaminated lines of mice.

PS10 Susceptibility of Neonatal Mice to Murine Norovirus Infection

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Murine norovirus (MNV) infection is endemic in many mouse colonies. MNV causes subclinical chronic intestinal infections in adult immunocompetent mice, and the fecal-oral route seems to the primary means of transmission. The hypothesis that MNV infection in neonatal mice is age-dependent and requires ingestion of feces was tested. In an initial study, 3- and 7-d-old male and female Swiss Webster (SW) mice (8 per group) were inoculated orally with 20 ul of a 10% intestinal stock of MNV-L. Homogenates of intestines collected on dpi 3 and 7 d post-inoculation (dpi) from mice inoculated at 3 d of age were all negative for MNV by RT-PCR. In contrast, MNV-RT-PCR of intestinal homogenates collect on dpi 3 and 7 from mice inoculated at 7 d of age indicated that half of the mice were infected (½ on dpi 3 and ¾ on dpi 7). A second study was performed to determine if cross-fostering of neonatal mice from MNV-infected to uninoculated dams could be an effective way to prevent MNV infection of neonates. Sixteen pregnant SW mice were inoculated orally with 30 ul of MNV-L 3 to 5 d pre-delivery. Four litters each of 1-, 2-, 4- or 6-d-old pups (approximately 48) from MNV-infected dams (infection was confirmed by RT-PCR) were transferred to uninoculated dams with similar aged litters and vice versa. On post-partum day (ppd) 21, feces from all MNV-infected dams and litters transferred to them were MNV RT-PCR positive. In contrast on ppd 21, feces from all uninoculated dams and litters transferred to them were MNV RT-PCR negative. Almost half of intestinal homogenates from 11- to 13-d-old mice fostered to MNV-infected dams on ppd 4 or 6 were MNV RT-PCR positive, whereas intestinal homogenates from 8- to 9-d-old mice fostered to MNV-infected dams on ppd 1-2 were MNV RT-PCR negative. In summary, 1- to 3-d-old pups were resistant to infection by oral inoculation and contact with infected dams. Infection of 4- to 9-d-old mice occurred following direct inoculation but not by contact with infected dams. Only mice older than 10 d were infected by contact with infected dams. Therefore, susceptibility to MNV infection is age-dependent, and cross-fostering may be an effective means of eliminating MNV from endemic infected populations of mice.

PS11 The Use of a Murine Model for the Investigation of Candidate Virulence Factors of Enterotoxigenic Escherichia coli Strains Isolated from Humans

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Diarrhea caused by enterotoxigenic Escherichia coli (ETEC) is a major cause of morbidity and mortality in developing countries. Despite ETEC’s importance, no protective vaccine is available, partly due to the lack of an appropriate animal model of infection. We recently identified EtpA, a large protein secreted by ETEC, which mediates adhesion to intestinal epithelial cells. To study EtpA’s role in intestinal colonization, we used a recently developed murine model of ETEC infection. Female CD1 mice (5-8 wk old) were housed in microisolator cages with sterile food, water, and bedding. Intestinal bacteria were eradicated by adding streptomycin to the drinking water. Animals were fasted 12 h prior to bacterial inoculation. Treated water was replaced with sterile water 4 h prior to inoculation and cimetidine was administered intraperitoneally. Mice were gavaged with human ETEC isolate H10407 in a total volume of 400 μl. As control, AAEC191A, an avirulent E. coli strain, or jf1289, an etpA deletion mutant, were used. For colonization studies, mice were gavaged with 1 x 10^7 colony-forming units (cfu) of bacteria on days 0, 14 and 68, and then challenged with either 6 x 10^7 or 6 x 10^6 cfu of wild-type H10407. Bacteria were isolated from the small intestine and quantified to determine the degree of colonization 24 to 72 h later. For immunogen studies, mice were inoculated intranasally on days 0, 22, and 42 with recombinant EtpA protein, while control mice received adjuvant alone. Serum antibody response was measured with an in-house ELISA and mice were gavaged with 8.5 x 10^4 cfu of H10407 on day 63. The degree of colonization was determined 24 h later. Our results showed that mice exposed to ETEC are protected against subsequent colonization. These mice also mounted an immune response to EtpA (≥1:1024), during the course of experimental infection. etpA-deletion mutant ETEC strains were impaired in colonization, and intranasal immunization of mice with recombinant EtpA protein conferred protection against infection with ETEC strain H10407. These experiments validate the use of this murine model of intestinal colonization for use in testing candidate ETEC immunogens, and suggest that EtpA may be a viable target for vaccine development.
Controlled lighting in animal facilities has long been a concern to both biomedical scientists and animal care personnel. Light-induced chronobiologic disruptions can occur in a variety of behavioral and physiologic venues and compromise the nature and outcome of scientific investigations. Recent evidence indicates that night-shift workers have an increased breast and prostate cancer risk. Previously, our laboratory demonstrated that dark phase “light contamination” with as little as 0.25 lux (0.08 μW/cm²) suppressed production of the neurohormone melatonin (MLT) and stimulated rodent and human breast tumor growth and metabolism. This occurs through an MLT receptor-mediated suppression of signal transduction, as demonstrated using MLT receptor antagonists, resulting in inhibition of linoleic acid (LA) uptake and conversion to the mitogenic metabolite 13-HODE. Therefore, using “tissue-isolated” human PC3 prostate cancer xenografts in male (250 g) nude rats (Hsd:RH-Foxn1rnu), given free access to water and standard chow, we tested whether the nocturnal MLT level suppresses, while constant light (24L) stimulates, tumor growth activity. Animal room light intensities were monitored daily using a radiometer/photometer (IL 1400a). Exposure of PC3 xenograft-bearing rats (n = 10/group) to 24L (300 lux;123 μW/cm²) during animal room dark phase (12L:12L) for 2 wk resulted in a 2-fold decrease in latency-to-tumor onset (time of implant to palpable mass) and 2-fold increase in tumor growth rate (24L non-stress related), compared to rats maintained on a 12L:12D light/dark cycle (controls). Tumor arteriovenous blood samples collected at midnight revealed, as expected, nocturnal MLT levels that cycle (controls). Tumor arteriovenous blood samples collected at midnight revealed, as expected, nocturnal MLT levels that cycle (controls). 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order to streamline the system for SOP review, a program to automate the process is now used. Initial training occurs in a face-to-face staff meeting that can include various numbers of the department in attendance. Those in attendance sign off that they received the training. Those not in attendance have an SOP training module assigned in the company-wide online learning management program for each employee. The module includes a link to the SOP along with a short quiz to demonstrate that the SOP was reviewed. An automated email message is sent to each employee notifying them of the required training and another to the employee and their manager if the training is overdue. Employees can access their assignment, review the SOP, take the test, and document review in one easy process. We provide a 2-wk period to complete the training, thus keeping effective dates on schedule.

PS16 In-house Assessment of the AALAS Learning Library as the Sole Method for AALAS Technician Certification Training
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Since 2003, the AALAS Learning Library (ALL) has provided subscribed users the ability to access online study modules for the 3 levels of AALAS technician certification (ALAT, LAT, LATG). A switch from didactic training sessions to the sole use of the ALL for technician certification training was made to decrease the time away from work for both trainees and trainers as well as decrease the workload for those individuals who taught classes but who were not full-time trainers. After offering the ALL as the sole training method for AALAS technician certification at all 3 levels to over 30 technicians for 1 y, a survey was sent to those technicians to assess their feelings on the ALL and online training, what steps could be taken to enhance the training process, and the effectiveness of the ALL as a training tool (that is, whether or not the technicians passed). Survey results indicate that there was a 78.5% pass rate on the certification examinations, and that technicians feel multiple in-person review sessions in addition to the online modules and reminders of milestones (such as completion of a certain number of chapter modules) would enhance their training experience and outcomes.

PS17 High- and Intermediate-level Disinfection in Decontamination Locks: Development of a Validation Method
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The decontamination of heat-sensitive equipment to be introduced into laboratory animal holding areas is an important contributing factor for operating efficiency. A number of chemicals suitable for decontamination are available and their use is facilitated by complex technologies, such as gas generators, or by more straightforward technical methods, such as simple fogging of ready-to-use liquid disinfectants. We compared the performances of two hydrogen peroxide (HP) generators, a chlorine dioxide (CD) generator and a fogging system for intermediate-level disinfectants (ILD) in a large (282.5 ft3) decontamination lock. Liquids disinfectants tested by means of the fogging system were a glutaraldehyde-based disinfectant with sporidical properties (HLD) and a second product made of a blend of peroxygen compounds, surfactant and organic acids with limited sporidical activities (ILD). Geobacillus stearothermophilus and Bacillus atrophaeus spores at a concentration of 10⁶ spores/ml were used as biological indicators (BIs) in addition to cultures of Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, and Candida albicans at 10⁴ CFU/ml. After the definition of standard loads, represented by a 4-shelf trolley loaded with irradiated diet bags, and once the expected levels of performance (HLD, ILD) were achieved, cycle parameters in terms of quantity of chemical to be used (ml for HP and liquid disinfectants, ppm for CD), were defined. Cycles were repeated 3 times to confirm results. All 3 generators for delivery of HP and CD achieved 100% sporidical activity during repetitions. The length of the cycle for the dry HP was shorter (1 h and 30 min) and comparable to the CD generator (1 h and 40 min) in contrast to the wet HP system (2 h and 20 min). The ILD performed at a higher level than expected showing intense sporidical activity comparable to the HLD; an obvious limiting factor was the difficulty in the assessment of glutaraldehyde residue. None of the chemicals repeatedly used in the trial had any impact on the integrity of gaskets, stainless steel surfaces, or nylon components of the decontamination lock.
boxes between rooms and racks within the room is difficult to monitor. Investigators often challenge the accuracy of the census. We have implemented a census tracking system using warehouse management software and a combination of radio frequency identification devices (RFID) and bar-coding to address these issues (Dynasys, Clearwater, FL). The coordinated use of policies and procedures with the software and hardware has provided a cost-effective approach. Animal care technicians use an RFID induction station to add cages to inventory when cages are received or when cages are otherwise added. The technician then places cages within a specified room on a specified rack, and uses a combination of RFID and bar-coding to identify cages to rack and room locations. Weekly, the technician uses a mobile tower with a reader, computer, and antennae to identify movement of cages and census errors. Investigators remove cages from inventory by dropping the cage card holder, which is equipped with an RFID tag, into a check-out station. At the check-out station, investigators receive a printed receipt with protocol and cage card numbers along with an email that provides the time and date the specific cages were removed from inventory. Real-time data is provided through all steps of the process by the use of wireless technology. The system has been validated and has repeatedly provided 100% accurate data. It has proven to be cost effective through the reduction of labor previously associated with using bar coding alone for monitoring rodent cage census. Reports are generated identifying movement of cages from previous to current locations. Because of the receipts and real-time inventory, investigators are confident that the census is accurate.

PS20 Refinement of a Vivarium Quality Assurance Program by Incorporation of Vaporized Hydrogen Peroxide
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Rodent parvoviruses (PV) are among the most common and environmentally stable agents causing outbreaks in otherwise pathogen-free animal facilities, and can be extremely difficult to exclude and eliminate. Our facility experienced multiple outbreaks of PV, including KRV, RMV, H-1, MPV, and MMV. We define an outbreak as the detection of an excluded agent in one or more animals by serology or PCR. Detection of an agent in multiple rooms which share investigators, animals, or laboratories is considered a single outbreak. During the process of re-evaluating our vivarium quality assurance program, we identified inadequate decontamination of investigator laboratories following PV outbreaks as a potential weakness. Our decontamination procedures were cumbersome for investigators, requiring extensive preparation and down-time, and on occasion had damaged sensitive electronic equipment. We chose to incorporate vaporized hydrogen peroxide (H$_2$O$_2$) into our quality assurance program based on its demonstrated efficacy against canine parvovirus and its ability to effectively decontaminate sensitive electronic equipment without damage. Biological indicators (Bacillus stearothermophilus) have been used in rooms and inside equipment to validate the decontamination. Disadvantages of H$_2$O$_2$ decontamination included the complexity of equipment operation, engineering challenges and malfunctions, and safety issues. We addressed these issues through training, correction or alternative methods for engineering problems, detailed standard operating procedures (SOPs) that include step-by-step usage instructions and a setup checklist, and communication through meetings, email, and signage. Since starting the use of H$_2$O$_2$ in December 2005, we have seen greatly increased investigator cooperation, allowing not only decontamination following an outbreak, but also preemptive decontamination of laboratories on a regular schedule. Although PV outbreaks continue to occur in our facility, the use of H$_2$O$_2$ has offered a reliable method of decontamination for animal rooms as well as laboratories that house extremely sensitive equipment, making it an asset to our quality assurance program.

PS21 Significant Employee Noise Exposure in Cage-wash Facilities
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The National Institute for Occupational Health and Safety estimated that workers exposed to a 40-y lifetime of noise at 80, 85, or 90 dBA had an excess risk for hearing impairment of 3%, 16%, or 29%, respectively. MIT’s Environment, Health, and Safety office (EHS) has established a Hearing Conservation Program for employees exposed to time-weighted average (TWA) noise levels equal to or exceeding 80 dBA. Using criteria more conservative than OSHA standards, our EHS categorizes noise exposure as action level, defined as TWA exposure to 80 dBA, or maximum exposure level, a TWA exposure to 85 dBA. As part of an annual assessment, noise exposure in the clean and dirty rooms of 7 cage-wash facilities was measured over a 7-h work day by having 18 employees wear Quest Micro-15 dosimeters with microphones attached to their shirt collars. Results for 50% of the monitored employees were classified as action level, measurements ranging from 78.54 to 84.93 dBA, and 50% were exposed to the maximum exposure level threshold, measurements ranging from 86 to 91.50 dBA. Although TWA noise levels on the dirty side of the cage wash (n = 10) were higher than the clean side (n = 8) (P < 0.02), all facilities posed a similar noise exposure risk despite differences in room design and materials, construction age, and type of equipment. Based on these results, all cage-wash employees are required to attend an annual EHS hearing conservation training course. In addition, an annual audiogram is performed to ascertain whether there has been a significant shift in the individual’s hearing threshold. For comfort and convenience, technicians are provided a choice of several types of ear plugs and ear muffs that reduce noise by at least 20 dBA; their use is required. To further reduce noise, we are evaluating sound attenuating materials and refining work practices, such as turning off equipment when possible and using scrapers and a pre-wash to avoid banging cages on the dump stations.

PS22 Assessment of Cage Temperatures with Direct Intra-cage Ventilation Provided by the Building HVAC System
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Our institution has opened 2 buildings designed to house rodents in ventilated cages with the supply air for the cages provided directly by the building heating, ventilation, and air-conditioning (HVAC) system. Rack supply air and room air temperatures (temps) are controlled independently. Assuming the rack supply air temp would control the intra-cage temp, the decision was made to maintain the room at 68 °F and the cages at 72 °F. A series of experiments were designed to test this assumption.
Temperature recording devices (Data Logger™, Edstrom Industries) were placed to simultaneously measure room temp, rack supply air temp, and temps inside 4 cages. For all tests, the room temp was varied from target temps of 60 °F to 80 °F. For study 1, cages were varied at different locations on the racks. Since no difference was observed between the cages, all cages were placed on the middle row of the rack for ergonomic reasons. In study 1, rack supply temp was held constant at 72 °F. In study 2, the rack supply temp was set at 72 °F, 74 °F, and 76 °F, while the room temp was varied at each set point. For study 3, study 1 was essentially repeated, but with 3 to 4 CD-1 mice housed per cage. Regardless of the rack temp set point, the intra-cage temps followed the room temp as it varied during the study. For studies 1 and 2, when the room temp attained its maximum of 75 to 76 °F, the intra-cage temp peaked 1 °F cooler than the room temp. When the room temp approached the minimum of 62 to 63 °F, the intra-cage temp remained 2 °F warmer. For Study 3, when the room temp reached the maximum level (this time 77 to 78 °F), the intra-cage temp was 2 to 3 °F warmer than the room temp. As the room temp reached its minimum, the intra-cage temp remained 4 °F warmer. These results indicate that intra-cage temps are more significantly influenced by the temp of the room than by the temp of the rack supply air. The body heat generated by the mice also influenced intra-cage temps. Thus, intra-cage temps cannot simply be predicted based on the temp setting for air delivered to the rack, as the room temp and mouse body heat have more significant influences.

PS23 Gastric Helicobacter Species as a Cause of Feline Gastric Lymphoma in Pet Cats: A Viable Hypothesis

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Gastric Helicobacter spp are associated with chronic inflammation and neoplastic transformation in humans as well as domestic and laboratory species. The present study examined the association of Helicobacter heilmannii (Hhe) infection in pet cats with feline gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Tissues were collected by gastric biopsy or at necropsy from 47 pet cats with clinical signs of gastrointestinal disease, including vomiting and inappetance, and classified as gastritis (14/47), lymphoma (31/37), or normal (2/47). The cohort consisted of 13 castrated males, 5 intact males, 24 spayed females, 2 intact females, and 3 of unknown gender. Ages ranged from 1 to 17 y with a median age of 12 y; 3 cats’ ages were unknown. Tissues positive for argyrophilic organisms with Warthin-Starry stain (29/47) were assessed by fluorescent in-situ hybridization (FISH) for the presence of Hhe strains 1-4 as well as with a fifth probe that detected H. salomonis, bizzozeronii, or felis. A significant association of positive Warthin-Starry status with Hhe infection was found in cases of sick cats (22/29; P < 0.05 by Chi-square; $\chi^2 = 7.034$). Interestingly, a significant association between Hhe status and a diagnosis of lymphoblastic or lymphocytic lymphoma was observed as well in a subset of 24 Warthin-Starry positive lymphoma cases: of lymphoblastic lymphoma cases, 13/17 were positive for Hhe ($P < 0.05; \chi^2 = 4.854$). Hhe strains 2 and 4 were most commonly found (18/29 and 17/29, respectively) among sick cats, although a higher than expected number of cats were also positive for Hhe1, which initial reports have described as rare in cats and common in humans. The association found between a positive Hhe status with the presence of feline gastric lymphoma, especially lymphoblastic lymphoma, argues for the need to conduct prospective studies to better identify the frequency and strain distribution of Hhe infection in both healthy and clinically ill cats, particularly those cats with gastric lymphoma.

PS24 Using Toyota Production System Tools to Standardize Rodent Health E-Alerts

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Occasionally, rodent health problems are not accurately recognized or the time involved to respond and resolve those problems takes too long. As a result, animal welfare, research data, and staff morale may suffer. In addition, many animal care programs involve at least 2 technical staff levels to identify and document a health problem before the veterinarian or investigator are contacted. We developed a faster and less expensive approach by using a Toyota Production System tool known as value stream mapping. Such a map was created to evaluate our process of rodent health problem identification and treatment. It revealed 2 key areas in which too much time was wasted: initial diagnosis and investigator response, with some clinical cases going as long as 12 d between initial detection and treatment initiation. Consequently, we developed standardized email message templates to be used by front-line animal husbandry staff as well as others to report rodent health problems directly and quickly to investigators. Those emails were simultaneously copied to facility managers and staff veterinarians. Initial versions were in a paragraph format that described the health problem and recommended treatment, but were not consistent. No response was received for 50% of these emails, resulting in additional communications and delays in treatment. A second version was designed in which information was emailed in a color-coded grid, including a clear definition of the particular health problem; the corresponding action required of the investigator was displayed at the top of the email alert. Also included was the course of action to be automatically taken by our department if there was no response within the stated time interval. Since employing the new standardized email templates, the response rate soared to 90% across 5 barrier facilities and reduced maximum delays in treatment initiation from 12 d to 3.

PS25 MRI as a Diagnostic Tool in a Research Setting: The Diagnosis of Intracranial Infections in Two Pigtailed Macaques (Macaca nemestrina) with Cranial Implants

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Intracranial infections are a potentially life-threatening complication of chronically instrumented nonhuman primates, for which an immediate diagnosis is required for a successful outcome. Diagnosis is complicated by the non-specific clinical signs. This is demonstrated by unrelated cases of intracranial infection in 2 pigtailed macaques (Macaca nemestrina). Animal 1, an 8-y-old intact male, presented 8 mo post-cranial implant placement with intermittent recumbency in the cage, weight loss, and decreased performance at behavioral tasks. Physical exam revealed no significant findings; body temperature was 88.
100.4 °F, and the CBC showed a slightly elevated WBC count. Despite supportive care, the animal continued to lose weight. A follow-up physical exam revealed a wound margin infection at the perimeter of the head-implant and further elevation of the WBC count. At no time were overt neurological signs observed. Animal 2, a 9-y-old intact male with a history of seizures which were controlled successfully with phenytoin, presented 4 mo post-craniotomy and recording chamber placement (20 mm), with chronic weight loss, and observation by the laboratory technician of a small fluctuant mass under the dura mater, only visible through the chamber. In both cases, magnetic resonance imaging (MRI) was used to elucidate the diagnosis (Siemens Trio 3 Tesla MRI system). MRI was possible as neither cranial implant contained ferromagnetic material. Animal 1 was diagnosed with an epidural abscess and underwent craniotomy and open evacuation of pus. Animal 2 was diagnosed with a brain abscess which was subsequently aspirated. Antibiotic treatment for each animal was based on results from abscess fluid cultures. MRI is the most sensitive imaging tool to detect focal or diffuse brain parenchymal infectious lesions. It allows for early diagnosis, accurate localization of the lesion, and better monitoring of the response to medical therapy, all resulting in significant improvements in the prognosis. This is demonstrated with these 2 cases, where rapid diagnosis resulted in efficacious treatment and positive outcomes.

**PS26 Necrotoxigenic E. coli in Macaques**

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Macaques (Macaca mulatta, Macaca fascicularis) received from 2 US-based vendors are routinely pair-housed and receive health screenings during quarantine and at quarterly intervals. Twenty-five fecal samples collected either rectally or from cage pans from a cohort of clinically normal macaques (n = 92) during 2005-2006 were culture positive for a β-hemolytic *Escherichia coli*. Six of these isolates were submitted for serotyping and 5 serotypes were identified: O1:H7, O88:H7, O6:H7, O6:H1, and O2:H1 (2 isolates). Although treatment was not instituted because of the absence of clinical signs, disk diffusion susceptibility testing demonstrated that these isolates were most resistant to cephalothin (5 isolates). Molecular characterization of the *E. coli* by PCR revealed 5 of the 6 isolates were positive for the cytotoxic necrotizing factor 1 gene (*cnfl*), a virulence factor associated with necrotoxigenic *E. coli* (NTEC), previously found in domestic animal species and humans. The CNF1 toxin activates Rho-GTPases, a family of molecular switches with several cellular functions, resulting in the reorganization of the actin cytoskeleton. In tissue culture, affected cells undergo membrane ruffling, form focal adhesions and actin stress fibers, and become multinucleate. Previously published studies from our laboratory have identified *cnfl*-positive *E. coli* from diarrheic feces and diseased tissues of ferrets, including mammary gland, uterus and brain. The ferret and macaque samples were all PCR-negative for CNF2, a related cytotoxin with similar in vitro effects that has been associated with NTEC isolated from ruminants. NTEC strains from humans and animals are closely related and animal strains could be reservoirs for human disease, which manifests as urinary tract infections and neonatal meningitis. Because of the pathogenic and zoonotic potential of NTEC and its high prevalence in the colony, future studies are planned to further characterize these strains of hemolytic *E. coli*.

**PS27 Sentinel Monitoring of Rodent Holding Rooms: An Alternative Approach**

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Principal investigators (PIs) often need to remove rodents from vivaria for tissue collection or imaging. Because neither our facility managers nor our IACUC have control over sanitation procedures in these areas, other animals in the vivaria are at risk when these animals return to our facilities. We have created “holding rooms” in each facility to segregate these possibly infected animals from clean animals. This experiment compared the costs and benefits of our typical sentinel monitoring program to one in which each investigator had a sentinel cage that was monitored monthly. In our largest holding room, we placed one dirty bedding sentinel cage holding 4 Swiss-Webster mice for each of 35 PIs. One sentinel was tested at a commercial laboratory every 28 d using a comprehensive profile (gross necropsy, oropharyngeal and fecal bacterial cultures, pelt exam, and examination of ileocecal contents and feces) and a level II serology panel of 16 viral agents. This was compared to our standard practice of testing each of 6 static racks every 8 wk with a level I serology panel of 8 viral agents and every 24 wk with a comprehensive/level II panel. Three new sentinels were added when 1 sentinel animal remained in the cage. During the 46-wk experiment, the cost of the more intensive monitoring was $34,962.30, compared to $1,941.00 that would have been spent with the usual sentinel program. One outbreak of MHV was identified and localized to 1 investigator. These animals were euthanized, and weekly MHV serology of other sentinel cages in the room for 8 wk was added to the scheduled testing to assess for any spread to other cages. No other sentinel animals tested positive, and monitoring other investigator cages during the outbreak cost $733.12. If our “typical” approach had been employed in this outbreak, the costs alone to test all cages in the room and culled positives averaged $46,000 per outbreak. This does not factor in any costs to investigators for lost animals, results or time. Therefore, an investigator-associated sentinel cage can more quickly identify and localize an outbreak at lower cost and with minimal disruption and loss to other investigators.

**PS28 Helicobacter Species Isolated from the Ceca of Wild Mice as a Potential Source of Infection for Mouse Colonies**

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Little is known about the colonization of wild mice with *Helicobacter* species and the potential for zoonotic transfer and source of infection for other wild and laboratory mice. Using culture techniques followed by PCR and 16S rRNA sequence analysis, *Helicobacter* spp. were isolated and identified from the ceca of wild-caught mice in the greater Boston area. Between 2004 and 2007, 21 mice were captured in 5 different residential locations (A-D). Location A is rural and 32 mi north of Boston, locations B1 and B2 are in a suburb 15 mi north of Boston, location C is in a suburb 2 miles south of Boston, and location D is in a suburb 15 mi south of Boston. Eighteen of the mice were *Peromyscus* spe-
cies and 3 were *Mus musculus* subspecies. Mice were euthanized using CO₂ and the cecum collected and submitted for culture. Culture results for *Heliobacter* spp. suggested that each mouse was colonized with a single *Helicobacter* species. Three novel species of helicobacter were isolated and identified by sequence analysis of the 16S rRNA gene. MIT 05-5294 was isolated from 3 *Peromyscus* captured at location A and MIT 06-7409 was isolated from 1 *Peromyscus* also in location A. MIT 04-6687 was isolated from 12 *Peromyscus*: 4 from location A, 3 from location B1, and 5 from location D. Two *Mus musculus* subspecies from location C were colonized with *H. rodentium*. One *Mus musculus* subspecies from location B2 and one *Peromyscus* from location A were culture negative for *Heliobacter* spp. Among those species common in laboratory mice, only *H. rodentium* was found in the mice tested (2/21 = 9%). The prevalence of *Heliobacter* spp. in these wild mice was 90%. This study indicates that most wild mice are colonized with diverse *Heliobacter* spp. and serve as a potential reservoir for helicobacter infection of laboratory and other wild mice in the Boston area.

**PS29 Clinical Signs and CNS Effects of Meloxicam, Buprenorphine and Morphine Administration in Male Rattus norvegicus**

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Meloxicam, a novel acidic enolcarboxamide NSAID, has gained popularity due to its high therapeutic index, high intrinsic activity, low ulcerogenic potential and long duration of action in rodents. We wanted to introduce meloxicam at our site as a post-surgical analgesic alternative to the opioid buprenorphine. Buprenorphine, a partial agonist at the mu receptor, has adverse effects in rodents. A major concern for neuroscientists is the CNS activity associated with buprenorphine, which can confound interpretation of behavioral assays. The frequency of dosing of buprenorphine (every 8 h) also presents a logistical problem in the research setting, which meloxicam’s once-daily dosing regime effectively eliminates. The scope of the present study was to show that there are no adverse CNS effects associated with intraperitoneal administration of meloxicam. To assess adverse CNS effects, 47 male Sprague Dawley rats weighing approximately 220 to 240 g underwent clinical observations followed by an accelerating rotorod assay at 1, 3, and 5 h after administration. Clinical observations were made over a 5-min period per group of animals and included normal status, pica, piloerection, ataxia, prostration, staring, startle, eye squinting, lethargy, depression, abnormal gait, circling, seizing, freezing, aggression and excessive grooming. Groups were compared to vehicle (negative) and morphine (positive) controls. Each clinical observation, other than “normal,” was given a score of 1 point. There was no statistical difference in mean clinical score or rotorod performance between meloxicam (0.3, 1.0, 3.0 mg/kg) (n = 8) and vehicle (n = 7) administered intraperitoneally. There was an elevated clinical score and a statistically significant motor deficit in the rotorod assay in the morphine group (n = 8) administered subcutaneously (10.0 mg/kg) as compared to vehicle. Buprenorphine administered subcutaneously (0.05 mg/kg, n = 8) showed an elevation in clinical score with the longest duration as compared with vehicle. Based on these data, we conclude that meloxicam is a suitable analgesic for once-daily administration without perceivable CNS-related side effects.

**PS30 Disease due to Acid-fast Bacilli Septicemia in a Colony of African Clawed Frogs (Xenopus laevis) and Japanese Medaka (Oryzias latipes)**

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The use of aquatic species in biomedical research has grown immensely in the pharmaceutical and toxicology field due to high-throughput capabilities. With the increased use of these species, clinical disease due to infectious agents can lead to high morbidity and mortality. These diseases can be challenging to clinicians accustomed to diagnostic and treatment approaches in mammalian species. In this report, we describe an outbreak of bacterial septicemia in a small colony of African clawed frogs (*Xenopus laevis*) in which 2 of 7 frogs developed clinical disease, including coelomic distension and necrotizing ulcerations that resulted in death or euthanasia. Culture of coelomic fluid from one of these frogs demonstrated the presence of *Aeromonas hydrophila*; however, skin scrapings and impression smears yielded numerous intracellular and extracellular acid-fast bacilli. Histological analysis demonstrated inflammation, vasculitis, and edema along with acid-fast bacilli suspected to be a *Mycobacterium* sp. in a number of different tissues. During the same time frame, a colony of approximately 300 Japanese medaka (*Oryzias latipes*), which were housed in the same room as the frogs, experienced a 26% (78/300) mortality rate over a 4-mo period with little to no clinical signs. Zebrafish (*Danio rerio*), which shared the same recirculating water supply as the medaka, had been introduced approximately 5 wk prior to the medaka deaths and had no mortality. Skin scrapings did not demonstrate any acid-fast organisms; however, numerous acid-fast bacilli were observed histologically in spleen, kidney, liver, heart, intestines, and skeletal muscle. Cultures of several medaka identified the organism as *Mycobacterium marinum* complex. The *Xenopus* and medaka colonies were euthanized. A new medaka colony has been established separate from the zebrafish. Studies are ongoing to determine via molecular analysis if the same acid-fast bacillus led to the disease in both *X. laevis* and medaka. This report is believed to be the first describing disease in Japanese medaka due to a natural infection with *Mycobacterium marinum*.

**PS31 The Challenges of Ovariectomy on the Gray Short-tailed Opossum (Monodelphis domestica)**

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The gray short-tailed opossum (*Monodelphis domestica*), a small (60 to 150 g) marsupial native to Brazil is currently used at Ohio State in a study of behavioral neuroendocrinology. This study requires females to be ovarioctomized and males to be castrated at 11 wk of age. Opossums were induced and maintained under isoflurane anesthesia. The scrotum of the male is quite pendulous and castration was easily performed using a fixed, double ligation with absorbable suture. Unfortunately, ovarioctomizing the female was a much more challenging task. Initially, a dorsal flank incision was attempted as used in rats, but the ovaries were not easily accessible. The ovary is tightly adhered to the dorsal wall, making access from a flank incision
nearly impossible. Opossums mature slowly; they weigh only 40 to 45 g at 11 wk of age, and the immature ovary is difficult to visualize. Using an ophthalmic retractor, we were able to create a window in which to work through a ventral midline incision. Curved micro-hemostats were used to isolate each ovary, clamping across the blood supply on both sides. At this age, the arterial supply is minimal. Momentary occlusion using hemostats was effective for hemostasis, and ligation of the vessel was not needed following removal of the ovary. On recovery from anesthesia, opossums would immediately begin removing sutures or wound dressings. Successful closure was finally achieved using a buried line of suture, followed by surgical glue, and a bandage. The opossums were also kept sedate using acepromazine (2.5 mg/kg) given subcutaneously for the first 12 post-op to prevent suture removal. Ibuprofen (10mg/kg) was given orally for 2 d to provide post-op analgesia. Recovery was uneventful and the bandage was typically removed around 4 to 5 d after surgery. Once perfected, this technique for ovariec-toomy and wound closure was efficient and effective for creation of this unique model.

**PS32 Urinary Alkalization in the Treatment of Traumatic Rhabdomyolysis in Rhesus Macaques**

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A 15-y-old, 9.1-kg female rhesus macaque (Macaca mulatta) housed in an outdoor breeding group presented with severe crush wounds inflicted by cagemates. Physical exam revealed a capillary refill time of 6 s, pale mucous membranes, and a heart rate of 192 BPM. Severe ecchymosis, edema, and lacerations were noted on the extremities, thorax, perineum, and face. Due to marked hypovolemia, an IV catheter could not be inserted. An intraosseous catheter was placed and blood samples collected. The animal was secured to a restraint board and a urinary catheter inserted. Lab results included: BUN 52 mg/dl, CREAT 3.7 mg/dl, CPK > 30K U/l, K+ 4.5 mEq/l, and WBC 18K /ul. An urine test strip indicated a pH of 6.0 and Blood +, in the absence of hematuria. These strips cross-react with myoglobin, a hemoprotein found in muscle and associated with acute renal failure 48 to 72 h post-crush injury. Current medical literature suggests urinary alkalization (to pH > 6.5) increases myoglobin byproduct solubility in urine, allowing renal excretion with minimal tubular damage. This principle has been demonstrated to increase survival in earthquake victims with analogous injuries. Fluid therapy was initiated with 0.9% saline plus 50 mEq/L of sodium bicarbonate; 20 ml/kg was infused over 30 min, followed by 30 ml/kg/h for a total volume of 110 ml/kg. Urine output was monitored to maintain 1 to 2 ml/kg/h and a pH above 6.5. For 2 subsequent days, IV fluids were administered at 20 ml/kg/h for 100 ml/kg/d. The myoglobinuria resolved, bicarbonate was discontinued, and lab values returned to normal ranges. In the 8 preceding months, 1 macaque with rhabdomyolysis (RM) failed to respond to conventional fluid therapy, and 4 others were found dead with signs of RM. Since implementation of this protocol, 4 of 4 macaques presenting with traumatic RM (CPK > 65K U/l in one case) have been treated successfully. Urinary alkalization, correction of metabolic acidosis, and the treatment of hyperkalemia often associated with RM make bicarbonate a rational adjunct therapy for the condition in the rhesus macaque.

**PS33 Novel Wireless In-cage Running Wheels Used to Record Mouse Wheel Running Activity in Ventilated Rack Home Cages**

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Running wheels are popular enrichment devices. Numerous studies have shown positive effects of running wheels on behavioral measures, such as conditioned responding and memory. Accurate quantitation of running levels has only been possible using custom cages, or shoe box cages large enough to accommodate a running wheel and associated electronics. Our goal was to develop a running wheel small enough to be placed in standard mouse ventilated rack cages and able to transmit data, without any exposed cabling or electronics, to a computer. Three groups of male mice (5 to 7 wk old) were used. Experiments were approved by the IACUC. One group consisted of Crl:CD1(ICR) mice singly housed in ventilated rack cages equipped with wireless running wheels. In another group, Crl:CD1(ICR) mice were singly housed in shoe box cages with a lab animal running wheel attached. The 3rd group consisted of C57BL/6J mice group-housed (4 per cage) in shoe box cages containing a “pet-store” running wheel and bicycle odometer. In this group, the total distance ran was divided by 4 to normalize running distance per animal. Crl:CD1(ICR) mice in ventilated rack cages ran 2.28 ± 0.69 km (n = 3 mice) during the first day on a wireless running wheel. Crl:CD1(ICR) mice having access to lab animal running wheels attached to the outside of shoe box cages ran 2.13 ± 0.14 km during the first day of wheel access (n = 3 mice). C57BL/6J mice group-housed in shoe box cages ran 1.86 ± 0.54 km during the first day of access to “pet-store” running wheels (n = 4 groups of 4 mice). Running distances did not differ significantly among any of the groups (P = 0.8, 1-way ANOVA). In conclusion, we have developed a novel running wheel that is small enough to fit within the confined space of a typical ventilated rack mouse cage, occupying a footprint of less than 10 in.². This wheel sends data to a computer wirelessly. The design is flexible so that every cage in a mouse ventilated rack facility could be equipped with a wheel. This device will be useful for scientific studies where it is important to quantitate running levels, as well as general husbandry where monitoring population activity is essential.

**PS34 Human Activity and Visual Contact with the Surrounding Environment Affect the Stress Response and Behavior of Laboratory Rats**

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Laboratory rats are typically housed in clear or opaque cages on racks with multiple shelves. Depending on location, clear cages allow a view of the room and facilitate visual social contact with neighbouring rats, but may also induce anxiety due to lack of visual cover. We hypothesized that partial visual cover would be most beneficial for rat welfare. To evaluate effects of visual cover and shelf height on physiological and behavioral responses of rats during routine handling and behavior testing, we used adult male Sprague-Dawley Rattus norvegicus [Sim:(SD) FBR Albino] found free of disease via quarterly serology and parasitology, and yearly necropsy, evaluations. Rats (n = 54 pairs housed in
adjacent cages with the same visual cover) were assigned to 1 of 3 cage cover types [no cover, solid, or partial (walls covered with vertical opaque bands)] and three shelf heights [top (143 cm from ground), middle (82 cm from ground) or bottom (21 cm from ground)]. Chromodacryorrhea was recorded following weekly body weight measurement and cage cleaning. Behavior during anticipatory reaction to handling (ARH) and elevated plus maze (EPM) tests was assessed during weeks 4 (ARH1, EPM1) and 8 (ARH2, EPM2). Body weight, growth rate, chromodacryorrhea, and behavior in ARH1, ARH2, and EPM1 were not affected by the treatments. In EPM2, rats from partially covered cages spent the most time in open arms of the maze ($P = 0.015$), suggesting that they were the least anxious. Increased distance of the cage from human activity areas was associated with increased chromodacryorrhea secretion after cage cleaning ($P = 0.003$) and a tendency for increased avoidance of open arms in EPM1 ($P < 0.1$). In conclusion, providing rats with partial visual cover and controlling for levels of human exposure within rodent rooms could improve rat welfare and external validity of behavioral tests used in biomedical research.

**PS35 Physiological Responses to Increased Housing Density in C57BL/6J Mice**

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The effects of housing densities on the welfare of inbred laboratory mice have not been systematically examined. We report the physiological responses of C57BL/6J mice housed at different densities for 4 mo. Male and female mice were single-sex housed from 4 wk of age in double-sided duplex pens (51.7 in$^2$) with 4, 6, or 8 animals per pen. Six pens were established by density and sex. At the outset, the average weight for male mice was 13.9, 13.3 and 12.9 g for those housed at 4, 6, and 8 per pen, respectively, and similarly 11.5, 11.7 and 12.3 g for females. At 6 wk of age, a male from 1 duplex pair of pens had an intra-abdominal telemetry device implanted to monitor heart rate (HR), body temperature (BT) and activity. All mice were weighed every 2 wk and had blood collected at 12 and 20 wk of age to assess hematocrit, serum biochemistry, and hormones. Fecal corticosterone metabolite (CM) concentration was measured at 10, 16 and 20 wk of age. At 20 wk of age mice were euthanized, body composition was determined by dual energy X-ray absorptiometry (DEXA), and selected organs were weighed and fixed for histology. All mice survived, with none removed due to poor performance or increased aggression. Weight gain differences were most marked between mice housed at 4 per pen which gained significantly more weight than those at 8 per pen, (females $P = 0.002$, males $P < 0.0001$). Throughout the study, mice housed 6 per pen were the most active and tended to have higher HR and BT. Conversely, mice at 8/pen were the least active and had lower HR and BT. Few blood parameters showed significant changes between densities. Serum calcium, cholesterol, HDL cholesterol, triglycerides, free fatty acids and T4 were higher in mice housed at higher densities. Females housed 4 per pen had significantly higher fecal CM levels than those at 8 per pen ($P = 0.0005$) and heavier adrenal glands ($P = 0.0392$). Males at the highest density had significantly smaller adrenals ($P = 0.0276$). For both sexes there were very few measured parameters that changed consistently with housing density. These data suggest that the welfare of mice housed at a density of 6 per pen is little different to that of mice at 4 per pen but that this may not be true at 8 per pen. Further studies are in progress to assess the effects of these differences.

**PS36 A Replacement Program for Damaged Caging and Equipment**

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All primary enclosures should be kept in good repair to prevent escape of or injury to animals, promote physical comfort, and facilitate sanitation and servicing. This statement from the Guide for the Care and Use of Laboratory Animals presents a challenge when large animal populations are involved. The Center for Comparative Medicine (CCM) at Baylor College of Medicine has an average monthly population of mice of 42,000 cages. To meet the Guide’s requirement on this scale required CCM to find a creative, easy, and economic solution for regular cage replacement. Storing large quantities of caging was becoming a significant problem due to limited warehouse space. Tracking the “in and out” inventory movements became increasingly more difficult. To address the problem, a cage replacement program was developed. “Just-in-time” is a term used in the business world. Equipment is received and put into service just in time to be used by the facility that needs it. It is a way to manage equipment without large on-hand quantities that require storage in a warehouse or animal facility. Each facility is required to maintain a damaged equipment report and submit this information each week. This information is logged in a spreadsheet and analyzed on a monthly basis. The data include a weekly cage count for each facility to track the increase or decrease in the mouse populations. From this information, standing purchase orders were established for 1 y with a caging manufacturer. Each month the manufacturer automatically ships the designated amount of equipment to CCM, reducing the set quantity of the standing purchase order. If changes in the monthly allotment are needed, a call to the manufacturer is made to adjust that month’s quantity. (The manufacturer agreed to ship more or less depending upon our monthly needs.) This equipment is then distributed to the facilities based on the damaged equipment reports. Storage of the equipment is eliminated with direct delivery to each facility. Using this program will make it possible to track incoming and outgoing equipment, maintain operational costs to a known monthly amount, and reduce the amount of damaged equipment in the facilities.

**PS37 Strategic Scheduling using Process Metrics**

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The integration of process metrics data into an efficient, attainable, and equitable mouse husbandry assignment is essential for optimal productivity, quality care, accountability, and planning. Our union-management joint departmental committee previously developed time factors for all mouse direct care activities (16 specific activities, such as cage check/change/water bottles) and indirect care activities [3 specific activities, such as room sanitation, equipment preparation, functional (global) roles]
derived from analyzing data collected from all technicians in a academic program with 37,000 rodent cages. The purpose of this project is to develop a flexible scheduling platform that will use set time factors. We determined that the scheduling platform is most accurately based on a room profile rather than cage count. Variables include cage count, service type, room size and type, and prevalence of special services. Room servicing times were then used to calculate technician schedules using a 6-h, 10-min work day. A 5,500 cage facility, including ventilated racks, ABSL-2, and satellite housing, previously scheduled husbandry assignments based on a 440 to 840 cages/d metric and required 11 full-time equivalent employees (FTE) for all activities. Strategic scheduling resulted in assignments of 600 to 1,000 cages/technician/d, requiring 8 FTEs for all activities (including coverage of benefit time and for technicians with other global responsibilities). Another 18,000 cage ventilated rack facility previously required 26 FTEs for all husbandry activities and now requires 23 FTEs. We expected strategic scheduling to redistribute the workload equitably but not significantly increase productivity because the "pace" for each activity was based on program-wide averages. We conclude that the numbers of variables inherent to large, complex programs require a sophisticated, objective approach to achieve fairness and efficiency. These findings also suggest that there was time spent on activities other than direct and indirect care tasks and that strategic scheduling allows discretionary effort to be leveraged as a schedulable, accountable activity.

**PS38 Positive Reinforcement Training To Enhance the Voluntary Movement of Group-housed Sooty Mangabeys (Cercocebus atys atys)**

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Positive reinforcement training (PRT) has been successfully used to train a wide array of species to execute a variety of tasks which are helpful in the everyday care and wellbeing of the animals. Since there is little information available about training sooty mangabeys (Cercocebus atys atys), we analyzed the use of PRT with a group of 30 adult males as they were trained to shift from one side of their enclosure to the other. Over a 4-mo period we conducted 57 training sessions totaling 26.5 h of training and recorded compliance information. In the first 5 training sessions, 76% of the monkeys cooperated with the request to move; during the last 5 sessions, 86% cooperated, indicating some progress but still falling short of what was intended. After 25 training sessions, problem-solving techniques were applied to help the 5 consistently noncompliant animals become more proficient. The techniques included providing additional enrichment to make the area they were shifting to more desirable, emptying the run that a noncompliant monkey was moving into of other animals, and applying a variety of food reinforcements. The 5 sessions immediately following implementation of these changes had 100% success, but this rate of compliance was not maintained over time. To determine whether social rank affected training success, a dominance hierarchy was derived based on 7 h of behavioral observations collected using a modified all-occurrence method. The mangabeys were categorized into high, medium and low dominance ranks. An analysis of variance was near significance (F = 6.52; P = 0.06), indicating greater compliance among the high-ranking mangabeys. While training this large group has proved challenging, 25 of the 30 animals consistently cooperated. Since it is likely that social factors are accounting for some of the lack of compliance, continued problem solving focusing on social issues will be implemented.

**PS39 Effects of Enrichment on Aggression in Male Mice**

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Although it is generally assumed that providing environmental enrichment to laboratory animals is beneficial, we found in a previous study that severe aggression was higher in groups of male CD-1 mice given a running wheel. We hypothesized that this effect might not be seen in different strains of mice, or if destructible rather than rigid enrichments were used. Adult male mice (n = 120) from 3 strains (Balb/cAnNCrl; C57/Bl6NCrl; Crl:CD1[ICR]) were housed in polycarbonate cages containing 5 same-strain mice. The enrichments provided were either destructible (Nestlet® [NESTLET], Shepherd Shack® [SS]) or rigid (perspex tunnel [TUNNEL], Fasttrac and Igloo® running wheel/shelter [WHEEL]). Each cage was presented with each enrichment for a 2-wk period using a Latin square design, and with a 2-wk control (no enrichment) period inserted between each enrichment period such that each cage served as its own control. Behavior was assessed from video recordings taken for 15 min every hour for 24 h at the end of each period. Data were analyzed using the general linear model; control periods did not differ from one another and were combined for the analysis. There were significant enrichment by strain interactions. CD-1 and C57 displayed significantly more (Tukey, P ≤ 0.001 for all comparisons) severe aggression during TUNNEL (7.3 and 9.3% of active periods, respectively) than during control, NESTLET or SS (1.4% and 2.14%, respectively) periods, and also significantly more during WHEEL (10.1% and 15.64%, respectively) than TUNNEL periods. There were no differential effects of enrichment on aggression in BALB/c, since they showed no serious aggression during any period. Thus, providing rigid enrichments to either CD-1 or C57 males increased severe aggression, possibly due to territorial competition, and thus has the potential to be associated with injurious interactions in these strains.

**PS40 Effects of Cage Size and Enrichment on Aggression and Stereotypic Behavior in Three Strains of Laboratory Mice**

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The Guide recommends providing 96.8 cm2 of floor space per 25 g mouse. However, there is limited scientific information about the effects of different space allocations on mice, particularly on their behavior. The purpose of this study was to examine the effects of both floor space and environmental enrichment on mouse behavior, in order to evaluate the importance of both quantity and complexity of space. Female mice from 3 strains (C57BL/6NCrl, BALB/cAnNCrl and Crl:CD1[ICR]) were housed in polycarbonate cages each containing 4 same-strain mice under 1 of the following conditions: small (58 cm2/mouse) enriched or non-enriched cage; medium (96.8 cm2/mouse) enriched or non-enriched cage; large (219.4 cm2/mouse) enriched or non-enriched cage; large (219.4 cm2/mouse) super-enriched cage. There were 5 replicate cages per treatment, and commercially available enrichments (Nestlet™, Mouse Tunnel, and Shepherd Shack®) for enriched groups; plus Mouse Igloo and spaceFast-Trac®, Guimabone and Mouse Hut for super-enriched groups) were used. Behavior was videotaped for 45 min/d at
0200, 0715, and 2115 h, from 10 to 16 wk of age. Data were analyzed using repeated measures ANOVA. Behavior was affected by enrichment, but not by cage size. Aggression was less frequent in enriched than non-enriched cages (6.0 ± 0.7 compared with 3.0 ± 0.7 bouts; F1,69 = 13.16, P < 0.001), particularly in C57BL/6 housed in super-enriched cages. There was an interaction between strain and enrichment for stereotypy (F2,69 = 3.64, P < 0.05), with CD-1 mice exhibiting less stereotypic behavior in enriched cages. There was a variable amount of stereotypy in the large cage treatments depending upon strain, but in the super-enriched cages no stereotypic behavior was observed in any strain. The results from this study suggest that, for female mice, increased complexity of space is more important than the quantity of space provided per mouse.

**PS41 Work Teams and Team Leaders in Laboratory Animal Care**

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Toyota Production System (TPS) is an operational approach that has improved quality while reducing waste in many manufacturing and service firms; we adopted it in 2004, with the goal of providing defect-free products or services on time, every time, at the level needed by the customer or researcher. Since 2004, we have expanded our program from 50,000 ft² of academic research space to 80,000 ft², while increasing rodent cage capacity from 13,300 to 33,000 cages. This was accomplished while maintaining staffing levels of approximately 100 full-time equivalent employees (FTEs). A contributing factor is the creation of exceptional teams from exceptional staff that understand and follow the TPS philosophy. We developed select individuals well versed in TPS to function as specially trained team leaders. These team leaders function as exemplary members of their 3- to 4-person team, coordinate the daily team responsibilities, ensure standard procedures are being followed, identify areas of waste and continuous improvement, and otherwise serve as role models for TPS practices. Last year, we piloted a new team leader training program which began with workshops that emphasized TPS management philosophy, techniques, tools, and team leadership skills. Included in the workshops were team projects geared towards practicing what they learned before its implementation in their own teams. We emphasized that teamwork and leadership are skills that must be learned, just as other skills in animal husbandry, a different approach from traditional animal care program strategies. While there were several obstacles in adjusting to a team setting, the advantages to both morale and productivity for our staff included sharing skills and knowledge with each other, rotating through tasks and gaining exposure to more areas of the facility, improving individual accountability, and avoiding fatigue and mistakes that can be caused by long periods of repetitive work.

**PS42 Establishment and Management of a Full-service SPF Mouse Barrier Facility to Reduce Personnel Traffic and Spread of Murine Disease**

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In an effort to initiate a program to control and eradicate endemic mouse parvovirus in several mouse facilities, a full service, “entry-restricted” SPF rodent barrier, focusing on breeding colony management and facilitating up to 4,500 mouse cages, was opened in 2004. Housing of animals in this unit was “by request,” and each PI (and their staff) requesting entry was “interviewed” prior to admission. It was explained that the main purpose of the facility was as a repository for breeding colonies. Access to animals in this facility by investigators and their staff was limited, and strict mouse import guidelines were instituted (redelivery or purchase from established vendors). An electronic breeding colony log was developed to reflect each investigator’s colony to serve as a method of communicating investigator’s needs regarding their animals to vivarium technical staff by email; information includes: pairing/mating requests, weaning, DNA sampling, mouse identification, culling and consolidation, copulatory plug checking for timed-pregnancies, mouse release to research areas and breeding/project planning. Investigators provide vivarium technicians with supplies and/or information necessary to assist them with experimental projects, including feeding special diets, fasting, blood collection, and injections. Our full-service barrier also includes routine cage care, a web-based animal health care program and minimal experimental support. Despite limited access to their animals, restrictions on imports, and increased per diem costs (24% higher than other facilities), investigators have been overwhelmingly positive regarding breeding success, freedom from disease, and reduction in their staff time to manage breeding colonies. After 3 y of operation there have been no virus outbreaks. Many more investigators have requested housing in similar facilities, prompting us to extend this “limited access” policy to 2 newly opened mouse facilities.

**PS43 Efficacy of Disinfectants Against MVM- and MNV-contaminated Surfaces**

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Successful environmental disinfection is an integral part of preventing the spread and introduction of pathogens in laboratory animal facilities. The purpose of this study was to evaluate common disinfectants used in laboratory animal facilities against 2 of the most prevalent viral pathogens in laboratory rodents: rodent parvoviruses and murine noroviruses (MNV). The disinfectants evaluated were bleach (10% solution, generic brand), Trifectant (1% solution, Vetoquinol), Clidox-S (1:5:1 dilution, Pharmacal Research Labs), and Spor-Klenz (undiluted ready-to-use solution, Steris). Minute virus of mice (MVM; from Dr. David Pintel, University of Missouri) was used as a representative of rodent parvoviruses and MNV-4 (a field isolate from our laboratory) as a representative of MNV. Viruses [4 x 10⁵ plaque forming units (PFU) of MVM or 2 x 10⁸ PFU of MNV] were dried onto glass slides to simulate contaminated impervious surfaces such as cage change hoods and cardboard squares to mimic rodent shipping containers. Disinfectants were sprayed onto virus-contaminated surfaces. After 10 min of exposure, viral titers were determined. All experiments were performed in quadruplicate. Disinfectants showing greater than a 2-log₁₀ reduction in cultivatable virus following a 10-min exposure were further assessed at 1-, 2-, 5-, and 10-min exposures to determine viral reduction with shorter contact times. For MVM on glass, bleach and Trifectant reached maximum efficacy at 2 and 5 min, respectively; Clidox and Spor-Klenz were ineffective. For MNV on glass, bleach, Trifectant, and Spor-Klenz all resulted in at least 2-log₁₀ reduction in viral titers and reached maximum efficacy by 1 min; Clidox was ineffective. On cardboard, none of the disinfectants were effective at killing either MVM or MNV.
PS44 Immune Factors Affecting MPV Shedding and Transmission

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Mouse parvovirus (MPV) is a highly prevalent and difficult-to-control infection of mice. Although there is no clinical disease associated with infection, MPV disrupts research by inducing T cell dysfunction. To better understand the role of immune deficiency in the shedding and transmission of MPV, we examined the impact of corticosteroid-induced immune suppression on MPV reactivation and determined the immune cell types and locations that contained high levels of MPV. To determine if immune suppression resulted in reactivation, 15 BALB/cByJ and 15 C57BL/6J mice (3 per cage) were inoculated orally with 30 infectious doses of MPV-1d; infection was confirmed by serology. On post-inoculation day (PID) 17 and 28, feces collected from all mice were negative for MPV DNA. Dexamethasone was administered in the water (100 ug/ml or 0.5 mg/mouse) to 9 BALB/cByJ (3 cages) and 9 C57BL/6J (3 cages) for 2 wk (PID 28 to 42). Six mice of each genotype served as controls. Two contact sentinels were housed in each cage with the 3 index mice from PID 28 to 35 and were replaced with new sentinels from PID 35 to 63. PCR of pools of feces from the 3 index mice in each cage indicated low levels of MPV DNA in 1 cage of immunosuppressed BALB/c mice at PID 42 and PID 63, 1 cage of untreated C57BL/6J mice at PID 42, and 1 cage of untreated BALB/c mice at PID 63 cage. Feces from the 2 sentinels in contact with immunosuppressed BALB/c mice in one cage between PID 28 to 35 were also MPV PCR-positive. All contact sentinels were seronegative. Reactivation of low levels of MPV shedding was observed regardless of immunosuppression, and while transmission to sentinel mice was seen, the virus dose transmitted was not sufficient to induce seroconversion. A second experiment used flow cytometry to determine which cell types (B, CD4+, CD8+, dendritic, macrophages, natural killer) contained high levels of MPV VP2 antigen during peak infection (PID 7) in BALB/c mice. MPV was detected in 2% to 15% of natural killer cells in the mesenteric lymph nodes. Significant levels of MPV were not detected in other lymphocytic cell types or in peripheral blood and spleen. These preliminary studies suggest natural killer cells have a significant role in MPV infections.
embryos generally led to a 10- to 100-fold reduction of MMVp. Independent of embryonic stage and time of virus exposure, recipients receiving in vivo embryos exposed to a minimum of 10^2 TCID_{50}/ml MMVp seroconverted by day 42 after ET, while 10 washing steps in the IVF-ET procedure were sufficient to remove the virus to a non-infectious dose. The results indicate that MMVp can be transmitted to recipients even after washing in vivo embryos 10 times prior to ET, but there is minimal risk of transmission of MMVp by in vitro-produced embryos to recipients if spermatozoa become contaminated with such viral loads as used in the present study.

PS47 Development and Validation of a Multiplexed Fluorometric Immunoassay™ for Serodiagnosis of Rabbit Infectious Diseases

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A Multiplexed Fluorometric Immunoassay™ (MFIA™) for serosurveillance of laboratory rabbits was developed using the Luminex xMAP® bead-based technology. Antigen coupled beads for adenovirus, CAR bacillus, C. piliforme, E. cuniculi, lymphocytic choriomeningitis virus, pneumonia virus of mice, reovirus-3, rotavirus-A, simian virus-5, and Sendai virus were part of the 14-member rabbit MFIA bead panel. Conventional antigens (whole virus or partially purified lysate) or purified recombinant antigens were individually coupled to the different color-coded bead sets. Internal tissue control beads were coated with the lysate of the wild baculovirus-infected Sf9 insect cells to determine the sample related nonspecific antibody binding. In addition, rabbit IgG and goat anti-rabbit IgG were added to the MFIA panel as system and sample suitability controls, respectively, to validate individual runs of the MFIA. Multiplex assays were run using high and low immune sera controls for the viral antigens and a non-immune serum was added as a negative control. In the validation study, 16 known positive sera from naturally or experimentally infected rabbits for one or more of the above mentioned infectious agents and 16 known negative rabbit sera from specific pathogen free colonies were tested using multiple technicians on multiple days. The antibody status of validation positive and negative samples was previously determined by indirect immunofluorescent antibody (IFA) test which was the only method used in the lab for serological analysis of rabbit sera. A total of 2442 assays were performed and analytical performance of the rabbit MFIA assay including selectivity and limit of detection was found to be comparable to or better than those obtained by IFA. Similarly, the diagnostic sensitivity and specificity of rabbit MFIA was ≥95% compared to ≥90% for IFAs of individual infectious agents indicating that MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of laboratory rabbits.

PS48 Enrichment Delays Gait Disturbances in BIO 14.6 and BIO TO2 Dystrophic Hamsters

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Enrichments to laboratory animal housing contribute to animal model phenotypes. Hamsters, for example, are known for their extensive voluntary wheel running, but most animal facilities do not routinely provide activity wheels. We hypothesized that access to activity wheels might affect motor function in the delta-sarcoglycan-deficient hamster strains BIO 14.6 and BIO TO2, models of muscular dystrophy. Accordingly, we compared the gait of BIO 14.6 and BIO TO2 dystrophic hamsters to healthy BIO F1B controls. We applied ventral plane videography (DigiGait) to quantify gait in 3 mo and 9 mo old male BIO 14.6 (n = 10), BIO TO2 (n = 10), and BIO F1B controls (n = 10) on a transparent treadmill belt walking 16 cm/s. Five hamsters from each group were provided voluntary activity wheels in their cages for 1 mo prior to study, though wheel activity was not measured. Gait indices were based on approximately 10 strides. We found kinematic and postural changes in BIO 14.6 and BIO TO2 hamsters not provided activity wheels, including shorter swing, stride, and stance durations (P < 0.05). Stride length was approximately 13% shorter P < 0.05) in BIO 14.6 and BIO TO2 dystrophic hamsters at 3 mo and 9 mo of age compared with BIO F1B controls. Propulsion duration of the hind limbs, an indicator of muscle strength, was shorter in 9 mo BIO 14.6 (236 ± 14 ms) and BIO TO2 hamsters (244 ± 8 ms) compared to BIO F1B (303 ± 11 ms; P < 0.05). Hind paw eversion, evidence of muscle weakness, was greater in 9-mo-old BIO TO2 than in BIO F1B control hamsters (20.1 ± 1.1 degrees compared with 6.7° ± 1.7 degrees; P < 0.05). Propulsive deficits were apparent in BIO 14.6 and BIO TO2 hamsters not provided activity wheels at 3 mo of age. Gait disturbances were absent in 3-mo-old, and blunted in 9-mo-old, dystrophic hamsters that had access to activity wheels for 1 mo. Our quantitative analysis demonstrates gait disturbances commence as early as 3 mo in male BIO 14.6 and BIO TO2 dystrophic hamsters that do not have access to activity wheels. One month of wheel activity may be sufficient to blunt motor dysfunction at 9 mo of age. These findings underscore the importance of laboratory conditions, animal activity, and quantitative gait analysis for developing therapeutic approaches to muscular dystrophy.

PS49 Differential Susceptibility of CD and SD Rats to Rat Theilovirus (RTV): A TMEV-like Virus Isolated from Rats

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It has long been recognized that rats can develop antibodies that react with antigens of Theiler’s murine encephalomyelitis virus (TMEV) of mice, but that rats are not infected with mouse strains of TMEV. Recently in Japan, a rat TMEV-like virus (NGS910) was isolated and passaged in rats that is genetically distinct from TMEV, documenting the presence of a TMEV-like virus of rats, hereafter referred to as rat theilovirus (RTV). Very little is known about RTV, yet based on serologic data, it is among the most prevalent viral pathogens infecting rats used in biomedical research. In this study, a RTV isolate was cultivated in BHK cells from the
HPLC analysis confirmed chow homogeneity and appropriate outcomes, yet the desired biological efficacy directed at COX-2 shown to be safe. Reducing the dose of CBX improves animal being given documented doses of CBX that were previously rate to 3 in 112 rats (3%, 36 ± 33 d). GI lesions developed despite ppm for subsequent studies, which decreased the complication Maryland rats (35 ± 8 d). The CBX dose was reduced to 1000 rats (28 d) experienced GI complications compared to 3 of 10 Fredericks, MD) of Harlan. After 60 d, only 1 of 10 Indianapolis plasma concentrations of CBX. A pilot study was conducted with GI flora and were negative for known rodent microbial entities. (31 ± 26 d). Microbiology and virology panels showed normal complications were more sporadic among intervention groups 13 animals (77%) expired within an average of 11 ± 3 d, while portions of the small bowel. In the prevention group, 10 of 13 animals either died suddenly (n = 9) or were euthanized (n = 16). Necropsies revealed typical findings of gastrointestinal (GI) perforations or obstructions in the jejunal-duodenal portions of the small bowel. In the prevention group, 10 of 13 animals (77%) expired within an average of 11 ± 3 d, while complications were more sporadic among intervention groups (31 ± 26 d). Microbiology and virology panels showed normal GI flora and were negative for known rodent microbial entities. HPLC analysis confirmed chow homogeneity and appropriate plasma concentrations of CBX. A pilot study was conducted with F344 rats obtained from separate vivaria (Indianapolis, IN and Frederick, MD) of Harlan. After 60 d, only 1 of 10 Indianapolis rats (28 d) experienced GI complications compared to 3 of 10 Maryland rats (35 ± 8 d). The CBX dose was reduced to 1000 ppm for subsequent studies, which decreased the complication rate to 3 in 112 rats (3%, 36 ± 33 d). GI lesions developed despite being given documented doses of CBX that were previously shown to be safe. Reducing the dose of CBX improves animal outcomes, yet the desired biological efficacy directed at COX-2 inhibition may become compromised. Although this investigation did not identify the basis of increased susceptibility and sporadic response of F344 rats, the dose relationship between CBX and the GI effect is apparent.

PS51 Evaluation of a PCR Microarray Platform for the Detection of Rodent Infectious Agents by Fluorogenic Nuclease PCR

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Nucleic acid microarrays traditionally depend on capture oligonucleotides that are bound to solid surfaces or polymer beads. The use of capture array technology for the detection of infectious agent nucleic acid has been hampered by poor analytical sensitivity, but can be improved by including a PCR step prior to hybridization. However, introducing a pre-amplification step necessitates either the use of multiplex PCR, which can lead to competitive inhibition and cross-assay primer interaction, or PCR product pooling, which promotes amplicon contamination. A recently devised PCR microarray platform permits the amplification of up to 3072 nucleic acid targets directly in the array. This platform eliminates the need to multiplex or pool PCR products and also accommodates the use of fluorogenic nuclease PCR technology, which has been documented to provide better analytical sensitivity and specificity over conventional gel-based PCR. To investigate the utility of this new platform, an array was produced which contained primer and probes representing 20 common viruses and bacteria. Each of the 48 64-hole subarrays contained each agent assay in triplicate holes so that 48 samples could be evaluated for all 20 assays on the same array. Ten-fold dilutions of each target nucleic acid were evaluated by both the PCR microarray and a standard 96-well PCR format (duplicate wells) to compare analytical sensitivity. All sub-arrays and duplicate 96-well sets were positive at 100 copies, and 60% of both sub-arrays and duplicate 96-well sets were positive at 10 copies. The PCR microarray was resistant to PCR inhibition associated with nucleic acid isolated from feces and also to high concentrations of test nucleic acid (600 ng/μl final reaction concentration). Tissues, fecal pellets, and nasal aspirates, which had each previously been determined to be PCR-positive for at least one agent by the 96-well platform, were also positive for the same and sometimes additional agents when evaluated with the PCR microarray. Potential applications for the PCR microarray include virus panels for biologics testing and select infectious agent panels for routine health monitoring.

PS52 A Nonhuman Primate Model for Evaluation of Pathogenesis and Immunity to Avian Influenza A (H5N1) Virus

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Avian influenza A (H5N1) virus infection in 2004 caused respiratory disease in 54 humans, resulting in 33 deaths. To better understand this viral disease, we established a nonhuman primate model to study the pathogenesis and immunity to H5N1 virus. Six male and 3 female rhesus monkeys (Macaca mulatta), 2.5 to 3 y old, were used in our study under ABSL-3 conditions.
Infected animals were injected with 7 ml (10^5 TCID_50/ml) of influenza virus A/Goose/Guangdong/NH/2003 (H5N1) intratracheally. Control animals received noninfectious allantoic fluid. After infection, clinical signs were assessed and blood samples were collected daily. Peripheral blood leukocyte counts were performed by microscopy. Antibody response was detected by ELISA. Dynamics of T lymphocyte subsets were analyzed by flow cytometry. On days 1, 3, 10, and 14 post-infection, 1 monkey was euthanized and tissue samples were either stored at –70°C for virological test or fixed in formalin for pathological analysis. Monkeys infected with H5N1 virus exhibited fever, dyspnea, lethargy, anorexia, and so forth. Gross lesions were mainly focal or extensively consolidated in the lungs. Lesions in consolidated pulmonary tissue involved alveoli and bronchi, with desquamation of respiratory epithelium, intra-alveolar hemorrhage, hyaline membrane formation and leukocytes infiltration. Virus isolation, RT-PCR and immunohistochemistry showed that the respiratory tract was the major viral target. A significant decrease in the total number of circulating leukocytes occurred during the infection period, but recovered to normal levels 7 d post-infection. The antibody increased rapidly from days 7 to 11 post-infection, and then slowly increased at least until day 50. The numbers of CD4+ and CD8+ lymphocytes significantly decreased during the infection period, and an upward trend returning to normal levels was seen after day 7. These results are similar to those found in patients infected with H5N1 virus. This is the first report that rhesus monkeys infected with a H5N1 virus isolated from a goose in the Guangdong province can be used as a model that resembles the respiratory disease in humans caused by H5N1 viruses, and will be useful for evaluation of novel vaccines as well as antiviral drugs against H5N1 viruses.

PS53 Passage of cDNA Expression Vector from Maternal Circulation into Rodent Embryo Germ-line Cells and Expression in Subsequent Generations
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Oocyte pronuclear injection is widely used for creation of transgenic mice, but the method is inefficient, resulting in euthanasia of many animals. For example, 10 or more females are euthanized as donors of fertilized oocytes, and 80% to 90% or more offspring are not transgenic and are euthanized. In the present study, 5 pregnant Cr:CD-1(ICR) mice at day 6.5 of gestation were injected in the tail vein (without anesthesia) with a GFP expression vector that included an ampicillin resistance gene (ampr). Thirty-five founder animals were analyzed by PCR for vector sequence in their DNA; 15 were positive for either GFP or ampr sequence, and another 12 were positive for both GFP and ampr sequences. Every founder litter included offspring positive for both GFP and ampr. Some founders positive for both GFP and ampr were mated to give 3 litters of F1 generation animals. These F1 animals were analyzed for ampr and GFP sequence in DNA. GFP and ampr sequences were detected in 7 of 11, 9 of 12 and 4 of 13 of the animals in the F1 litters. Southern blot analysis of F1 animals showed hybridization of a 32P-labeled GFP sequence probe to a band the size of the vector that had been injected into the pregnant grandmothers of the F1 animals. F2 and F3 generations of animals were produced. All tested animals of subsequent generations were positive for the presence of vector gene sequences. Although GFP protein was expressed, as indicated by western blot, the level of GFP protein was inadequate to give animals that were visually fluorescent green. Animal use could be reduced by 50% to 80% or more using this method because it does not require euthanizing oocyte donor females and its efficiency would require fewer offspring to be euthanized because they were not transgenic. Possibly, this method may allow difficult strains of mice, as well as other rodent species and even larger animals, to be made transgenic. The ability to make transgenic animals from a greater range of strains and species could contribute to improved models for the study of human disease, to better models for drug discovery and toxicity testing and to more accessible platforms for commercial production of bio-pharmaceuticals.

PS54 Use of Fluorogenic PCR to Estimate the Prevalence of Diarrheagenic Bacteria in Asymptomatic Nonhuman Primates
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Idiopathic diarrhea in nonhuman primates remains a significant colony health concern. Asymptomatic carriers of bacteria associated with diarrheal disease may be a potential source for outbreaks. Fluorogenic TaqMan PCR assays specifically targeting the ipaH gene from the virulence plasmid of Shigella species; the invA invasion gene of Salmonella enterica strains; the 16S rRNA gene from Campylobacter coli, C. jejuni, C. lari, and C. upsaliensis; and the 16S rRNA gene from a wide range of Helicobacter species were performed on DNA extracted from fecal specimens obtained from groups of apparently healthy cynomolgus macaques (Macaca fascicularis, a total of 93 from 4 colonies) and olive baboons (Papio anubis, 25 from a single colony). Bacteriological culture screening for Shigella, Salmonella, and Campylobacter was also performed on replicate specimens from 2 of the macaque groups (53 animals) and from the baboons. Results of the PCR testing indicate that Helicobacter and Campylobacter sequences are virtually ubiquitous in the feces of NHPs: 100% of macaques and 96% of baboons tested PCR-positive for Helicobacter, while 99% of macaques and 92% of baboons tested PCR-positive for Campylobacter; no samples tested positive by the Salmonella PCR assay. DNA sequence analysis of positive samples showed the presence of sequences identical to those of H. macacae, H. cinaedi, C. coli, C. jejuni, and C. upsaliensis. PCR testing found the presence of Shigella sequences in 14% of all macaque samples, varying among the 4 groups of macaques from 0% to 28%. 44% of the baboon specimens tested positive by Shigella PCR. Sequence analysis confirmed the presence of the Shigella virulence plasmid in PCR-positive samples. Only 23% of macaque specimens and 12% of baboon specimens were positive by Campylobacter culture, and none were positive by Shigella culture, although Salmonella isolates were obtained from 1 macaque specimen and 1 baboon specimen. These results suggest that common bacteriological methods may significantly underestimate the prevalence of potentially diarrheagenic bacteria in asymptomatic animals.
Poster Sessions

P1 Bilateral Abnormal Eruption of Deciduous Teeth in a Cynomolgus Macaque (Macaca fascicularis)
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Physical examination of an adult male cynomolgus monkey revealed bilateral gingival masses (0.5 by 0.5 cm right and 1.0 by 1.0 cm left) ventral to the gap between the first and second mandibular premolars. On each side, fistulous tracts drained purulent material to the gingival surface adjacent to the second premolar. CBC and serum chemistry results were in normal range. Radiographs revealed small, sparsely mineralized structures bilaterally. The right second premolar and associated mass were surgically removed. Two wk later, the left second premolar and associated mass were also removed. After each extraction, a long-acting penicillin G procaine and benzathine (40 IU/kg dose) was administered pending preliminary histopathology findings; this is the antibiotic that we regularly use in our primate colony. Analgesia prior the extraction was initiated and maintained for 3 d with buprenorphine HCL (0.03 mg/kg) and flunixin meglumine (1 mg/kg). We provided fruit, juice, soaked biscuits and pellets for a minimum of 3 d. The right and left lower second premolars were unremarkable. Each mass contained a single small, unerupted deciduous tooth. The right deciduous tooth had marked, suppurative pulpitis and chronic gingivitis with fibrosis. Dense mats of gram-positive and gram-negative bacterial and fungal hyphae were present on the tooth surface. The fungal hyphae extended to the enamel. The left deciduous tooth had slight to mild pulpitis, gingivitis, and was covered by a dense mat of mixed bacterial colonies. Despite these findings, efforts to culture for microbiological evaluation were unsuccessful. The bilateral masses were retained deciduous teeth complicated by gingivitis and pulpitis. Inflammation of the gingiva and pulp was secondary to colonization with oral bacteria and fungi. Surgical removal was curative. Abnormal eruption of deciduous teeth is not uncommon and bilateral presentation may suggest a genetic possibility that trauma can be the possible cause.

P2 Sudden Onset of Mortality within a Colony of FVB/n Mice
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Five breeding pairs of FVB/n mice, less than 23 d of age, were obtained from a commercial vendor, housed in a semi-barrier animal room, and produced 37 FVB/n offspring (17 male, 20 female) in 3 litters. One female FVB/n mouse was found dead with no premonitory symptoms 12 wk later. No gross lesions were noted at necropsy. Over the next 4 wk, an additional 15 female mice and 1 male mouse (3 to 4 mo old) were found dead. All mice were noted to have wet fur below the mandible and on the ventrum of their neck. One clinical report described abnormal behavior in 1 mouse after a cage change, and is the only report of an observed seizure. A serology panel was submitted from a remaining mouse and found to be negative for all major mouse pathogens. All mice were housed in a room with multiple mouse strains, including an immunocompromised strain on the same IVC rack. None of the other strains developed any morbidities or mortalities during this time period. Differential diagnoses for such acute mortality with no clinical signs were acute toxicity, degenerative disease, seizures, iatrogenicity, or vascular disease. Histopathologic examination was performed on all major organs from 4 mice, and revealed multifocal areas of neuronal necrosis and loss in cerebrum. Gliosis was also detected in brain sections stained for GFAP in 1 of 2 affected mice. This presentation is reminiscent of a syndrome with sudden death and variable CNS lesions that was reported within the last decade in both transgenic and wild-type populations of FVB mice and named “space cadet syndrome.” This syndrome may have arisen as a mutation first reported in the FVB/NCR subline and lines derived from NCR stock. While both sexes are affected, females are predisposed. The behavioral manifestation of this syndrome, including withdrawal from social interaction, lead to the memorable name. The condition is thought to result from neuronal necrosis in the brain due to seizure activity leading to the behavioral changes in survivors. The vendor that supplied these mice described this condition as “consistent with FVB strain-related neurological problems,” indicating the incidence of this condition may be underdiagnosed and should be considered in evaluation of FVB/n wild-type and Tg phenotypes.

P3 Refinement in the Management of the Denervated Canine Urinary Bladder Model Using an Abdominal Vesicostomy
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Bladder management in canine models of spinal cord injury requires manually voiding the animal using external pressure. Vescicostomies are routinely used in humans but not in canines. In a study of bladder reinnervation in dogs, sacral nerves mediating bladder contraction were identified with nerve stimulation and transected under isoflurane anesthesia. Denervation was confirmed by disappearance of bladder contraction with electrical stimulation of the conus medullaris. Vescicostomy was created by opening the bladder through a midline abdominal incision at the urachal remnant. The mucosa was everted and fixed to the skin. Analgesia included preoperative fentanyl patch, IV morphine during and 18 h post-op and ketoprofen for 5 d post-op. Animals returned to the normal housing 48 h post-op. Topical antibiotic was applied daily for 7 d post-op. Animals were followed twice daily for vescicostomy patency, inflammation, and fever for post-op periods up to a full year. In 1 animal, the vescicostomy narrowed 3 mo post-op and a repair was done before stenosis developed. One other animal removed the stitches prior to complete healing, requiring subsequent surgical procedures. In a few animals, irritation ensued from the constant contact of urine with the skin of the lower abdomen and inner thighs; daily topical application of petrolatum ointment circumvented this symptom. None of the canines in this study developed stenosis of the vescicostomy after the initial procedure. Contractility studies of bladder muscle strips from these animals showed no evidence of changes associated with bladder hypertrophy. Immediately before euthanasia, pelvic nerve stimulation failed to induce bladder contraction in the 2 nerve root transection-only animals but induced bladder contraction in 5 of 9 root transected and immediate repair, 3 of 3 coccygeal to sacral root transfer, 3 of 4 immediate genitofemoral nerve transfer (GFNT), 3 of 4 GFNT 1 mo after denervation and 5 of 6 GFNT 3 mo after denervation. This study demonstrates other neurologic problems,” indicating the incidence of this condition may be underdiagnosed and should be considered in evaluation of FVB/n wild-type and Tg phenotypes.
vesicostomy as a refinement method for management of the neurogenic bladder in canines. This avoids the distress and possible bladder hypertrophy induced by multiple daily interventions to empty the urinary bladder.

**P4 Nonhuman Primate Model of Severe Heart Failure Associated with Dilated Cardiomyopathy**

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Heart failure is the leading cause of human morbidity and mortality. However, the critical events that impair myocyte performance are poorly understood. Therefore, large animal models of spontaneous heart failure that reliably mimic human disease are required so that the causative mechanisms can be understood and lead to the development of novel drugs, diagnostic procedures and therapies. The present study evaluates spontaneous heart failure associated with dilated cardiomyopathy in a breeding colony of cynomolgus monkeys (Macaca fascicularis). We identified 10 affected monkeys (8 males, 2 females) using noninvasive electrocardiography (ECG), radiography, echocardiography, blood pressure measurements, electrocardiography (ECG), blood pressure measurements, echocardiography, blood pressure measurements, and histological evaluations, and compared these data with 123 clinically stable monkeys. The affected monkeys were 5.9 to 28.4 y old (mean, 17.5 ± 8.4 y) and weighed 4.1 to 8.1 kg (mean, 5.9 ± 1.2 kg). Echocardiography revealed dilated ventricles, decreased fractional shortening, and ejection fraction, and radiographs showed heart enlargement compared with that of clinically stable monkeys. Blood ANP and BNP levels were significantly increased in the affected monkeys. Wall motion abnormalities and myocardial viability were revealed by ECG gated MRI. Overt symptoms of heart failure such as loss of left ventricle function, hypertension, dyspnea, pleural effusion and ascites were evident in 5 of the affected monkeys. Two of the animals experienced sudden death with heart failure; histological analysis showed interstitial fibrosis and complicated myocardium. Five of the affected animals showed familial occurrence of the disease. We analyzed human mutations of the R9C in phospholamban and S151A, DK238, R97Q in δ-sarcoglycan loci in affected and unaffected animals in the pedigree. We identified a single nucleotide polymorphism of R97Q in the affected animals. We thus identified a novel, spontaneous, and hereditary nonhuman primate model of heart failure associated with dilated cardiomyopathy that closely resembles the human syndrome. This model should represent a useful research resource for the development of novel therapies, drugs, and diagnostic techniques.

**P5 Prevalence of *Giardia intestinalis* Infection and a New Treatment Option for Giardiasis in a Colony of Common Marmosets (Callithrix jacchus)**

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*Giardia intestinalis* is a common protozoan parasite causing diarrhea in humans worldwide. It has been described in many laboratory animal primates including rhesus macaques, squirrel monkeys, and common marmosets. While its role as a possible contributor to the induction of gastrointestinal disease in these species remains unclear, it does represent a zoonotic risk to animal handlers. Currently, treatment of giardiasis in nonhuman primates is a challenge because of low drug efficacy and taste aversion. This study sought to investigate the prevalence of *Giardia* in a colony of common marmosets using a *Giardia* fecal antigen capture assay and to address the possible eradication of this infection with tinidazole, a human-approved antiprotozoal similar in action and palatability to metronidazole, but requiring only 1 to 2 doses. Pharmacokinetic data is available for humans but not other primate species. Interactions with other drugs commonly used in nonhuman primate medicine are not reported; side effects are minimal but can include neurological effects, dizziness, anorexia, and vomiting (not seen in this study). Tinidazole should not be used in pregnant animals or those prone to seizures. Results indicated that 12 of 31 (38.7%) singly housed colony marmosets were positive for *Giardia*. No statistical difference was noted between species or age of monkeys. Two doses of oral tinidazole treatment (30 to 60 mg/animal, based on human dose), administered by oral gavage 4 d apart, resulted in elimination of infection from all animals as assessed on 5 subsequent antigen capture tests up to 20 d post-treatment. While long-term follow up on this cohort of animals will be necessary, we provide evidence that elimination of *Giardia* from a closed colony of common marmosets may be possible. Oral gavage is a reasonable method to overcome taste aversion to the drug, and tinidazole may be considered for treatment of other nonhuman primate species because of the favorable dosing schedule.

**P6 Polypoid Cystitis in a Research Beagle Dog**

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A 4-y-old intact male beagle (*Canis familiaris*) presented with a history of intermittent hematuria over a 3- to 4-wk period. The animal was not being used in a research study. Dietary regime consisted of a standard canine laboratory diet ration and reverse osmosis water ad libitum. Findings on physical examination were within normal limits. Hematology and serum chemistry panel results were within normal reference ranges. The urinalysis was positive for blood (250 Ery/μl), with a pH of 7.5, and a sediment consisting of red blood cells and aggregates of transitional epithelial cells. No crystals were observed. A culture and sensitivity was not performed. A double-contrast cystogram was diagnostic for cystic calculi. This was the first such case seen in our colony. While a cystotomy and surgical removal of the stones was considered, the high rate of recurrence in dogs despite prescription dietary management precluded this therapeutic plan in a research setting. Therefore, the animal was euthanized and a necropsy was performed. Necropsy findings included 12 dark tan, irregularly shaped uroliths, approximately 1 to 10 mm in diameter. Crystallographic stone analysis identified the composition as calcium oxalate dihydrate. (The fact that calcium oxalate crystals are usually found in acid urine may explain their absence in the urine sediment.) Multiple irregular masses with mucosal proliferation of the urinary bladder were also observed at necropsy. Chronic, polypoid cystitis was diagnosed by histology. These findings were consistent with chronic irritation and likely a result of the urolithiasis. Polypoid cystitis is a rare lower urinary tract disease of dogs characterized by chronic inflammation of the bladder mucosa and development of a polypoid mass or masses without histopathological evidence of neoplasia. The cause of polyp formation in dogs...
remains unknown, but surgical removal has been reported to be the most efficacious treatment. Whether mutations in benign polyps can lead to the development of malignant neoplasia is unknown. Additional studies are needed to determine the relationship between chronic bladder irritation and inflammation, polypoid cystitis, and the development of transitional cell carcinoma.

**P7 Hemipenal Plugs with Secondary Abscessation in Laboratory-housed Anoles (Anolis cristatellus)**

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Wild-caught anoles (Anolis cristatellus) from Puerto Rico were housed in polycarbonate cages with cob bedding and a wooden dowel perch. Males were housed singly, females either singly or in pairs. Cages were housed in an environmental chamber maintained at 78 to 82 °F with a 12:12 light:dark cycle and an average relative humidity of 60% until after December 2005, when low humidity spikes were noted (the lowest was 32%). Humidity in the anole’s natural environment is consistently 70% to 80%; the recommended humidity for captive anoles is 60% to 80%. Humidifiers were added to the room, and cages were misted daily with water to mimic the natural water source. Anoles were fed waxworms and crickets supplemented with Reptavite. Dyseccysis (abnormal shedding) was evident in some anoles. In the following winter months, approximately 20% to 30% of the male anoles developed distinct cloacal swellings with dark green/brown discoloration. Rarely, ulceration of overlying skin was noted. Urates were often adhered to the cloacal skin. Three male anoles developed chronic swelling near the cloacal fold, with the diagnosis of hemipenal plugs. Hemipenal plugs should be suspected in captive anoles showing chronic swelling near the cloacal fold, especially in times of lower relative humidity. The plugs were difficult to remove without damage to the hemipene. Cultures showed heavy growth of Proteus mirabilis and Escherichia coli. Histology revealed large masses composed of concentric layers of keratinized epithelium, eosinophilic amorphous debris, macrophages, erythrocytes, and heterophilic. There was extension of the inflammation into the adjacent skeletal musculature with myocyte necrosis. Following diagnosis, recommendations to the investigator included treatment with hydrotherapy and gentle manual removal of the hemipenal plugs followed by systemic antibiotic treatment (Baytril, if needed). No further mortality was reported in the colony. Seasonal low humidity within the environmental chamber is implicated in dehydration. Subsequent thickening of any reproductive exudates (semenal fluid) is suspected to inhibit their ejection. Additionally, an absence of suitable substrates for rubbing and plug removal are suspected in the formation of this condition. Hemipenal plugs should be suspected in captive male anoles showing chronic swelling near the cloacal fold, especially in times of lower relative humidity.

**P8 Cutaneous Mycobacteriosis in a Cynomolgus Macaque (Macaca fascicularis)**

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An 8-y-old breeder male cynomolgus macaque, group-housed outdoors, presented with a 4- by 8-cm, full-thickness skin wound on the ventral abdomen. Initial wound care included cleaning, debridement, topical applications of silver sulfadiazine, and hydroactive dressings. Systemic therapy was initiated with cefotiofur sodium for 10 d and flunixin meglumine for 3 d. After 10 d of treatment, portions of the lesion remained edematous, erythematous, and produced serosanguineous exudate. The antibiotic was subsequently changed to enrofloxacin, resulting only in partial improvement after 5 d, at which time surgical excision of the affected tissue was elected. Enrofloxacin regimen was extended and the frequency of administration increased to BID. The wound responded favorably until wk postoperatively. Inguinal and axillary lymph node abscessation and a fistulous draining tract originating from the cranial extent of the incision site were noted. Bacterial culture of material recovered from affected areas yielded Staphylococcus aureus and an acid-fast bacteria, later identified as Mycobacterium chelonae (no sensitivity was provided). Additional diagnostic tests, including TST (tuberculin skin test), CBC, serum chemistries, and blood culture were within normal limits; abdominal and thoracic radiographs were unremarkable. TST was also performed on the cagemates; results were negative. Due to potential colony health ramifications and humane concerns, the macaque was euthanized. On gross examination the incision site was partially healed but ulcerations with crusting were present. Axillary and inguinal areas had umbilicated, raised lesions with draining tracts. Histopathology revealed a pyo-granulomatous/necrotizing dermatitis with acid-fast bacteria; lymph nodes sections revealed diffuse moderate lymphoid hyperplasia with no evidence of neoplasia. To the authors’ knowledge, this is the first reported case of cutaneous mycobacteriosis in a nonhuman primate. In contrast, in the human literature, atypical mycobacteriosis is commonly reported, usually associated with natural and processed water sources, with the potential to contaminate post-traumatic wounds and cause chronic skin and soft tissue infections after surgical procedures.

**P9 Infertility and Hermaphroditism in Phenotypically Male Chimeric Mice**

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Generation of chimeric mice through genetic engineering technologies has become a common procedure in the field of biomedical research. Here, the efficiency of germline transmission in a transgenic core facility was established through the production of embryonic stem cell chimeras using microinjection of unmanipulated 129/E6 wild-type embryonic stem cells into C57BL/6 blastocysts. A total of 59 chimeric mice were produced including 52 males and 7 females. Germline transmission occurred in 34 males and 1 female (59.3%) and did not occur in 2 males and 5 females (11.9%); the remaining 16 males and 1 female (28.8%) did not produce offspring. In total, 65.4% of male chimeras were germline. At 5 to 6 mo of age, 16 phenotypic males and 1 phenotypic female were determined to be infertile when housing them with C57BL/6 females or males, respectively, for at least 2 mo did not yield offspring. The reproductive tracts from 11 of these phenotypic males were available for histopathologic analysis. Seven of these were diagnosed as true hermaphrodites based the presence of unilateral ovaries and testicles. Four mice had only testes bilaterally. Ovaries were well differentiated and characterized by follicles in variable stages of development, presence of eggs, aggregates of lutein granulosa cells admixed with cystic corpora hemorrhagica, and multifocal aggregates of hemosiderin-laden macrophages. All but 1 testis had spermatogenesis with spermatooza in the epididymis and variable hyoplastic and dysplastic changes. The reproductive
tracts from 3 of these males contained a tubular organ resembling a distended uterus with minimal gland development, and 2 of these presented as large scrotal swellings prior to necropsy. The pathologic findings are consistent with other published reports indicating that hermaphroditism is not an uncommon phenomenon in the production of chimeric mice and may be associated with infertility in transgenic colonies.

**P10 Methicillin-Resistant \textit{Staphylococcus aureus} (MRSA) Pneumonia in a Rhesus Macaque**

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A 2-y-old breeder female rhesus macaque, group-housed outdoors at an AAALAC International-accredited facility, exhibited signs of respiratory distress during a routine biannual physical examination. The animal was transferred to the hospital for a comprehensive evaluation and medical care. Blood was collected for a complete blood count and serum chemistries. Respiratory tract specimens were obtained via tracheal lavage for bacterial culture and sensitivity. Thoracic radiographs and tuberculosis skin testing were performed. Physical examination and thoracic auscultation revealed moderate dyspnea, intermittent coughing, and markedly increased respiratory sounds. Treatment was initiated with penicillin-G and ceftiofur sodium for 10 d. The CBC revealed marked neutrophilia (17,658, 81%) and a relative lymphopenia (3052, 14%). There was marked leftward cardiac displacement on the ventral-dorsal radiograph. Tuberculin skin tests were negative (24, 48, and 72 h readings). Cultures from the tracheal wash yielded a heavy growth of \textit{Escherichia coli} and methicillin-resistant \textit{Staphylococcus aureus} (MRSA). The animal’s condition waxed and waned during a period of more than 3 wk of continuous therapy before totally deteriorating. The macaque was humanely euthanized, and a postmortem examination was performed. The left mediastinal lymph nodes were enlarged. The left cranial and caudal lung lobes were dark and had a purulent exudate on cut section. Histopathology revealed severe multifocal to diffuse purulent bronchopneumonia with intraseional gram-positive bacteria and bronchus-associated lymphoid tissue (BALT) hyperplasia. Additional gram stains of the lung revealed occasional small aggregates of gram-positive cocci intermixed with short gram-positive rods. A culture taken directly from the lung parenchyma at necropsy also yielded a heavy growth of MRSA. In human medicine, MRSA infection is a serious global problem of epidemic proportions, with an increased prevalence among soft-tissue infections in the HIV-infected human population, newly admitted patients, and premature infants in community hospitals over the past years.

**P11 Possible \textit{Escherichia coli}-associated Hemolytic Uremic-like Syndrome in a Rhesus Macaque**

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An 18-y-old female rhesus macaque, group-housed outdoors at an AAALAC International-accredited facility, was presented for lethargy. Physical examination revealed a grade IV/VI systolic heart murmur. Three foci of alopecia and pyoderma were noted on the thorax. An ulcer was present on the tail, superficial to a healed fracture site. A complete blood count, serum chemistry, and skin cultures were collected, and the animal was placed on enrofloxacin. Initial blood work indicated a mildly elevated BUN (31 mg/dl), mild bilirubinemia (0.5 mg/dl), a hematocrit of 35.1%, and 3 nucleated red blood cells (nRBCs). Skin cultures were positive for coagulase-negative \textit{Staphylococcus} spp., sensitive to enrofloxacin. The skin lesions responded to treatment, and a partial caudectomy was performed. Ten d later, the animal became anorectic, lethargic, and unresponsive. Lab work revealed severe azotemia (BUN 278, CREAT 15.1) and a regenerative microcytic normochromic anemia with marked red blood cell fragmentation (HCT 28.8%, 63 nRBCs). A blood culture was positive for \textit{Escherichia coli}. The animal died shortly thereafter. On postmortem exam, both kidneys had dark black-red foci throughout the parenchyma and diffuse discoloration. The myocardium was pale, the heart valves appeared mildly thickened, the left atrium was dilated, and there was left ventricular enlargement with a diminished lumen. Histologically, the most significant lesions were observed in the kidneys. These included acute tubular necrosis with hemoglobin-cast deposition, glomerular thrombosis, fibrinoid necrosis and vasculitis. Similar renal lesions have been described in cases of hemolytic uremic syndrome (HUS) in humans and domestic animals. This syndrome is associated with a shiga-like toxin produced by certain strains of \textit{Escherichia coli} (O157:H7). Prognosis is usually poor because there is no treatment of proven value other than supportive care. Differential diagnoses include acute renal failure, disseminated intravascular coagulopathy, and other causes of microangiopathic intravascular hemolysis such as vasculitis, malignant hypertension, and systemic sclerosis. To the authors’ knowledge, this is the first report of spontaneous \textit{Escherichia coli} infection-induced HUS-like syndrome in a rhesus macaque.

**P12 An Accessible and Humane Approach to Mouse Intubation**

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With several surgeons operating a complicated model of coronary occlusion/reperfusion requiring mechanical ventilation and using inhalant anesthesia, we required a novel approach to rapid, accurate mouse intubation that did not require specialized peripheral equipment. The method that we developed incorporates common laboratory implements and greatly reduces anesthesia time, user error, and tracheal damage. Mice anesthetized with isoflurane are placed supine on the crook side of the vertical leg of an L-shaped plexiglass support (fabricated in-house, each leg 2” x 5”). The animal is restrained with a rubber band placed beneath the animal’s incisors and around the support port. Trans-illumination is accomplished by aiming a horizontal microscope light at the mid-tracheal level. The animal’s tongue is held aside with a pair of forceps shielded with PE tubing and the tongue is flattened against the lower jaw with the bent end of a small weighing spatula. The backlit larynx is easily visualized and a 20-gauge, 1.25-in. Teflon i.v. catheter is inserted into the trachea. The animal is placed supine by rotating the plexiglass support ~90 degrees about the vertex and endotracheal tube placement verified by presence of condensation on the tube and actuation of the lungs with a small disposable pipette. The animal is removed from the apparatus, and the endotracheal tube attached to a ventilator. With this technique, the animals move from induction chamber to supplemental ventilation and
P13 Strategies for Elimination of Mouse Parvovirus
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Eliminating mouse parvovirus (MPV) from our continuously occupied multi-user facility has been difficult. Sentinels (Hsd:ICR, CD-1® mice) were group-housed in non-filtered polycarbonate cages on room floors, exposed to soiled bedding weekly and sampled every 6 wk. Detection sensitivity by the sentinels was likely compromised by a low prevalence of infection in resident mice and poor transmission to sentinels. From 2001 to 2005, our sentinel program indicated 30% of our rooms had a history of MPV-positive sentinel infections. The incidence of MPV was essentially unchanged during the period of time, in spite of depopulation and decontamination of MPV-positive rooms. Specific epidemiological traits were observed in these rooms; the eradication strategy was modified to focus on rooms with these traits. MPV-positive rooms usually had one of the following characteristics: breeding colonies, multiple users, or rooms where mice were frequently transferred to or from testing laboratories outside the vivarium. Therefore, the strategy was modified to periodically depopulate and decontaminate rooms with these characteristics, regardless of negative sentinel results. Advance notice was given to researchers to stop receiving animals into a room scheduled for decontamination and to complete their studies in that room. The schedule was flexible to avoid interfering with research projects. Since MPV persistence is also affected by its resistance to environmental inactivation, decontamination procedures were modified from liquid chemicals only to an additional method for decontaminating rooms, laboratories and equipment using vaporized hydrogen peroxide (VHP). VHP is a process that maintains vapor concentration below the condensation point to protect sensitive instrumentation. The number of breeding rooms was also decreased. Subsequently, MPV transmission and physical exam, and good body condition. The fertile mouse was housed with 2 females in a harem breeding scheme to produce progeny for malarial studies. Differential diagnoses included otitis externa, otitis media, or central vestibular disease (including brainstem or cerebral disease). The mouse was euthanized at the request of the attending veterinarian; subsequent necropsy revealed no gross lesions. Microscopic findings consisted of necrotizing vasculitis in multiple medium-sized arteries of several tissues, including skeletal muscles, heart, mesentery, and renal hilus, with focal (unilateral) hemorrhage and necrosis of the cerebral medulla, corresponding to the clinical signs. Vascular lesions included expansion of the subintimal and medial laminae with increased numbers of neutrophils, spindled cells, and necrotic cellular debris. In some vessels, a thick layer of hypereosinophilic material replaced the lamina media. Multifocal, thick layers or nodular collections of plasma cells and lymphocytes obscured the adventitial layer and perivascular tissues. Necrotizing polyarteritis is a chronic degenerative disease which has been identified in several mammalian species, particularly rats of the spontaneously hypertensive (SHR) strain and rats with chronic glomerulonephropathy. Mouse strains known to develop necro- tizing polyarteritis include SL/Ni, (NZBxNZW) F1, MRL, and PN. Some institutions recognize these lesions as spontaneous findings in C57BL/6j mice, yet a review of the literature revealed little published descriptive information. Although direct causal relationship between the brain and vascular lesions remains unproven, the cerebral lesions potentially developed as a sequel to prolonged tissue hypoxia. Thus, we report spontaneous necrotizing polyarteritis and associated encephalomalacia in a C57BL/6j mouse; the lesions are similar to necrotizing poly- arteritis reported in other species and strains.

P15 Mice Consume More Drinking Water with Liqui-gel Ibuprofen than Drinking Water with Children’s Ibuprofen
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A previous publication and abstract have described the effectiveness of oral children’s ibuprofen for post-surgical analgesia. Ibuprofen is a non-steroidal anti-inflammatory (NSAID), non-selective COX1 and COX2 inhibitor commonly used for its analgesic, anti-inflammatory and antipyretic properties. Ibuprofen is available in multiple forms: children’s elixir and liqui-gel capsules. When forced to use the liqui-gel capsules due to the unavailability of the children’s elixir, we found that mice consumed more medicated water and had a more distinct clinical response. Therefore, we proposed to compare the palatability and efficacy of oral children’s berry-flavored ibuprofen elixir (1 mg/ml) and oral ibuprofen liqui- gel (1 mg/ml) on wound repair. Mice (n = 14 to 15 per group) with various sized wounds were given children’s ibuprofen elixir or ibuprofen liqui-gel and fed a standard rodent diet. Liqui-gel capsules were cut and the contents added to the drinking water; the contents dissolved easily without changing the color or consistency of the water. The addition of the children’s ibuprofen elixir to the drinking water resulted in a cloudy, pale orange-colored water that developed a detectable odor after 2 d and left a significant amount of white residue in the water bottles. Water and food consumption was measured over a 9-d period; other factors were monitored such as locomotor activity, grooming frequency, and hydration status. Mice consumed more oral ibuprofen liqui-gel (mean 11.7 ± 2.27 ml/d, P = 0.0001) over a 9-d period than the children’s ibuprofen elixir (mean 6.83 ± 1.47 ml/d, P = 0.0001). In addition, the mice on oral ibuprofen liqui-gel consumed 2 times the amount of food and were more alert, active, and well groomed than when given children’s ibuprofen elixir.

P14 Spontaneous Necrotizing Polyarteritis and Encephalomalacia in a C57BL/6j Mouse
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1Univ. Res. Animal Resources, 2College of Veterinary Medicine, University of Georgia, Athens, GA

This report describes the finding of multifocal necrotizing polyarteritis and encephalomalacia in an 8-mo-old C57BL/6j male mouse with a 2-mo history of progressive head tilt, normal mentation and physical exam, and good body condition. The fertile mouse was housed with 2 females in a harem breeding anesthesia very rapidly and with minimal equipment. Thus, the window for hypoxia and laryngeal trauma is reduced, addressing Refinement (3 R’s). As of May 2007, this technique has been employed by the surgical team for nearly 4 y, and has increased survivability by approximately 50%. The nature of this technique requires both mouse handling and anesthesia familiarity, and thus is ideally taught to an experienced technician. Additionally, this technique may be more easily taught with the use of injectable anesthesia, reducing the input required to maintain a surgical plane as the surgeon develops mechanical proficiency.

P10 M15 Mice Consume More Drinking Water with Liqui-gel Ibuprofen than Drinking Water with Children’s Ibuprofen
PC Ezell1, GW Lawson
DLAM, UCLA, Los Angeles, CA

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P16 Micro-CT and Histopathology of Mus musculus Hair-induced Mandibulofacial Abscess Demonstrates Severe Periodontitis and Alveolar Bone Destruction

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Coagulase-positive Staphylococcus aureus is commonly found on the skin of animals and has been reported as a primary cause of facial abscesses in mice. Though an oral route of infection has been suggested, the actual route is unknown. It has been theorized that Staphylococcus aureus is introduced into the oral mucosa by pelage or vibrissa hair via grooming or barbering activity. At our institution, histopathology has identified hair in a plethora of mandibular abscesses, further supporting the theory that hair is forcibly introduced into the periodontal space following barbering and mastication and results in periodontitis. A 4-mo-old male FVB/NCr1BR mouse singly housed in the largest SPF mouse facility at UCLA was found with an abscess extending from the anterior right mandible. The mouse was euthanized, and a culture of the abscess material indicated coagulase-positive Staphylococcus aureus as the causative agent.

Other pathology noted at necropsy included splenomegaly likely due to extramedullary hematopoiesis. The head of the mouse was fixed in 10% formalin and submitted for a micro CT scan to determine if periodontitis existed. The scan showed severe localized periodontal bone loss around the buccal right mandibular third molar, left buccal and lingual 2nd and 3rd molars. To determine if hair was the causative agent inducing the periodontitis, the skull was decalcified and 5μm transverse sections were collected from the rostrom to the caudal aspect of the mass and stained by hematoxylin and eosin. The abscess was multiloculated with each lobule separated by dense fibrous connective tissue typical of Staphylococcus aureus abscesses. The tongue had multiple hair-induced foci of chronic inflammation. A small fragment of hair was present in the dense fibrous connective tissue adjacent to the abscess base and the tooth root. The micro-CT scan was invaluable in determining the severity and the extent of periodontal bone loss, whereas histologic examination was invaluable in identifying the hair fragments and the amount, type, and location of inflammation.

P17 Diagnosis and Treatment of Pyometra in a Squirrel Monkey (Saimiri sciureus sciureus)
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A 12-y-old female, non-bred, 700 mg squirrel monkey used in behavioral studies presented with foul-smelling, mucopurulent, and sanguineous vaginal discharge. On physical examination, the uterus was firm, enlarged and nodular. The animal was not painful upon palpation and remained bright, alert, and responsive during the whole episode. Differential diagnoses included pyometra/metritis, vaginitis, and neoplasia. Complete blood count and chemistry levels showed lymphocytosis and mild monocytosis. Cytological examination and bacterial culture of the vaginal discharge showed more than 50 types of bacteria (gram-positive and -negative rods and cocci) overgrown by Proteus mirabilis. Antibiotic therapy with enrofloxacin (5 mg/kg, IM, SID) was initiated and continued for a total of 5 wk. The animal was examined following 7 d of treatment; the uterus size had decreased, but the vaginal discharge was still present. Radiographs and ultrasound examinations revealed a cystic mass 3 cm in diameter. Based on the minimal response to treatment, likely due to poor antibiotic penetration of the thickened uterine wall, the researcher elected to perform a hysterectomy (more invasive than a biopsy but curative as well as diagnostic). The laparotomy revealed a firm uterus with indistinct transition at the cervix between the uterus and the vagina. The uterus was adherent to the rectum, bladder and the body wall. The ovaries were enlarged, and a cystic structure was present on the right ovary; therefore, a partial ovariecomy was also performed. The animal survived and is currently doing well. Histological analysis of the uterus showed a mild lymphocytic and neutrophilic endometritis. The ovarian structure was within normal limits. In most species (for example, humans, dogs, cats), sex hormones influence the immune system and can predispose the uterus to infection. In humans, endometritis usually occurs during the follicular phase of the menstrual cycle while uterine infections in most other species occur during diestru.

P18 Administering a Constant Rate Infusion of Post-op Medications to Domestic Sheep (Ovis aries) with an Elastomeric Balloon Infusion Pump
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Managing the delivery of a constant rate infusion (CRI) of post-op medications to domestic sheep (Ovis aries, Dorset-cross) is difficult using traditional mechanical fluid pumps. Mechanical pumps require frequent monitoring and creative ways to keep the lines from becoming obstructed. Therefore, bolus dosing is a common alternative. A major disadvantage of bolus dosing is the difficulty of maintaining the drug concentration within the therapeutic range. To solve these problems, an elastomeric pump was adapted to deliver intravenous CRIs to free-moving sheep. This technique was developed in our facility to help control the post-op arrhythmias experienced by our cardiac ischemia models. Prior to this technique, the animals were administered bolus dosing of lidocaine if arrhythmias were detected. Four sheep have been managed with this pump. We hypothesized that the CRI offers better control of arrhythmias. The device is practical and reliable. The elastomeric pump is currently marketed to provide CRIs of antibiotics to septic joints in large animals. This pump operates based on the material properties of the balloon. The flow rate can be controlled by attaching specific flow control tubing to the infusion line. Several flow rates are available making the pump adaptable to different drugs and dosages. The pump holds up to 100 ml of volume and is a single-patient, one-time use, disposable product. It can be refilled to extend the length of treatment for the patient. Both the pump and the line can be secured to the animal by modifying a lamb tube (a form-fitting jacket) to contain the system. The flow control tubing from the pump attaches directly to a standard catheter placed in the jugular vein. It is recommended to individually house these sheep to protect against another sheep disrupting the pump. This technique may offer a good alternative to a fentanyl patch because it avoids the skin contact issues caused by the natural production of lanolin in sheep. The major benefit of this pumping device is that it allows for the CRI of medications in many situations where it was previously not practical and can therefore increase the level of animal care provided. There are many possibilities for this elastomeric pump in the research environment.
**P19 Cystic Mammary Adenocarcinoma Associated with a Prolactin-secreting Pituitary Adenoma in a New Zealand White Rabbit (Oryctolagus cuniculus)**

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A 44-mo-old, female, nulliparous New Zealand White rabbit (Oryctolagus cuniculus) presented with bilaterally enlarged mammary glands having enlarged, discolored teats which exuded brown, mucoid discharge. The discharge was submitted for culture along with a complete blood cell count (CBC) and a serum chemistry panel. A presumptive diagnosis of mastitis was made and the animal was placed on enrofloxacin (5 mg/kg IM once a day for 10 d) and ketoprofen (3 mg/kg IM once a day as needed). The CBC and serum chemistry panel were within normal limits, the culture was negative, and the clinical signs did not resolve with treatment. The original diagnosis was abandoned, and mammary dysplasia due to a prolactin-secreting pituitary adenoma was considered. Computed tomography (CT) imaging was performed antemortem on the affected rabbit and on an age-matched, normal female rabbit. Serum prolactin measurements were also taken antemortem from the affected rabbit and from 2 age-matched normal females. The affected rabbit was euthanized due to the primary experimental endpoint, and a full diagnostic necropsy was performed on the affected rabbit. The CT images confirmed an enlarged, irregularly shaped pituitary gland in the affected rabbit compared to the normal rabbit, being 2 to 3 times normal size. Three-dimensional imaging software allowed enhanced viewing of the irregularities. The lesion was later identified grossly at necropsy. Serum prolactin concentration was consistent with the diagnosis, at 19.2 ng/ml compared to the normal rabbits’ values of 2.7 ng/ml and 4.0 ng/ml. Histological analysis revealed a pituitary adenoma, diffuse mammary dysplasia, and cystic mammary adenocarcinoma. Prolactin immunostaining on the pituitary gland is pending. While mammary dysplasia associated with prolactin secreting pituitary adenomas is documented, this is the first report describing neoplastic transformation from dysplasia to malignancy, as well as antemortem diagnosis using CT.

**P20 Comparison of Two Heating Devices for Use during General Anesthesia of Rats and Mice**

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Rodent species are highly susceptible to hypothermia during general anesthesia making thermal support important. This study examined 2 devices for their effectiveness in maintaining normothermia in rats and mice during general anesthesia: the SnuggleSafe® disc, a reusable, commercially available, microwaveable heating disc, and a circulating hot water blanket. Sprague-Dawley rats and CF-1 mice (n = 6 per treatment) were anesthetized for 60 min using isoflurane, during which time heat source surface temperature and animal body temperature were monitored every 5 min using a thermistor probe. Additionally, a negative control group of animals was anesthetized with no heat source. Both devices were found to carry minimal risk of causing thermal burns. The SnuggleSafe disc temperatures ranged from 102.1 to 108.9 °F for rats and 96.5 to 100.5 °F for mice when heated for 1.5 min in a microwave oven. The circulating hot water blanket temperature ranged between 99.1 to 100.7 °F with rats and 96.5 to 100.5 °F with mice. Furthermore, both devices maintained body temperatures very close to initial temperatures (T0) throughout all experiments. For rats on the circulating water blanket, there was an insignificant decrease (P > 0.05) of 0.38 ± 0.40 °F from the T0; with the SnuggleSafe disc, there was a significant increase (P < 0.001) of 1.83 ± 0.39 °F. For mice on the circulating water blanket, there was a significant (P < 0.001) decrease of 0.82 ± 0.09 °F from T0; on the SnuggleSafe disc, there was a significant increase of 1.79 ± 0.23 °F from the T0 temperature. With no heat source, body temperatures of rats and mice significantly decreased (P < 0.001) by 8.13 ± 1.44 °F and 17.82 ± 0.62 °F, respectively. Although statistically significant deviations from T0 body temperature occurred with both heat sources, no changes were considered physiologically significant, and both heating devices were considered safe and effective. Finally, SnuggleSafe discs are inexpensive and very portable making them potentially more desirable for use with rodents during anesthesia compared to the hot water circulating blanket.
P22 Metastatic Squamous Cell Carcinoma in a Sooty Mangabey (Cercocebus atys)

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A 30-y-old, female sooty mangabey (Cercocebus atys), an unassigned, retired breeder, was presented to the clinical staff with an ulcerated, cutaneous mass in the right nipple. A biopsy identified the mass as squamous cell carcinoma; the mass was completely resected. Three months later, she presented with hemorrhagic, fluctuant cutaneous mass (approximately 5 cm in diameter) was present in the right mammary gland. Adjacent to the gastric-duodenal junction at the greater curvature of stomach the pyloric mucosa was focally ulcerated, necrotic, and the wall was segmentally thickened to about 4 times its normal width. Histologically, skin and gastric masses had non-circumscribed nests of neoplastic squamous epithelial cells extending from the ulcerated epidermis or gastric mucosa to the subcutis and submucosa, respectively. Rarely, the neoplastic cells invaded the gastric serosa. These cells were polyhedral, with intracellular or extracellular keratinization and intercellular bridges. They were supported by a dense connective tissue stroma. Often foci of central keratinization (keratin pearls) were found within concentric layers of neoplastic squamous cells. Since squamous cell carcinomas may develop in many organs of the body, the primary site of origin is unknown. Squamous cell carcinoma has been reported only once previously in a sooty mangabey. This mass was also resected. Two months later, she was accessed and the animal was euthanized. At necropsy the animal was emaciated and had kyphosis of the spine. An ulcerated, hemorrhagic, fluctuant cutaneous mass (approximately 5 cm in diameter) was present in the right mammary gland. Adjacent to the gastric-duodenal junction at the greater curvature of stomach the pyloric mucosa was focally ulcerated, necrotic, and the wall was segmentally thickened to about 4 times its normal width. Histologically, skin and gastric masses had non-circumscribed nests of neoplastic squamous epithelial cells extending from the ulcerated epidermis or gastric mucosa to the subcutis and submucosa, respectively. Rarely, the neoplastic cells invaded the gastric serosa. These cells were polyhedral, with intracellular or extracellular keratinization and intercellular bridges. They were supported by a dense connective tissue stroma. Often foci of central keratinization (keratin pearls) were found within concentric layers of neoplastic squamous cells. Since squamous cell carcinomas may develop in many organs of the body, the primary site of origin is unknown. Squamous cell carcinoma has been reported only once previously in a sooty mangabey.

P23 Chyloperitoneum Associated with Hepatitis in a Transgenic Mouse (Mus musculus) Strain Beta-catenin Gene Floxed

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An 11-mo-old female mouse (strain B6.129-Ctnnbltm2Kem/ Knw) was examined due to apparent pregnancy with no delivery. No gravid uterus was detected at palpation. Differential diagnoses included peritoneal effusion or mucometra. To confirm, radiographs were taken which revealed a greatly distended abdomen with a homogeneous ground glass appearance. The presumptive clinical diagnosis was ascites. The animal was euthanized. Upon necropsy examination, the animal weighed 59.6 g. Her peritoneal cavity contained approximately 25 ml of a pink-white fluid. The liver was atrophied and the surface was roughened. The spleen was severely enlarged. Bacterial culture of the peritoneal fluid was negative. Smears of the peritoneal fluid were Gram-negative rods. The portal area had moderate granulocytes. The lamina propria of the gallbladder had a diffuse moderate to severe infiltration of lymphocytes, plasma cells, and macrophages. Differential diagnoses for the hepatic lesions included bacterial infections; viral infections including mouse hepatitis virus (MHV), adenovirus, and herpes virus; or toxic conditions including a lead toxicosis or iron overload. The animal was diagnosed with chylous ascites secondary to choanal hepatitis. Serology was negative for 15 different pathogens, including MHV. Special stains for infectious agents including bacteria and fungi were negative. Electron microscopy revealed margination of chromatin and large intranuclear inclusion bodies. Viral particles were not observed. The organelles appeared to be decreased in numbers. The mitochondria were swollen with loss of cristae. This animal had no prior history of illness. She had previously delivered 4 healthy litters. In addition, no other cage mates or individuals from the same strain had history of identical or related clinical diseases. To our knowledge, this is the first case of hepatitis associated with chyloperitoneum in this strain of mice.

P24 A Case of Marek’s Disease in Japanese Quail (Coturnix japonica)

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Japanese quail (Coturnix japonica) are commonly used in research as a model for atherosclerosis and brain-stereoid interactions. Three Japanese quail (CBT Farms, Chestertown, MD) engaged in a reproductive behavioral study focusing on neuronal and hormonal pathways presented with lethargy, anorexia, weight loss, soft stool and lime-green urates. The 3 birds were group-housed on the top level of a triple-deck 30 x 18 x 18 in. breeding cage. Fecal samples and cloacal smears were collected from the sick birds for microscopic examination to isolate parasites and culture and sensitivity for bacteria. Treatment was started with enrofloxacin (15 mg/kg) and oxytetracycline (50 mg/kg) pending receipt of test results. Parasitology was negative; culture and sensitivity indicated the presence of a heavy growth of E. coli sensitive to both enrofloxacin and oxytetracycline. PCR for Chlamydophila psittaci was also negative. Due to the lack of response to treatment, the 2 remaining birds were euthanized and submitted for necropsy. The most prominent gross lesion in 1 of the birds was an enlarged liver with a focal area of necrosis. Histopathology revealed lymphoproliferative infiltrates in multiple organs. The differential diagnoses for lymphoproliferative disease in birds include Marek’s disease and avian leukosis. Enlarged sciatic nerves are pathognomonic for Marek’s disease in the chicken; however, that lesion usually does not occur in the quail. Serum samples from another male bird that was in contact with the affected females tested positive for Marek’s disease virus based on immunofluorescent antibody. Results of immunohistochemical staining of tissue samples submitted for virus-specific antigen will be reported. Marek’s disease virus can naturally infect Japanese quail; nevertheless, the incidence is quite rare. Unexpectedly, even though the disease is highly contagious, none of the other birds housed in the room became infected.
P25 Characterization of Clinical and Pancreatic Islet Pathology in Captive, Spontaneously Diabetic Vervet Monkeys (Chlorocebus aethiops)
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We have observed spontaneous obesity and diabetes in a pedigreed colony of vervet monkeys (Chlorocebus aethiops) and are in the process of characterizing this species as an animal model for diabetes. Based on prior analysis of the amylin gene sequence corresponding to the amyloidogenic amino acid region (residues 20-29) in vervets, we predicted pancreatic islet pathology of spontaneous diabetics would involve amyloid deposition consistent with humans and macaques, which have similar amyloidogenic sequences. Necropsies were performed on 3 spontaneously diabetic vervets; 1 died and 2 were euthanized from diabetic complications. Clinical diagnosis was based on presence of hyperglycemia and glucosuria, with or without ketonuria. Subsequent confirmation of impaired glucose tolerance (IGT) by intravenous glucose tolerance test (IVGTT) was performed in 2 of the 3. All 3 were started on insulin therapy after diagnosis and were fed an identical chow diet throughout their lives. Pancreases were collected at necropsy, and histologic examination included hematoxylin and eosin staining, Congo Red staining for amyloid, and immunohistochemistry for insulin and glucagon. Pancreatic islet lesions ranged from minimally to mildly hypocellular, and individual islets were multifocally, mildly to moderately expanded by acellular pale eosinophilic proteinaceous material which exhibited green birefringence under polarized light (amyloid). Immunohistochemistry for insulin revealed strong positive cytoplasmic staining within large numbers of islet cells. Compared to well-characterized Type 2 diabetic cynomolgus macaques and humans, the vervet lesions seem to have less amyloid and more insulin, possibly explaining why insulin requirements of our diabetic vervets are far less than our diabetic cynomolgus macaques. These results suggest that diabetic vervets may have different pathophysiologic characteristics compared to diabetic macaques and humans. Assuming a classic polygenic-environment basis for diabetes in vervets, one possible explanation is that islet amyloidosis occurs later in the pathogenesis of Type 2 diabetes. Further study is required to confirm these results and explore alternative explanations.

P26 Atypical Presentation of Mycobacteriosis in a Zebrafish (Danio rerio)
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A 12-mo-old female zebrafish (Danio rerio) from an open research colony was reported for having raised scales. Five additional fish from the colony exhibited similar clinical signs. Gross findings included abdominal distension and focal epidermal hemorrhage. Differential diagnosis included parasitic infestation, fungal colonization, and bacterial infection. Microscopic evaluation revealed granulomas disseminated throughout the coelomic cavity, visceral organs, and soft tissues. Review of Gram-, periodic acid Schiff-, and Ziehl-Neelson-stained sections revealed numerous indistinct Gram-positive rod and occasional coccioid-shaped bacteria, abundant PAS-positive material that was negative for fungal organisms, and numerous acid-fast (red) rod-shaped bacilli within granulomas and macrophages. The bacilli were slender and less than 4 μm in length. Protozoa or ectoparasites were not identified. Gross and microscopic findings were consistent with a diagnosis of systemic mycobacteriosis. Further speciation of the organism was not performed. Mycobacterium marinum and M. fortuitum are noted piscine and zoonotic pathogens. Classic presentation of disease in fish may include shallow to deep skin ulceration, decreased growth rate, chronic wasting, decreased reproductive rates, scale edema, scale petechiation, and abdominal distension. Profound scale edema causing a “porcupine” appearance was the predominant clinical sign in this case. While this sign has been reported, it is often absent in cases of mycobacteriosis. Knowledge of classic and atypical clinical signs, allowing early detection of zoonotic diseases, is paramount. Disease in humans is caused by direct contact with contaminated animals or fomites and usually confined to cutaneous digital lesions, with generalized disease reported in immunosuppressed individuals. Strict quarantine of new zebrafish to a colony is the best strategy for disease control. Elimination of mycobacteria species from aquatic habitats is difficult since these organisms are ubiquitous and become resident in biofilms. Depopulation and disinfection of the system may be necessary to control an epidemic. The zoonotic potential of Mycobacteria spp. necessitates appropriate risk management procedures in facilities housing aquatic species.

P27 Scrotal Herniation of the Bladder in a Squirrel Monkey (Saimiri sciureus)
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An 8-y-old male squirrel monkey (Saimiri sciureus) assigned to a behavioral pharmacology study was presented to the veterinary clinical staff for severe scrotal swelling. The differential diagnosis for a swollen scrotum in a squirrel monkey includes herniation, epididymitis, orchitis, torsion of the spermatic cord, and neoplasia. On examination, the scrotum was firm, edematous, and bilaterally enlarged approximately twice its normal size. A non-reducible cordlike structure was palpated within the scrotal sac, extending from the right inguinal ring to the cranial aspect of the right testicle. Radiographs were non-diagnostic, revealing a diffuse soft-tissue opacity within the scrotum. On ultrasound, a fluid-filled structure was observed cranial to the right testicle. Urine was aspirated, confirming scrotal herniation of the bladder. The bladder was emptied via cystocentesis and manually reduced through the inguinal ring. Repair of the hernia was accomplished by surgically reducing the size of the neck of the parietal vaginal tunic and the external inguinal ring. The scrotal edema diminished rapidly postoperatively, and herniation has not recurred. To the authors’ knowledge, this is the first reported case of a scrotal herniation of the bladder in a squirrel monkey.

P28 Effects of Time and Water pH on Antibacterial Activity of Enrofloxacin and Timethoprim/Sulfa in Drinking Water for Mice
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Oral administration of small volumes of drugs is technically challenging and stressful to small rodents. Therefore, rodents are often given antibiotics in drinking water. We found that routine dosing regimens were either anecdotal or untested sci-
The purpose of this study was to test the biological activity of 2 antibiotics, enrofloxacin and trimethoprim/sulfadiazine (TMS), in drinking water supplied to mice. Antibiotics (injectable enrofloxacin and an oral suspension of TMS) were added to bottles that contained either municipal, HCl acidified (pH = 2.8) or municipal water (pH = 6.1). Enrofloxacin concentrations were selected to deliver 0, 100, or 200 mg/kg of drug daily or TMS concentrations that deliver 0, 240, or 960 mg/kg of drug daily, based on an average daily consumption of 5 ml of water per mouse. These dosages were based on currently used dosages from our facility. Three bottles for each combination of antibiotic, water type, and concentration were analyzed (n = 36 water bottles). On day 0, the water bottles were vigorously shaken to thoroughly mix the antibiotics and were placed on static microisolator cages with no mice in the cage. At days 1, 2, 4, and 7, samples from each bottle’s sipper tube were plated on Mueller-Hinton agar containing Escherichia coli lawns. Antibiotic efficacy was measured by zones of inhibition (ZOI) of bacterial growth. Data was analyzed by repeated measures ANOVA. Results demonstrated that enrofloxacin at low and high concentrations retained consistent antibacterial activity in both water types for all 7 d and that the acidity of the water did not affect ZOI. The lower dose of TMS retained bactericidal activity over 7 d; however by day 4 (in municipal water) and day 2 (in acidified water), the higher dose of TMS did not leave a ZOI. To determine if drug activity had changed due to precipitation of drug, TMS bottles were vigorously shaken after day 7 and samples were plated. Interestingly, the ZOI returned to the same diameter as the initial time point, indicating that the drug was biologically active, but unavailable at the level of the sipper tube after 48 to 96 h. Based upon our findings, we feel the animal failed to respond to treatment, developed sepsis, lost 20% of its body weight, and was euthanized. MRSA was administered enrofloxacin to treat infection. Unfortunately the animal failed to respond to treatment, developed sepsis, lost 20% of its body weight, and was euthanized. MRSA was

P29 Hematological Changes in Vervet Monkeys (Chlorocebus aethiops) During Eight Months Adaptation to Captivity
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Vervet monkeys (Chlorocebus aethiops) are widely used as induced laboratory animal models of infectious diseases. The present study determined the hematological reference ranges for juvenile (with permanent incisors, up to 4 to 5 y old) and adult (with permanent molars, over 5 y old), male and female vervet monkeys and examined the changes in these from capture to habituation under laboratory conditions. We investigated fluctuations in hematological values of 50 wild-caught vervet monkeys during habituation to captivity. The monkeys were categorized into 4 groups according to age and sex: adult males, adult females, juvenile males, and females. The erythrocyte values were significantly higher (P < 0.05) in the adult males than in the other animals. There was an increase in most of the erythrocyte parameters studied during the monitoring period, with the most significant being hemoglobin (14 ± 1.6 to 15.1 ± 1.2 g/dl), hematocrit (44.9 ± 6.2 to 50.6 ± 6.2%) and mean corpuscular volume (72.8 ± 6.3 to 77.2 ± 3.1 fl). However, the red cell distribution widths, which were higher in adult females, declined. The total white blood cell (WBC) counts, which were higher in adult females than in the other animals, were closely correlated with granulocyte counts. The WBC levels decreased in all the animals throughout the 8-mo study, indicating acclimation, but they were relatively stable in males.

The platelet counts in adult females declined significantly at the fourth month from 420 ± 96.3 to 317 ± 19.64 (P < 0.05) before a general rise up to 8 mo. At 8 mo post-capture these counts were higher in females than in males. The juvenile female platelet counts were relatively stable during the monitoring period. The maintenance of the monkeys on an improved stable diet and in environment-controlled housing combined with progressing psycho-physiological adaptation may be important factors for the gradual improvements of the hematological values recorded. There were wide variations in these between individual animals emphasizing the need for long adaptation combined with establishment of individual baseline values prior to experimental studies.

P30 Hydroureter and Hydronephrosis Following Ilio-femoral Arterial Prosthetic Bypass
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This case report describes hydroureter leading to hydronephrosis in a canine following an ilio-femoral bypass graft placement. A 2-yr-old female mongrel canine (Canis lupus familiaris, 22 kg) was intubated under general anesthesia, placed on a mechanical ventilator, and monitored for depth of anesthesia consistent with the Guide for the Care and Use of Laboratory Animals. Following abdominal and femoral cut-down incisions, two 6.0-mm polytetrafluoroethylene (PTFE; non-reactive synthetic polymer tube with an extremely low coefficient of friction) bypass grafts were implanted proximally onto the common iliac arteries in an end-to-end manner and then tunneled retroperitoneally to the femoral artery, posterior to the ureters and inguinal ligaments. After completion and recovery from surgery, the canine returned to an observed normal daily activity and completed the experimental study without any documented change in daily habits. Upon terminal surgery at 5 mo, a necropsy revealed a dense fibrotic reaction involving the left ureter at the site of crossing over the graft. The ureter, proximal to the fibrotic reaction, and the kidney were enlarged consistent with hydroureter and hydronephrosis. There was no evident infection or trauma to the ureter; the reaction appeared to be all inflammatory. The contralateral right kidney and ureter were normal, with no sequelae from graft placement. These findings suggest that despite an otherwise uneventful graft placement, renal sequelae may develop. With normal contralateral kidney function, asymptomatic hydroureter and hydronephrosis can occur following ilio-femoral prosthetic graft placement.

P31 Investigation of Methicillin-resistant Staphylococcus aureus in Pigs (Sus Scrofa) Used for Research
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In August 2005, methicillin-resistant Staphylococcus aureus (MRSA) was unexpectedly isolated in a naïve pig (Sus scrofa) used for streptozotocin-induced diabetes research. The animal had a skin abscess from a surgical incision site and was administered enrofloxacin to treat infection. Unfortunately the animal failed to respond to treatment, developed sepsis, lost 20% of its body weight, and was euthanized. MRSA was
isolated from a bile specimen sent for bacterial cultures taken post mortem. To investigate the possible source of the MRSA isolate and to determine if there was an outbreak in the animal research facility, samples were taken from Landrace/Yorkshire crossbreed of mixed ages and sex (40 to 180 kg). Nasal swabs were obtained from the animal herd (n = 50), animal holding rooms (n = 14), in another animal laboratory center (n = 8), and from a slaughter house (n = 50) wa housing pigs of the same source as the facility. Human nasal swabs were also obtained from the veterinary staff (n = 23), research staff (n = 6) and animal laboratory staff (n = 3). The swabs were plated onto MRSA screening plates containing Mueller-Hinton agar with 6 mcg/ml oxacillin and 4% NaCl. The bacteria was identified by positive slide and tube coagulation tests, API Staph and universal 16s rDNA amplification and sequencing. Swab isolates were similarly typed using pulsed-field gel electrophoresis (PFGE), staphylococcal cassette chromosome mec (SCCmec) typing, and multilocus sequence typing (MLST). Overall, 4 MRSA isolates were cultured from 3 pigs and a clinician-scientist. Two were ST22-MRSA-V, a human strain type associated with epidemic spread. The other 2 were ST398-MRSA-V, a strain type associated with pigs. The isolation of the 3 strains from a pig provides evidence that MRSA is a problem not only in hospitals but in the veterinary field as well. It demonstrates that possible risks are involved in pigs used for experimental studies, as they may be potential recipients and sources of MRSA. This also has wider implications for research and the development of treatment modalities in humans as pigs are widely used in many types of biomedical research.

P32 Optimization of a Pig Model for Predicting Performance of Oral Formulations in Humans

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Pigs are attracting attention as alternate animal models for pharmacokinetic evaluation of pharmaceuticals due to similar gastrointestinal physiology to humans. We describe the optimization of a vascular access port (VAP) pig model for evaluation of clinical formulations. A jugular VAP minimizes animal stress levels and ensures accuracy of timed sample collections. To support clinical programs, retaining port patency for prolonged periods is important. Conventional pigs gain weight rapidly, which compromises port patency over time. Mini-pigs were chosen for the current model due to their reduced propensity for weight gain. Yucatan mini-pigs were chosen to approximate weight of adult humans. All animals were obtained surgically implanted with subcutaneous VAPs from an external vendor. Animals were 7 to 8 mo old and 30 kg upon implantation. Age and weight were selected to account for potential gain during the studies. Maximum weight reached was about 60 kg. The model was optimized over 2 consecutive periods with 2 groups of 10 intact and 8 castrated animals. Veterinarians recommended using castrated animals for the second period to improve animal behavior during port maintenance and dosing. A strict protocol including routine maintenance was followed in both periods to maximize the longevity of patency. VAPs were accessed weekly using a Huber needle with an injection cap on the end. Ports were flushed using heparinized 0.9% saline solution (10 IU), followed by blood withdrawal (to check for patency) and injection of 0.9% saline solution flush. After flushing, ports were locked using of taurolidine-citrate catheter solution. Studies involved oral dosing and plasma sample collections for up to 24 h. The day before the studies, an infusion line was connected to the VAPs via a modified Huber needle for ease of collection. The procedures resulted in successful use of both groups for formulation screening. We achieved 100% patency for 5 mo post-implantation with the 1st group and for 9 mo with the 2nd group. Increased experience with the model and the placidity of the animals during studies likely account for the increase in patency between groups. This optimized pig model for screening of clinical formulations allows for use of the same animals for a period of up to 9 mo.

P33 Jugular Venipuncture and Caudal Tail Vein Injection Techniques in Sprague Dawley Rats using Manual Restraint

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The Sprague Dawley rat (Rattus norvegicus) is widely used in pre-clinical drug safety studies. Frequently, a large amount of venous blood is required during the in-life portion of a study to accommodate investigator needs for hematology, clinical chemistry, coagulation and pharmacokinetic evaluation. Obtaining these samples with physical as opposed to chemical restraint is important for preclinical safety studies to avoid confounding variables created by anesthesia in assessing the toxicologic effects of the compounds. Secondly, drug studies frequently require repeated injection of a compound into a lateral tail vein using manual restraint. Many institutions have developed techniques for blood collection and injection, but these are not widely distributed in the literature. At the University of Texas MD Anderson Cancer Center, we have developed a 2-person technique for efficiently acquiring up to 2 ml of blood from 200-g Sprague Dawley rats that is well tolerated. In this procedure, 1 person firmly grasps the nape of the neck and places the rat in dorsal recumbency in the palm of the same hand, using the other hand to extend the neck and apply pressure to the jugular vein. The 2nd person then removes the hair with clippers and inserts the needle in a cranio caudal direction, lateral to the sternoclavicular junction near the point of the shoulder at approximately a 30° angle with the skin. Another 2-person technique was developed for administering compounds intravenously through the caudal tail vein. After initially submerging the tail in water heated to 40°C for 15 to 20 s, 1 person creates a cone with both hands in which the rat is positioned head-first and gently supported. After rotating the rat 90° into lateral recumbency, the 2nd person gently grasps the tail and injects the compound into a lateral tail vein as close to the tip of the tail as possible, leaving the majority of the vein unaltered for subsequent injections. To date, we have used these procedures on 200 to 300 rats and have found that minimal to no acclimation was required.

P34 Developing a Cost-effective and Efficacious Method of Pinworm (Aspicularis tetraperta) Treatment for Large Colonies of Mice

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Through our routine sentinel screening program, our facility identified pinworms in a colony of over 500 cages. The colony consisted of a breeding population of transgenic, knockout and immune compromised mice. We evaluated the available
methods of elimination, their effectiveness, and their associated costs. Past treatments used by the facility included ivermectin applied topically, ivermectin provided orally via water bottles, and fenbendazole provided orally via the feed. The ivermectin methods had lower material costs, but were labor intensive (drug, $20; labor, $3,000). The fenbendazole had higher material costs and required a delay in obtaining the special order feed (feed, $3,200; labor, $3,000). Both methods have been reported to be efficacious in the literature. The potential for potential ivermectin- or fenbendazole-related toxicity was evaluated with feedback from the investigators and determined to be of insignificant concern in this colony. In order to start treatment immediately, but cost-effectively, carboys were modified to hook up to the automatic watering system. Each rack had a 5-gal carboy containing 1% ivermectin at 3.2 cc/gal of filtered water installed on its top shelf. After flushing the racks with the treated water, gravity was used to pull the treated water through the system. The treatment consisted of 4 d on and 3 d off of ivermectin water for 7 consecutive weeks. Weanling mice who were naïve to the watering system were given treated water bottles on the same schedule as each rack. Tape tests and fecal flotation were used to confirm elimination of pinworms from the colony through testing of every 10th cage every 2 wk during and after treatment. This method was effective in the treatment of the pinworm outbreak with the advantage of significant reductions in the associated material and labor costs (drug, $20; carboys, $800; labor, $500).

P35 withdrawn

P36 Abdominal Distension in a Colony of Congenic Mice
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Two 15-mo-old mice (chromosome 9 D2.B6 [D9Mit90,18] congenic strain), 1 male and 1 female, presented for distended abdomens. Mice were SPR for common rodent viral, parasitic, and bacterial pathogens. Both animals were euthanized, but blood collected prior to necropsy revealed elevated liver enzymes, monocytesis, hypernatrema, and hyperkalemia. One mouse had a mild anemia and a leukocytosis, while the other had a mild leukopenia. Differential diagnoses included infectious, dietary, toxic, genetic, or congenital causes of hepatitis. On necropsy, the male’s spleen was double the normal size but was of normal color and consistency. His liver was approximately 4 times normal size, pale tan, mottled, and had several small cystic lesions in the caudal lobe. Other abdominal organs appeared unremarkable. The female’s spleen and liver were normal in size but the liver was also pale tan with 1 lobulated cyst in the caudal region of the left lateral lobe. The rest of the abdomen was unremarkable. Organs from both mice were submitted for histopathology. There was periportal hepatitis and cholangitis with cystic bile ducts. Some of the bile ducts contained cellular debris and degenerate neutrophils. There were no organisms detected on histopathologic evaluation. This mouse colony has developed an approximately 60% incidence of hepatic cysts. To the best of our knowledge, this is a unique manifestation in congenic strains with this background. This congenic strain was originally developed to identify potential locations in the genome that influence alcohol withdrawal and consumption. The diagnosis was familial hepatic cysts, hepatitis, and cholangitis.

P37 Metastatic and Immunohistochemistry Pattern in Mammary Gland Adenocarcinoma in a Gerbil (Meriones unguiculatus)
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Mammary gland adenocarcinoma in a the gerbil (Meriones unguiculatus) is not very frequent and there are few cases described in the literature. Liver and lymph node metastasis were not described, as well as the immunohistochemistry characteristics of this tumor. The spontaneous occurrence of mammary gland adenocarcinoma in a non-treated laboratory-reared gerbil is reported. The tumor affected a female breeder (39 mo old). No sign of disease was noticed at the time of euthanasia. The upper right mammary gland showed a yellowish and hardened nodule, measuring 4 mm and surrounded by hemorrhagic halo. One regional lymph node, as well as cervical, hepatic, and mesenteric lymph nodes, was enlarged and hemorrhagic. The histopathology showed tubular cords of neoplastic epithelial cells and was consistent with the diagnosis of well-differentiated mammary gland adenocarcinoma of tubular type. Metastasis was seen in liver and regional lymph node. Immunohistochemistry expression of p53 and c-erb B was positive; receptors for estrogen, androgen and progesterone were negative. This case represents the only mammary gland adenocarcinoma found in a colony of gerbils (males and females; n = 387) studied for evaluation of naturally occurring diseases. The present report describes the metastatic and immunohistochemistry pattern of an uncommon tumor in the Mongolian gerbil.

P38 Impact of Dietary Fenbendazole and Supplemented Vitamins on Growth of Implanted Lymphomas in SCID Mice
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We report that feeding an unautoclaved sterilizable rodent diet containing 250 ppm fenbendazole caused significant growth inhibition of a human P493-6 B cell line (lymphoma) in CB17/ Icr-Prkdcscid/Crl (SCID) mice. Initially we noted unexpected tumor growth inhibition in controls during a facility pinworm treatment with dietary fenbendazole. Here we report the results of a follow-up study. We hypothesized that fenbendazole may target tumor microtubules (like the anti-tumor agent vincristine) or act like the related mebendazole, which reduces tumor-induced neovascularization. Increased vitamin intake may also have played a role: the treatment diet was vitamin-supplemented with vitamins A, D, E, K, and B to compensate for loss during autoclaving, but was not autoclaved. Vitamins E and A both have anti-tumor properties by virtue of being anti-oxidants. Vitamin E causes anti-tumor and anti-metastatic effects in several cancer animal models and suppresses the nuclear transcription factor NF-kappaB in prostate cell lines. NF-kappaB regulates target tumor microtubules (like the anti-tumor agent vincristine) or act like the related mebendazole, which reduces tumor-induced neovascularization. Increased vitamin intake may also have played a role: the treatment diet was vitamin-supplemented with vitamins A, D, E, K, and B to compensate for loss during autoclaving, but was not autoclaved. Vitamins E and A both have anti-tumor properties by virtue of being anti-oxidants. Vitamin E causes anti-tumor and anti-metastatic effects in several cancer animal models and suppresses the nuclear transcription factor NF-kappaB in prostate cell lines. NF-kappaB regulates pro-apoptotic and pro-metastatic proteins; thus, its suppression has anti-tumor effects. Twenty vendor-supplied 4-wk-old SCID mice were randomly assigned to 4 treatment groups: standard diet, diet plus fenbendazole, diet plus vitamins, and diet plus both vitamins and fenbendazole 2 wk prior to subcutaneous flank implantation with 30 million lymphoma cells. Tumor size was measured by caliper at 4-d intervals until the largest tumors reached a calculated volume of 1500 mm3. Our results showed no significant difference in tumor growth between the standard diet and diets supplemented with vitamins or...
fenbendazole alone. However, the group supplemented with both vitamins and fenbendazole exhibited significant inhibition of tumor growth, and tumors were significantly smaller at the study endpoint at 22 d \((P = 0.009)\). We suggest that dietary treatment with 250 ppm fenbendazole or feeding unautoclaved sterilizable diet did not impact tumor growth. However, feeding diet supplemented with both 250 ppm fenbendazole and extra vitamins inhibited tumor growth, perhaps by synergism between Vitamin E and fenbendazole, and may similarly affect other tumor models.

P39 Use of Body Condition Scoring as an Adjunct Endpoint for Tumor Growth Studies

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When mice are used in studies where wasting and death are potential complications, practical, rapid, and noninvasive methods for assessing health status and establishing endpoints are needed. Tumor growth studies are often very challenging as the growth of the tumor may mask one of the more commonly used endpoints—loss of weight. Body condition scoring (BCS) has been proposed as a potential mechanism to quantify muscle loss on these studies, but controlled scientific investigation supporting this application has not been reported in the literature. This project evaluated BCS as an endpoint for tumor growth studies, used in conjunction with other common endpoints such as weight loss, general body condition, attitude, tumor size, and the presence of ulceration on the tumor. In keeping with the principle of reduction of overall animal use, this project characterized the use of BCS in collaboration with ongoing cancer research at our institution. C57BL/6j and transgenic mice at this facility that were used on skin tumor (papilloma and subcutaneous breast cancer cell implantation) and intra-coelomic (lymphoma and melanoma) tumor studies by other investigators were monitored with a battery of clinical parameters, including weights, tumor characters, and BCS. The BCS technique used was previously described. Approximately 100 animals were monitored over the course of this study. This project showed that BCS can be a beneficial addition to the health monitoring for animals that are used in studies with tumors that are intra-coelomic, such as ascites and other bulk producing tumors of the abdomen and thorax. However, this project also demonstrated that BCS is not appropriate for all studies of tumor growth. The skin tumor growth studies that we monitored had no significant changes in body condition scores that were predictive of morbidity or mortality, and more traditional clinical signs were found to be reliable for this model. This project confirms that body condition scores are a valuable adjunct to clinical health monitoring.

P40 A Modification to a Common Bronchoalveolar Lavage Technique in Nonhuman Primates

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Bronchoalveolar lavage (BAL) is a diagnostic tool often used for clinical and research purposes in nonhuman primates. Although many institutions use this procedure, the technique is not standardized. This study was intended to characterize and evaluate differences between 2 mechanical aspiration techniques. One technique studied is a more conventional method (cBAL) of attaching a syringe directly to the biopsy channel of a flexible bronchoscope. A modification to this technique (mBAL) was produced by adding a section of sterile standard intravenous tubing between the syringe and the biopsy channel of the flexible bronchoscope. Bronchoscopy and BAL fluid collection were performed on 2 groups of 10 rhesus macaques (Macaca mulatta). Each group had 2 BAL procedures performed at 2-wk intervals; each animal served as its own control. Data collected for comparison included heart rate, SpO\(_2\) levels, temperature, volume of returned fluid, total cell count, cell viability, differential cell count, and flow cytometry. Animal morbidity and human ergonomics were also evaluated. Data revealed an 8.3% increase in fluid yield using the mBAL versus the cBAL method of aspiration. No significant differences were noted in cellular makeup, indicating the mBAL does not negatively affect normal components of BAL fluid. A greater number of cells per microliter of fluid recovered was present using the modified technique. While results showed no appreciable differences in animal morbidity, it is postulated the mBAL may lead to decreased risk of complications due to diminished turbulent fluid flow and less tissue damage in the lungs. The mBAL method was found to be more ergonomically designed by a closed-ended questionnaire administered to the veterinarian and the surgical technician performing procedures. Though not statistically significant (n = 2), both respondents clearly preferred the mBAL method. All described results would likely produce improvements to clinical relevance of diagnostic sample collection, animal morbidity, and staff health as compared to the conventional technique.

P41 An Anesthetic Comparison of Ketamine-medetomidine Based on the Route of Administration and the Reversal Effects of Atipamezole in Dutch Belted Rabbits (Oryctolagus cuniculus)

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Forty male Dutch belted rabbits (Oryctolagus cuniculus) enrolled in a minimally invasive pharmacokinetics study were used to compare the efficacy of an anesthetic combination delivered via 2 injection routes. Ten rabbits were randomly assigned to 4 groups (n = 10/group) to determine the sedative and physiological effects of ketamine-medetomidine based on route of administration. Each rabbit received ketamine (25 mg/kg) and medetomidine (0.5 mg/kg) either intramuscularly or subcutaneously. Palpebral, pedal, ear pinch and righting reflexes, as well as cardiovascular parameters (heart rate, respiratory rate, and arterial blood oxyhemoglobin saturation), were recorded every 5 min. In addition, the reversal effects of an intravenous dose (1 mg/kg) of atipamezole, an alpha-2 adrenoreceptor antagonist, were assessed by comparing the return of the righting reflex in rabbits given the aid of the reversal agent versus rabbits allowed to recover spontaneously. We demonstrated that ketamine-medetomidine given subcutaneously at the described dosages effectively induces chemical restraint with less than a 2-min difference in the onset of anesthesia and significantly less resistance (such as flinching and kicking) during the injection as compared to the intramuscular route. In all groups, the anesthetic regimen, regardless of the route of administration, provided an adequate level of anesthesia. The rabbits induced using the intramuscular route seemed to recover more rapidly after being reversed with atipamezole. However, when the subcutaneous route was used...
to induce anesthesia, there was no benefit in reversing the rabbits since the difference in the time the animals reached full recovery yielded no statistical significance ($P = 0.07$).

**P42 Efficacy and Toxicity Combined Study of Human Adipose Tissue-derived Mesenchymal Stem Cells in a Rat Femoral Segmental Defect Model**

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Human adipose tissue-derived mesenchymal stem cells (hATMSC) have the potential to differentiate into multiple lineages of mesenchymal tissues. The treatment of critical size bone defects is still a challenging problem in orthopaedics. This study was performed to evaluate efficacy of bone regeneration and toxicity of hATMSC for 16 wk in a femoral defect model with athymic nude rats (Hsd:RH-Foxn1

**P43 Relocating an Existing Zebrafish Colony to a New Facility**

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In recent years, the zebrafish (Danio rerio) has become an increasingly useful research model. Zebrafish have short life cycles and breed efficiently, making them a financially attractive alternative to other species. Recent growth in zebrafish research has lead to an analogous growth in housing needs for aquatic species at many institutions. At the University of Pittsburgh, expansion in our program lead to the construction of an additional animal facility that included an improved aquatics area. The new facility, however, necessitated that our existing colony be relocated with the minimal impact on the program’s valuable transgenic fish. Prior to relocating the colony, we prepared the new systems by seeding the fluidized beds with sand from the old systems. Along with water parameter checks, sentinel fish from the original system tested the readiness of the new system. The colony move was scheduled to occur during 2 separate 1-wk periods. The PIs were assigned 1 d from each of the 1-wk segments and moved 50% of their fish population on each day. Maintaining a reserve in the original facility prevented the loss of any transgenic lines, and it also allowed for an incremental load increase on the new filtration system. The transition was largely successful, with less than 0.05% mortality. Some minor complications included temporary behavioral changes in the zebrafish such as hyperactivity, and we also observed a small rise in bacterial illness. Necropsy of the affected fish identified the bacteria as Aromonas hydrophila, which was caused by failure of a UV sterilization lamp.

**P44 Sodium Lighting as Viable Alternative to Red Lighting within Vivaria**

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Experimental parameters such as activity and feeding behavior have been shown to have profound circadian rhythms. For this reason, many pharmacological studies are carried out during rodents’ active phase (the dark phase), commonly under red lighting. However, it can be difficult to carry out tasks that require visual accuracy such as welfare checks, detailed clinical observations and intravenous dosing under these conditions. We explored the effects of sodium lighting on behavioral parameters as an alternative to red light (wavelength of 600 nm). Sodium light wavelength (589 nm) is within the human visual field (380 to 700 nm), allowing experimenters and animal care staff to see clearly but at the margin of rodent vision (200 to 580 nm). Quantification of spontaneous locomotor activity in rodents is known to be a robust system for detecting side effects of standard and novel compounds. A study observing 6 Sprague Dawley male rats over 4 wk was conducted to assess the activity differentials between alternate light sources during the rodent’s active phase. The locomotor activity recorded included assessment of both horizontal and vertical movement and was collected using an open-field photobeam monitoring system. Throughout the study the animals were singly housed under “normal” white fluorescent lighting set to a 12-h light/dark cycle (7 a.m., lights on; 7 p.m., lights off) with the light levels set to approx 150 lux during the light cycle. Data on the number of beams broken was collected in 1-min segments throughout with disturbance to the animals kept to an absolute minimum. During the first wk activity was monitored throughout the light and dark phase. On subsequent weeks a light source was set to come on during the rodent active phase for 4 h (7 p.m. to 11 p.m.). White, sodium, and red lights were all tested. The collated rat data showed that sodium light had no adverse impact on the behavioral parameters of locomotor and rearing activity as compared to red light and darkness. Sodium lighting is therefore a viable alternative to red light, improving ability to carry out husbandry and welfare tasks and therefore improving animal welfare.
P45 The Evaluation of a Novel Water Delivery System with a Transgenic Alzheimer’s Disease Mouse Model
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To identify an ergonomic, efficient, and economic method of water delivery for an Alzheimer’s disease mouse model that does not readily use an automatic watering system, a 28-d crossover study was performed with 5-amo-old male PSAPP (Fl hybrid: B6; SjL-Tg [APPswe] 257K6a X B6.129-Psen1tm1Mmp) transgenic mice. Two groups of 10 singly housed mice were provided water with either a water bottle or the novel water delivery system (a plastic bag containing up to 450 ml of water with a drinking water valve attached) for 14 d and then switched to the other method of water delivery for 14 d. Water consumption was measured by weighing the water bottles and the novel water delivery system daily. The animals’ weights were measured 3 d/wk. The amount of water consumed and body weights were compared using a 2-way ANOVA. Mice were housed in standard polycarbonate shoebox cages with wire bar lids and alpha cellulose bedding. Cages were changed every 14 d. PSAPP transgenic mice that were on water bottles consumed significantly greater (P < 0.05) amount of water than mice on the novel water delivery system. However, mice on the novel water delivery system consumed a volume consistent with a normal mouse for their weight range, 4 to 6 ml/d. The increased water consumption with water bottles 11 to 13 ml/d may have been due to spillage during weighing or the mice playing with the water bottles. There was no significant weight gain or loss during the study and the trends in body weight changes were very similar between the 2 groups. The results confirmed this to be a feasible alternative water delivery system for male PSAPP transgenic mice in this age range.

P46 A Practical and Reliable Method for Administration of Test Substances in the Drinking Water to Nonhuman Primates
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A research study at our site required delivery of H2O (Deutero- rium) supplemented drinking water to 12 male Macaca fascicularis primates for 7 consecutive days. We developed a novel water delivery system consisting of a pressurized, stainless steel (316L) 16-gal reservoir tank and a 3-gal air compressor which delivered treated drinking water to the lixits of multiple individual animal cages by a distribution manifold at a pressure of 3 to 5 psi. The tank was placed on the floor and was attached to a 12-ft piece of stainless steel tubing with 3 flex hoses connected to it. The tubing was mounted on the wall and the flex hoses connected the manifolds of the cages to the wall mounted pipe. The system was checked daily to make sure there was an adequate water supply in the reservoir tank. Using the standard cage-mounted automatic watering system to deliver the treated drinking water to study animals allowed us to eliminate concerns regarding animal acclimation to an alternate method of obtaining drinking water, and avoid the increased labor and problems associated with the use of water bottles with nonhuman primates. Additionally, this system supports efficient facility space ergonomics by supporting isolated delivery of treated drinking water to specific study animals housed in the same room with untreated colony animals that remain connected to the standard facility water supply. The single drawback of most concern was the increased noise associated with the sporadic operation of the small air compressor used to pressurize the reservoir tank. However, the primates acclimated to this noise quickly. In conclusion, this technique of delivering treated water to standard cage-mounted automatic watering devices proved to be a simple, cost-effective, and reliable method to deliver treated water to select nonhuman primates.

P47 Post-approval Monitoring Enhanced by Use of a Slate/ Tablet PC
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The Office of Animal Research Education and Compliance at our institution is responsible for ensuring that researchers are fully trained in all relevant aspects of animal use. The program’s compliance officer visits laboratories to monitor animal use activities, share information with research personnel, and observe procedures to verify competence. The office maintains a database of training and compliance activities that is integrated with the animal protocol database. Entering information from laboratory audits into the database was time consuming, requiring transcription of written notes into the system. To streamline this process, a slate tablet PC was purchased. Not only has this tool increased efficiency of data entry, it has become a valuable asset to the inspection process. A general laboratory checklist and survival surgery checklist were developed specifically for the slate. During inspections, items are checked off by simply touching the screen. The handwriting recognition software allows additional comments to be written on the screen, and automatically converts them into type. This data is later uploaded to the compliance database and is used in various ways such as identifying common areas where more training might be useful, IACUC policies that should be updated or clarified, documenting a laboratory’s progress over time, and providing historical data for IACUC program reviews. Having the tablet PC available during laboratory visits has proved invaluable in many ways. Various important documents and forms, particularly IACUC policies, are available to reference with the lab personnel. The slate’s wireless internet access allows immediate retrieval of protocols in real time; helpful internet links, such as training information and schedules, are kept on the desktop. The completed checklists as well as other useful information are immediately emailed during the visit, making the entire post-approval monitoring program more efficient and modernized. The scientists and research staff have responded very favorably to this tool, particularly the instant sharing of information and transparency of process. In conclusion, a slate PC has been a very effective implement in enhancing our post-approval monitoring program.

P48 Refinements to Rabbit Group Housing
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Refinement in animal housing is an ongoing goal in lab animal science. Years ago, our facility sought to develop an alternative to single housing that allowed us to house female NZW rabbits in social groups. We developed a method for group-housing rabbits directly on the floor. Today, after many refinements, the result has been the creation of a room within a room. We started with a conventional animal housing room measuring 15 ft 4 in. by 9 ft 4 in., constructed with epoxy-coated CMU walls, epoxy-
coated cement floors, and stainless steel sink located near the entry door. Using a Plexiglas barrier, we modified the room to keep rabbits away from the sink and door, keep bedding confined to the housing area, and allow care staff to service the room without disrupting the animals. The housing area with the addition of the barrier measures 11 ft 6 in. by 9 ft 4 in., which allows us to house a maximum of 25 rabbits (weighing up to 5.4 kg each) in a room. After exploring drinking water options, rooms were retrofitted with gravity-operated systems, designed and constructed in house. We’ve combined sanitation and health monitoring, allowing us to minimize handling and decrease labor while ensuring animal health and adherence to regulatory guidelines. Rabbits are transferred to holding cages and visually examined for health status during weekly room sanitation. They are weighed biweekly, and nails are trimmed bimonthly. We adjust feed levels based on weight trends for the entire group. Individual animal weights are monitored to address potential health issues. We use easily visible methods to identify individual rabbits. Our experience with rabbit social hierarchies has proven different from information available in the literature. We've been successfully integrating female rabbits into established social groups without major incident, and in fact have significantly reduced the occurrence of minor injuries as a result of changing group dynamics. We have since modified many rooms and have housed up to 500 rabbits at a time using this method. Our refinement in housing has resulted in improved animal welfare, has decreased boredom, and has increased species-typical behavior. It is a safe and efficient method for housing rabbits, and can be easily established at most institutions.

P49 Redesign of Water Bottle Handling Equipment to Improve Efficiency in a Static Microisolator Facility
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The process of washing and refilling water bottles is generally a labor-intensive part of running a static microisolator facility. Our current practice was to place dirty water bottles into commercial wire baskets, which were in turn sent through a tunnel washer. Upon removal from the tunnel washer, the wire baskets were placed onto a bottle-filling station, filled with tap water, and sealed with a stopper by a staff member. Finally, the bottles were transferred into sterilizable containers constructed of either stainless steel rectangular tubs or polycarbonate microisolator rat cages, both of which were covered with filter-top lids. Our current business needs require 1200 to 1400 bottles per week to be processed. The current process was reevaluated in order to decrease labor and increase efficiency. With assistance of a local fabricator, we reengineered our existing tubs to simplify the process by reducing the amount of handling of each basket. These modifications included a fabricated grid placed on the bottom of the tub to hold bottles in alignment during the filling process, a snap-on lid that enabled the tub to be flipped over and processed through the tunnel washer, and an extension added to the bottom of each basket to allow for easy stacking. The new process during cage change-outs would entail placing the dirty bottles directly in the modified bins to be used for the entire cleaning/filling/autoclaving process. Through a time study, we estimated that implementing this design and process into our current practice could save 45 min to 1 h per day. Additionally, the risk of repetitive motion injuries will be greatly reduced. There were no modifications needed on the bottle-filling station or the tub transport carts to implement the newly modified tubs, and the cost of these 3 modifications was easily recovered in the labor saved.

P50 A Refinement of Oral Dosing in the Common Marmoset (Callithrix jacchus)
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Forty-two common marmosets were successfully trained, using positive reinforcement (PR) techniques, to accept various per os dosing solutions by blunt-tipped syringe. This refinement was adapted, as a necessary dosing strategy, in an effort to minimize the stress and potential complications when using these ostensibly delicate nonhuman primates when subjected to conventional gastric gavage methods. Development of this dosing paradigm involved PR conditioning of marmosets to willingly come to the front of their cage or, while hand-caught, drink various aqueous solutions as offered by the technician. The solution volumes offered ranged from 0.5 to 1.5 mL. Flavor masks were employed to facilitate acceptance of dosing solutions and, specifically, to mask test compound solutions that would have otherwise been rejected due to unpleasant taste. These flavor masks, during the 3- to 5 wk training sessions, were of varying sweetness concentrations containing maple, blueberry, orange or raspberry flavors. Additionally, dosing efficacy could be visually assessed and compatibility pre-screening of a flavor mask with a selected test compound could be readily conducted to verify compound integrity and availability. Merit was also found in the ability to customize various dosing solutions and flavor masks to meet study designs. To date, acute and chronic (1 mo) studies have been successfully performed using this enhanced oral dosing technique without adverse effects. This refinement resulted in appreciable reduction of animal handling and stress in the marmosets used for drug discovery studies.

P51 Novel Nonhuman Primate Puzzle Feeder Reduces Food Wastage and Provides Environmental Enrichment
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We designed and built a novel, inexpensive puzzle feeder for several species of macaques (M. mulatta, M. fascicularis, M. nemestrina) that provides enrichment and reduces food waste. The feeder dispenses monkey chow and fits on nonhuman primate group four quad rack cages or similar style caging. Before we developed these feeders, we had a problem with food wastage and the associated need for repeat feeding and frequent clean-up to avoid attracting vermin. The original J-feeders dispensed 18 to 20 biscuits. At feeding time, the macaques removed all the biscuits within 3 min, and those that were not eaten or stored in cheek pouches were pushed back through the feeder onto the room floor or dropped through the cage floor grid. We encountered several design challenges: the feeders had to use the existing cage feed slot, hold the daily biscuit supply, dispense 1 biscuit at a time, not interfere with the squeeze mechanism, and be secure from the monkeys yet easily movable to allow access for observation or sedation. They also had to be easy to clean. We solved these problems with vertical puzzle feeder boxes built from cage wash-compatible 0.25-in. polycarbonate, 0.25-in. PVC sheets, and 0.5-in. PVC tubes attached to the cage with stainless steel double-ended snap clips. To obtain biscuits the monkey had
to push them one by one through a switchback channel via finger holes. Although we could easily remove the feeders, the primates could not reach the snap clips. Daily cleaning was accomplished by running water through them; it was not necessary to remove them from the cage for sanitation in the rackwasher. Each feeder took approximately 1 h to make and cost approximately $60 in materials that were readily available from the hardware store. We documented that puzzle feeder implementation increased time spent foraging (approximately 20 min per biscuit), reduced food wastage, and decreased clean-up time. Because most biscuits were eaten (actual consumption per macaque is 6 to 10 biscuits) we could also monitor food intake by counting the number of biscuits dispensed. We have successfully used this feeder on single- and pair-housed macaques, and on baboons (Papio sp.). We suggest this feeder is widely applicable, both for enrichment and to reduce husbandry costs.

P52 Two Novel Swine Enrichments
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We describe 2 novel pen enrichments for swine that promote rooting, gnawing, and scratching behaviors. Free-ranging swine spend 80% to 90% of their waking time rooting for food, but reproducing this in the animal facility is difficult. Natural materials are hard to clean and block drains, and animals quickly stop using balls on the floor or hung from pen sides due to fecal soiling or boredom. We solved these problems by suspending toys from an H-shaped frame attached as a roof over the pen several feet above the pig, and by threading a 12-in. polyethylene plastic ball onto a second bar at pig height. The 2 enrichments can be used together or separately. Enrichments were attached to the pen using double-ended stainless steel snap clips. They were readably incorporated into the daily cleaning schedule and easily removed for sanitizing in the rack washer. The H-frame was constructed from various sizes of PVC tubing that slide so that suspended toys can be moved throughout the pen and caretakers can move the central bar aside for cleaning. The pig-height bar, also constructed from PVC pipe, was attached front to back on one side of the pen and a 12-in. hollow polyethylene plastic ball containing multiple holes was threaded onto it. We loaded the ball daily with food or cooking spices so that they were released when the pig pushed and rotated the ball. Swine also used the pipe for back scratching and the stainless clips at either end for gnawing. Materials costs (excluding hanging toys) were $40 from the hardware store, and both enrichments took less than 1 h to make using a jigsaw and drill. We found that the enrichments remained clean, and by rotating the suspended toys and using food or cooking spices in the ball, we sustained interest. Enrichment devices were introduced to 10 individually housed pigs and monitored 5 d/wk for 3 mo. During the entire period, pigs continued to interact with the balls repeatedly during the day and the enrichment sustained no damage. We suggest that these devices are valuable and inexpensive additions to a swine environmental enrichment program.

P53 Suitable Housing for Japanese Quail (Coturnix japonica)
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Levels of aggression were unacceptably high between Japanese quail (Coturnix japonica), including breeding pairs, housed in standard wire battery cages. It seemed likely the birds were stressed because of insufficient space and opportunities for species-specific behavior such as foraging. Importantly, a literature review revealed that an artificial enclosure should be at least 30 cm in height to allow for a quail’s vertical flight response. Another consideration was the different quail social units required by the research protocol, which involves transgenic birds and disease resistance. To address these concerns, 3 housing systems were put in place, each using a cage type already on hand. Small breeding groups (1 or 2 females to 1 male) occupy commercial rabbit cages (height, 45 cm) with ample floor space per bird. The perforated floor is covered with cardboard overlaid with 1 in. of hay. Crockery bowls are used as water and feed dishes, while dust baths and plastic huts for refuge are available. Although ambient light may be reduced for cages at the bottom of the rack, a decrease in egg production has not been evident. A second housing option is to keep groups of female quail in flight cages (height, 51 cm) designed for finches. The female quail live compatibly together compared to co-housed males, where fighting injuries were common. Again the stocking density is kept low for the all-female cages, and environmental enrichment is like that described for breeding cages. Birds that must be singly housed under ABSL-2 conditions comprise a third housing variant. For these quail, large rodent microisolator cages have proven very successful. The individual bird has room to groom and to forage through scattered hay, while visual and auditory contact with conspecifics is maintained. Since switching to these caging options, facility and research personnel have been able to work with a more healthy quail colony.

P54 A Team-based Approach to Compliance: Being Ready for an Inspection at All Times
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Constant attention to compliance is critical for all contemporary laboratory animal facilities. Compliance is complex and needs to be managed daily. It ranges from federal regulations regarding safety and animal welfare to standard operating procedures (SOPs) which describe animal husbandry and care. In our facility we were experiencing recurrent findings during ACUC semiannual inspections. These findings were primarily related to sanitization procedures and intervals. In response to these trends, we created a team to monitor compliance in our facility. Five staff members representing husbandry, veterinary care, and operations formed the core team. The objective was to ensure the facility was inspection-ready at all times. The team employed several different strategies to improve compliance. One was bimonthly supervisor walkthroughs of all areas within the vivarium. Supervisors used a checklist which included items such as sanitation tags, room logs and animal checks. The team also performed informal post-approval monitoring of our departmental animal protocols. Any identified discrepancies were brought to the attention of the ACUC. Another approach
the team used was reviewing department SOPs for compliance concerns and improved practices that could be incorporated into existing SOPs. Thirty-five SOPs including room sanitization, husbandry, and sentinel programs were reviewed in 2006. A mock disaster drill involving stakeholders including environmental and occupational health, and security was performed to review the disaster-planning SOP. Key issues including emergency vivarium access were identified and addressed. Compliance concerns were addressed either on an individual basis or with department wide retraining sessions if trends were noted. To reinforce key points, a video SOP which reviewed room inspections was created by videotaping staff performing proper room inspection procedures. This video has been used for training new employees and as a refresher for current staff. There are plans for creating additional video SOPs based on its success. This team approach resulted in no compliance issues in our recent semiannual ACUC program review, USDA inspections, and AAALAC site visit. Innovative approaches for ensuring compliance continue to drive the team.

P55 Development and Assessment of a Domestic and International Rodent Shipping Program

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Transfer of rodents, most notably genetically engineered mice, from non-commercial sources (domestic and international) poses significant challenges for academic institutions. Health status of the animals, timeliness of the transfer process, communication, and the shipping of the animals are each, on their own, a major concern. When an institution ships large numbers of animals, these concerns are often compounded. In an effort to improve our processes, a subcommittee of management was created to review and implement process improvements for the approximate 340 annual shipments. Improvements included the creation of new standard operating procedures (SOPs), assigning dedicated effort for a shipping coordinator (SC) position, and integrating a transportation company into the process. These modifications to the process improved efficiency (measured as total days for completion of transfer/shipping) and reduced complications (measured as lack or delay of response of institutions, cancellations, and transit issues). Improvements were obtained by an integrated communication system between the dedicated transportation company and our SC (measured as response time/labor spent on collection of health data). The results of the subcommittee’s effort including the integration of the SC, formal SOPs, and the dedicated transportation company were responsible for improvements in many of the measurements. The total days for completion of outgoing shipments were decreased from 90 to 42 d and incoming shipments decreased from 82 to 49 d. The lack or delay of response from other institutions decreased from a total of 34 to 24 incidents. The number of cancellations increased from a total of 24 to 35, some of which were a result of shipments that were started before implementation of the new SOPs. Time for implementation of these changes was approximately 2 mo, with subsequent alterations implemented after the initial change. Cancellations resulting from health risks increased from 8 to 19. It is apparent that these efforts readily increased the efficacy of the shipping program at our institution, and may be used as a model for other institutions.

P56 Temperature and Humidity Levels within Individually Ventilated and Static Cages: A Comparison of the Macro- and Micro-conditions of Animal Rooms

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A comparison of the cage micro-environment as it relates to the macro-environment of the room has not been sufficiently studied to provide a conclusive answer as to how external room conditions affect the animal’s micro-environment. Manufacturers, care providers, and researchers need a better understanding of the interplay between the 2 environments in order to make informed decisions. Over a 4-wk period, 8 individually ventilated cages and 4 static cages were monitored continuously (including during sanitation procedures) at 1-min intervals, with a stand-alone data logger positioned within the cages, in 3 different animal rooms. Each cage and room was monitored for temperature and humidity within the cage, room, and external ambient conditions. Ambient temperature and barometric pressure were collected from a news source located 1 mi from the animal facility. Two rooms were full service rooms monitored by the university’s environmental monitoring system housed within the ventilation system of the room. One room was a basic service room monitored by a wall-mounted sensor. Each room was maintained at a temperature of 72° ± 3°F and humidity was set at 50%. During the recording period spikes in temperature of 5° were observed 6 times and fluctuations of 25% in humidity were observed 5 times. These spikes occurred at different times of the day and did not correspond with room sanitation. The results indicate that while the macro-environment of the animals may vary, the micro-environment is very stable. IVC caging experienced less fluctuation in temperature and humidity than animals housed in static cages. IVC cage temperatures varied 0.25 °F from room set temperature during spikes of 5, while static cages temperature increased by 1.5 °F. IVC cage humidity varied 0.5% during spikes of 25% humidity, while static cages increased by 3%. Humidity at the cage level is approximately 3% higher than at room level. External ambient conditions affect room conditions; a direct correlation from external humidity levels and room ambient conditions was observed. IVC cage level temperatures and humidity do not fluctuate significantly with room temperature and humidity fluctuations in the animal room. The position of the sensors affects the recorded values.

P57 Alternative Use of a Lateral Vascular Access Port and Modified Caging to Support Large Animal Tethered Infusion Systems

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Chronic catheterization for vascular infusion is commonly performed in preclinical biomedical research. The combination of a subcutaneous lateral vascular access port (VAP) and a percutaneously placed, externalized catheter positioned within the port lends an alternative to standard externalized implanted catheters. A complication often associated with externalized catheters is tract infection, potentially resulting in bacteremia and sepsis. Use of the Cath-in-Cath™ VAP system (CIC) can help minimize infections by providing a more advantageous infusion model as compared to the standard externalized catheter. The CIC externalized catheter can be easily relocated to different
exteriorization sites above the implanted port to minimize irritation/inflammation often associated with a single catheter exit site over time. Efforts to repair a compromised CIC system are generally less invasive as compared to those required for repairing a standard externalized catheter and simply require the externalized CIC catheter to be removed from the port and replaced. For periods when infusion is not required, the CIC catheter can be removed and the port can be maintained appropriately for future use. When a long-term continuous infusion is required, the externalized CIC catheter can be left in the port for extended periods of time. Tethered large animal infusion models are continually being improved to better incorporate equipment and minimize technician-to-animal induced stresses. A simple cage door modification involving the removal of a small section of the lower horizontal cross member between 2 vertical cage bars allows external access to all major components of the infusion system thus minimizing technician-to-animal interactions. This presentation describes use of the CIC system in IACUC-approved protocols in canines and nonhuman primates over the course of 12 mo, its advantages over a standard externalized catheter, and its incorporation into a tethered infusion model.

P58 Optimizing Mouse Fostering Protocols to Maximize Pup Survival
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When breeding animals, complications that lead to loss of valuable litters can arise. These complications can include the death of the mother, lack of milk production, or poor mothering skills. In these situations, it is often beneficial to foster the affected litter to another mother who can continue to nurse them until weaning. Current veterinary practices describe the use of fostering within 72 h of birth to foster mothers with age-matched litters. In our facility, we have been forced to foster under other clinical circumstances (such as 48-h-old pups to a foster mother who is due to give birth in a couple of days, 3-d-old pups to a foster mother with 10-d-old pups) and have found that these alterations can lead to successful recovery of the valuable litter. In this study, we intentionally bred litters to create combinations of fostered litters of a variety of predetermined ages under controlled circumstances. The predetermined ages included less than 48 h, 5 to 7 d, and 10 to 12 d. Fostering was performed with age-matched and non-age-matched litters and consisted of partial and total litter replacement. The fostering technique followed published references where the recipient dam is removed and pups to be fostered are mixed with bedding or pups belonging to the recipient dam (in order to “mark” the fostered litters and minimize pup rejection). Our results found that fostering can successfully be performed at a wide variety of ages and with large discrepancies in age between the fostered and birth litters. This information will assist research and technical staff that are attempting to save valuable orphaned mouse pups.

P59 Using Toys as a Means of Environmental Enrichment for Purpose-bred Canines (Canis familiaris)
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Clients using canines for biomedical research desire calm, well-socialized animals that come to the front of an open cage door. It has been our experience that pups begin to demonstrate a decrease in desire to come to the front of the cage at 12 wk of age. In an effort to supplement our current socialization program and to satisfy client demand for increased environmental enrichment for our canines, we have instituted the use of toys while socializing 10-, 11-, 12-, and 13-wk-old puppies. Our current canine socialization program begins at 4 wk of age and continues until the time of sale. Stainless steel and utility carts, nail clippers, trimmers, and leashes are used during the socialization process. Historically the implementation of toys during socialization has been difficult, as dogs at our facility showed no interest in manipulanda but preferred playing with one another. We choose toys based on durability, ease of sanitization, and how well puppies respond to them. Through trial and error we have discovered 3 types of toys to which our puppies respond favorably. These toys are Boingo Balls, Clutch Balls, and Mini Teasers. Puppies are exposed to each of the 3 types of toys during the 4 wk of toy socialization. Typically toys are placed in cages containing 3 puppies. The puppies are allowed to play with the toys for a minimum of 20 min. A technician then opens the cage and interacts with the puppies using the toys. Toys are removed from each cage, washed in a bleach water mixture, and examined for signs of wear. Toys that appear to have been damaged (chewed through, have hanging flaps of plastic, are broken, and so forth) are discarded and replaced. Most toys last 3 mo before needing to be replaced. Since adding the use of toys to our socialization program, the percentage of puppies that come to the front of their cages has risen 10%. It is hoped that the addition of toys to our socialization program will increase the confidence our puppies have when facing new situations and will help reduce stress during shipment and acclimation to new environments.

P60 Use of Commercial Microchips for Permanent Identification of Wild-caught Little Brown Bats (Myotis lucifugus) in Long-term Group Housing
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Medical management of long-term animal populations necessitates permanent individual identification for tracking and clinical purposes. Little brown bats (Myotis lucifugus) provide a challenging model for traditional identification such as foot bands, and number tags due to their physical size and anatomy; little brown bats typically weigh 7 to 9 g. Thus, it was critical to find a safe, permanent and easy-to-apply identification marker. Twenty-nine bats received microchip injections using pre-packaged sterile syringes in which single 2 x 12 mm microchips were loaded into luer-lock 12-gauge needles. Bats were manually restrained, given a subcutaneous injection of medetomidine (1 mg/kg) in the shoulder and placed into a small anesthetic chamber. Once the bat was at an appropriate level of sedation, it was removed from the chamber and manually restrained with the head pointed towards the individual performing the restraint. The technician tented the skin of the dorsum, lateral to the midline, and inserted the needle until the bevel was covered by tissue. The technician applied pressure at the site while the needle was withdrawn to ensure closure of skin around the microchip. Following microchip insertion, the bat was scanned by a microchip reader to confirm placement. The bat was placed into a darkened recovery cage, on a heat disc to maintain body temperature. All 29 bats recovered uneventfully within 15 min without reversal. Bats were placed into a holding cage for observation for 48 h post-procedure. After examination it was determined that the bats could return to the colony.
Seven months after microchip placement, the chips remain functional and well tolerated in the remaining bats. Application of a commercial microchip product proved to be a successful and relatively non-invasive form of identification and has been used in domestic and exotic species. The use of a commercial microchip as permanent identification in our long-term bat population has proven beneficial for accurate record keeping and tracking for regulatory and husbandry requirements.

P61 Current Microbiological Status of Mice and Rats in Experimental Facilities in Japan

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The ICLAS Monitoring Center performs microbiologic and genetic monitoring of laboratory animals in animal breeders, pharmaceutical companies, universities and other research institutes in Japan. This is an outline of the results of microbiologic monitoring in mouse and rat experimental facilities in Japan were mainly opportunistic pathogens such as Staphylococcus aureus, intestinal protozoa Pasteurella pneumotropica, Pseudomonas aeruginosa, Pneumocystis carinii, Helicobacter hepaticus, mouse hepatitis virus (MHV), Aspiculuris tetrapiera, Syphacia spp., Mycoplasma pulmonis, ectoparasites, and Sendai virus (SV). The total positive rate for one or more items in PC was lower (7.9 %) than that in U/I (16.4 %). Positive items in rat facilities were S. aureus, P. aeruginosa, Syphacia spp., P. pneumotropica, intestinal protozoa, P. carinii, A. tetrapiera, M. pulmonis, CAR bacillus, Closstridium piliforme, sialodacryoadenitis virus, and SV. Also in rat facilities, the total positive rate for one or more items in PC was lower (4.6 %) than that in U/I (18.9 %). On necropsy, 182 animals from 112 facilities (8 mouse and 2 rat facilities in PC, 98 mouse and 4 rat facilities in U/I) showed gross lesions. Although no relation with infection was suspected in most cases, a few cases were suspected to be due to infections by S. aureus, P. pneumotropica, and P. aeruginosa, and so on. These results suggested that the prevailing agents in Japan were mainly opportunistic pathogens such as S. aureus, P. pneumotropica, P. aeruginosa, and P. carinii.

P62 Special Care and Rearing Techniques to Successfully Raise GULO KO Mouse Pups

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1-Gulono-gamma-lactone oxidase (GULO), a critical enzyme present in most mammalian species is required for the terminal step in vitamin C biosynthesis. Our facility supports a colony of mice in which the GULO gene has been knocked out (KO) of the genome. These mice cannot synthesize vitamin C, which must be provided as a supplement in their water. Breeders are heterozygous for the gene; the dam produces sufficient vitamin C to support pups until weaning age at which time GULO KO pups are provided supplemental ascorbic acid in drinking water (0.33 g/l). As the research need for wild-type and heterozygous offspring diminished, new breeders were established with homozygous KO pairs. The breeder cage was provided water supplemented with ascorbic acid. Breeding and parturition occurred with no problems, producing litters averaging 6 to 7 pups at birth. However, within 1 to 2 d of birth, high mortality of pups was seen and surviving pups were weak, cool to the touch and less active. Milk spots were clearly visible in all pups. By P3-4, surviving pups developed large swollen joints. The clinical signs of the surviving members of the litter were consistent with scurvy. The parents appeared clinically normal, consuming ascorbic acid in their drinking water. Providing extensive supplemental care, facility care staff were able to increase the survival rate of these litters from 1 or 2 pups per litter to the entire litter. The breeder cage was placed on supplemental heat and facility staff hand-nursed pups 3 to 4 times/d with a concentrated mixture of ascorbic acid (33 g/l) in water. Signs of scurvy either did not develop with this intensive care or were quickly resolved. Breeding pairs in which the dam and sire were both KOs were separated to stop production of unhealthy offspring. We hypothesized that the dam, even with an increase in the amount of ascorbic acid consumed, was unable to support the needs of her offspring. Future breeders were established with the dam being heterozygous for the GULO gene and the sire remaining the KO genotype. Although some unneeded heterozygous offspring are produced, the KO pups have thrived with no problems related to a deficiency in vitamin C.

P63 Establishing an Ergonomic and Safety Committee

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Our husbandry department has actively tried to reduce injury rates over many years through one-time training programs and one-on-one medical or engineering consultations. Our husbandry management made a decision to support a more robust approach to minimize injuries and pain resulting from the repetitive nature of the job tasks animal care workers are required to do as well as pinpoint safety issues. Based upon this decision, a diverse group of co-workers teamed up with the University Occupational Health Program to form an ergonomic and safety committee. The safety committee is comprised of management and supervisory staff, a purchasing support member, animal care technicians, unionized cagewash staff, and an occupational therapist. Each group member brings different ideas, experiences, expertise, and workplace perspectives to the committee. Committee objectives are to use the current ergonomic resources, decrease rates of injury, minimize workplace discomfort, and maximize the health and productivity of the workforce by identifying and correcting problem areas. The committee has relied upon 6 important risk factors used in ergonomic endeavors to identify problems and reduce workplace injury rates: repetitive motion, awkward or sustained postures, excessive force, contact stress, vibration, and temperature extremes. These factors help the committee prioritize, evaluate, and change the work behavior, administrative issues, or the environment when possible. The committee tracks ideas, interventions, and solutions, and re-evaluates the final outcome, adjusting as needed for success. The committee emphasizes to the staff that it is their personal responsibility to ensure safe behavior and to be aware of the variety of environmental factors that may affect their ability to work both safely and ergonomically within their job classification. Overall, the committee’s commitment to minimize the effects of ergonomic risk factors
combined with the preventative education and training has made our department an ergonomically safer place to work. The committee has been in place for 1.5 y and has already seen a 30% reduction in injury rates from 2005 to 2006.

P64 Percentile Curves of Body Weight and Body Mass Index for Cynomolgus Monkeys (Macaca fascicularis) from Birth to 14 Years Using Retrospective Data

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The use of cynomolgus macaques (Macaca fascicularis) in research has increased over the last few decades, creating the need for body weight (BW) and body mass index (BMI) norms, based on age. The Wake Forest University Health Sciences (WFUHS) Primate Information Retrieval System (PIRS), a computer database, provides the opportunity to retrospectively assess all important events in the life of a monkey. Data collection continues until the animal is transferred to another institution or dies. This report of cynomolgus monkeys from a WFUHS breeding colony includes 337 males with 1,549 data entries and 322 females with 1,644 entries. Primate age was categorized into 1-y intervals. Descriptive statistics and percentiles for BW (kgs) and BMI (kg/m2) were done separately for each gender and age within gender. Per age group of males, BW and BMI means are birth, 1.4 (0.2), 36.1 (3.1); 1 y, 1.8 (0.3), 38.2 (5.6); 2 y, 2.4 (0.4), 39.8 (4.2); 3 y, 3.2 (0.6), 43.5 (6.3); 4 y, 4.1 (0.9), 48.4 (7.6); 5 y, 5.1 (1.1), 54.0 (9.1); 6 y, 5.7 (1.3), 60.1 (11.8); 7 y, 6.5 (1.4), 64.1 (10.4); 8 y, 7.0 (1.6), 68.6 (14.1); 9 y, 7.7 (1.9), 73.6 (13.4); 10 y, 7.7 (1.8), 71.2 (12.0); 11 y, 7.8 (1.6), 73.0 (14.4); 12 y, 8.1 (1.5), 75.3 (9.9); 13 y, 8.6 (1.9), 79.6 (19.0); and 14 y, 9.3 (2.3), 76.5 (16.5). Per age group of females, BW and BMI means are birth, 1.2 (0.2), 34.4 (5.5); 1 y, 1.6 (0.3), 35.0 (4.8); 2 y, 2.1 (0.3), 37.3 (4.4); 3 y, 2.8 (0.6), 40.1 (6.9); 4 y, 3.3 (0.8), 43.0 (7.9); 5 y, 3.7 (1.0), 46.1 (10.4); 6 y, 3.9 (1.1), 48.7 (11.1); 7 y, 4.0 (1.1), 50.7 (11.0); 8 y, 4.3 (1.0), 51.3 (9.8); 9 y, 4.4 (1.2), 54.9 (11.9); 10 y, 4.6 (1.5), 54.8 (13.9); 11 y, 4.9 (1.2), 57.1 (12.9); 12 y, 4.9 (1.0), 58.2 (11.6); 13 y, 4.7 (1.0), 56.5 (11.3); and 14 y, 5.5 (1.6), 68.3 (15.7). In addition, BW and BMI were sorted into crude percentiles at each year up to 14 y of age. These data were then used to provide smoothed percentile curves. The tables and graphs provide useful tools to evaluate the growth of individual monkeys compared to means.

P65 Using Your Organization’s Available Resources to Provide Cost-effective Enrichment for Nonhuman Primates

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In recent years, providing environmental enrichment to research animals has become accepted as an integral part of animal care, particularly for nonhuman primates. Enrichment increases species-typical behaviors and can help give animals control over their surroundings, resulting in a better research model. Providing enrichment is also mandated by the Animal Welfare Act. Still, the cost of providing can be exceedingly high. At the Oregon National Primate Research Center, we wanted to increase the enrichment of our group-housed Japanese macaques (Macaca fuscata) by adding trees and climbing structures to their 2-acre enclosed corral. As with most projects of this type, it was given to our physical plant, which sent it to local professional building companies. However, the price from the contractors was prohibitively expensive (about $2,200). Instead of giving up, we decided to build the enrichment internally. We turned to resources available at our center, including some apple trees scheduled to be removed due to construction of a new building, and our animal care staff. Many of our technicians were excited to help build enrichment structures for the monkeys. Further, some technicians had experience working in zoos or construction, so we had a great deal of expertise in addition to the enthusiasm. We “planted” approximately 9 trees in the corral using concrete, and attached additional poles cut from tree trunks to create a complex structure for the monkeys. The main part of the structure was approximately 61 ft long, 43 ft wide, and 14 ft high. We added fire hose (donated from local fire departments), chain, and PVC tubing to create swings, a bridge and other perching and climbing opportunities. The total cost of the project, including equipment rental, cement, and other supplies, came to less than $600. In the end, this project was not only enriching for the monkeys, but also brought out teamwork amongst caretakers. By using resources already available and tapping into the expertise of people with a vested interest in helping the monkeys, we were able to improve the environment for our monkeys in a cost-effective manner.

P66 A Novel Method for Lifting Weanling Pigs in Biomedical Research: The Ventral Scoop

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Swine are frequently used models in medical and veterinary surgical instruction classes. One may modify lifting techniques in small laboratory pigs (Sus scrofa domestic) to reduce fear response, strengthen the human-animal bond, and improve welfare. We hypothesized that recently weaned pigs lifted with a ventral (belly) scoop method would show less fear of new humans and less fear during treatment than pigs lifted vertically by their hind limbs, when each method is introduced as a new treatment. Thus, 31 Yorkshire cross pigs (average age, 3 wk) were acquired and divided into 7 groups of 4 and 1 group of 3. All pigs were acclimated to humans for 11 d and enriched with a scratch pad, large plastic balls, and red Kong toys prior to any lifting tests. Pigs were randomly assigned to 1 of 4 different combinations of ventral and/or vertical lifting techniques. Each combination dictated the lifting method executed on 2 consecutive days (1 ventral-vertical; 2 ventral-vertical; 3 vertical-vertical; 4 vertical-vertical). Each day data were collected regarding pigs’ aversion to lifting and willingness to be caught multiple times. Two hours later, their time to approach a new person and proportion of time spent hiding from the new person were also measured. Aversion scores were assigned based on duration of squealing, shaking, and freezing responses during lifting. Pigs that were lifted using alternate methods on consecutive days showed a significant decrease in aversion when being scooped ventrally (P = 0.008) as compared to lifted vertically by their hind limbs. Several behavioral trends were also identified during the study, such as: On day 1, pigs lifted first and second in each pen were more likely to return to the handler (P = 0.003), and allowed themselves to be caught more times (P = 0.001) than pigs lifted 3rd or 4th in order regardless of the lifting treatment, suggesting social facilitation influenced the flight response. We thus determined that the ventral scoop method is less fear-inducing than the vertical lift method based on standardized aversion scores. This is also true when the ventral
scoop is introduced after a bout of vertical lifting, showing that it is beneficial to make this change in handling even if the vertical lift is currently employed.

P67 How to Start a Technical Services Team
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The Unit for Laboratory Animal Medicine (ULAM) consists of animal care staff (ACS) and licensed veterinary technicians (LVTs), each group looking to broaden their daily tasks and use more of their trained technical skills such as blood draws, injections, and gavage. To pursue this goal, a survey was sent to ULAM customers and potential customers to see if there was any interest in us providing technical project support. The survey gave us a measure of interest from our customers before training and marketing our staff. Of 128 responses out of 300 labs surveyed, large percentages were in support of having procedures covered by ULAM staff. From the responses, we created a list of services that we would advertise. We met with the LVTs to identify which tasks the ACS could perform and which the LVTs would perform. The ACS were not included since we needed to establish the duties first and then gauge interests. Advertisements were posted, and a group email account was created to allow all members of the technical service team to receive requests. University Committee on Use and Care of Animals staff trained us become proficient in the procedures, and a protocol was created to cover the animals (donated from other investigators) used in the wet-labs. In 6 mo we have had 13 requests and logged 31 h of technical service time that we recharge back to the investigator on a per-minute time interval at a pay rate of $32.60/h. When request arrives, the coordinator will send out an email to find someone that has time to do the service within their schedules. A follow-up email is sent to the customers to check on the service. The ACS has been appreciative to have this opportunity to do the service.

P68 Innovative Methods and Ergonomic Advantages for Using Sipper Sacks™ in Rats
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Sipper Sacks™ is an animal watering system that has been available for 2.5 y and is becoming more popular for numerous reasons. The water filler can be hooked directly up to the facility watering system, and the patented drinking valve design does not allow back flow into the Sipper Sack, ensuring optimal water quality. The drinking valves do not drip or leak, so the cage stays dry. When using the Sipper Sack, you eliminate the ergonomic risks that are associated with filling water bottles. According to OHSA and the National Institute for Occupational Safety and Health it is estimated that one-third of work related musculo-skeletal problems severe enough to require time off work are a result of repetitive motions. These injuries can be directly linked to water bottles and create health issues that may directly interfere with job performance. Historically, the Sipper Sack has only been used in mice; due to the many benefits, however, we wanted to use this system in rats. Using creative problem-solving and partnering with Edstrom Industries, we were able to come up with a solution that showed Sipper Sacks are a viable and effective option in rats. When using the original mouse design we found that the rats were able to chew on the bag, causing them to leak. After trying different designs to eliminate that problem we came up with an insert that is made of clear plastic. The plastic insert is higher on the sides and back to make it virtually impossible for the rats to chew on them. The insert will also fit any style wire top. The disadvantage in using this system in rats is the length of the drinking valve can make it difficult for debilitated rats to use. We are currently collaborating with Edstrom Industries to rectify this problem. In 2003, our department had 3 OSHA-recordable injuries. We wanted to be proactive in keeping these numbers as low as possible. After implementing the use of Sipper Sacks for rats, our OSHA-recordable injuries dropped to 1 in 2006. This presentation will show the modifications made to the inserts and highlight the advantages and disadvantages of using Sipper Sacks in rats.

P69 A Novel Cutter Device for Uniform Preparation of Custom Diets
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The preparation of uniform rations of custom diet is an exacting but tedious process required for many chronic feeding studies. In our laboratory, Mazuri® Callitrichid diet, a diet designed for support of feeding regimes, is prepared daily in 2-kg bulk batches. The ingredients are mixed together and are placed in the refrigerator overnight to solidify into a semi-solid block. The block must then be cut into appropriate size and weight rations of 1.5 x 6 cm. Because we require precise 35-gm cubes, hand-cutting rations to these specifications is very labor-intensive. It typically requires 45 min to prepare uniform rations for each animal. A customized cutting device was developed to produce multiple identically sized portions in a much shorter time frame. The cutter was constructed of a stainless steel plate and handle with an anodized aluminum base receiver and 2 guided Delrin pins. It proved to be very simple and safe to handle. Cleaning was easily accomplished by any staff member using approved disinfectants. We have been able to decrease our manpower cost in the preparation of the diet by 80%. The cutter provides uniformity of rations and has increased productivity of the staff. It has delivered simplification of the repetitive task while providing an ergonomic and safety benefit. The design of the cutter can be universally applied to any semi-solid diet that requires precision preparation. The cutter is rigid in design; thus, a new cutter must be designed for different cutting dimensions.

P70 Motion Detectors as the Cause of Poor Reproductive Performance in Mice
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Environmental sources of noise and vibration are frequently suspected and sometimes proven causes of reproductive disturbance, physiological stress, and other adverse outcomes in laboratory rodents. The hearing range of mice ranges from approximately 1 kHz to 85+ kHz, with demonstrated peak sensitivities of about 14 to 16 kHz. Sounds above about 20 kHz are ultrasonic in that they are beyond the range of human hearing capabilities. We undertook a comprehensive noise, ultrasound, and vibration analysis of our vivarium to elucidate possible causes of maternal neglect, cannibalism, and reduced...
P71 Encouraging Staff to Set Their Own Goals Improves Facility Audits
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Animal care and use programs rely on managers to assure compliance with Federal and internal policies to maintain animal welfare, safety and quality science. Traditional management programs rely on individual assessment and individual counseling to identify deficiencies and training requirements. We designed a working group based monitoring system which maintains compliance, measures individual and group performance, confirms technical ability, and ensures the provision of quality animal care. We adopted this program to encourage work groups to self-identify unit-wide improvement goals and monitor progress towards this goal. Our program incorporates elements of a procedural monitoring system with those of an employee performance evaluation program. Managers audit rooms using a standardized form. Group findings are discussed at management meetings and individual deficiencies are discussed privately. Managers and technicians jointly identify areas for improvement. Audits consist of 3 areas; documentation compliance, room organization / cleanliness and animal health concerns. The animal care staff is audited at the animal holding room level and each audit is rated as “excellent,” “good” or “needs improvement”. Of 152 baseline room audits conducted in Q3 and Q4 of 2005, 36% were “excellent,” 56% “good” and 8% “needs improvement”. Animal care staff then set team goals for each quarter. Of 85 audits in Q4 2006, we saw improvement in ratings to: 88% “excellent,” 10% “good” and 2% “needs improvement”. Since establishing this program, the Department of Comparative Medicine staff has become motivated to improve their quality of work and has welcomed the process. As a result, this system has reduced the number and severity of deviations and animal welfare concerns as technicians self-monitor their work to raise the performance of their work group.

P72 Meeting the Challenge of Maintaining Healthy Rodent Colonies in an Animal Facility
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Maintaining healthy animal colonies is important in any research facility. Clinical disease may result in high morbidity and mortality, while sub-clinical infections can alter research data. Zoonotic diseases pose human health risks. Facilities monitor the health status of rodent colonies with sentinel programs using serological, microbiological, and molecular techniques to detect a standard group of pathogens. Because an increased number of animals originate from noncommercial sources, institutions must take more precautions to verify health status of incoming animals and must monitor animals closer to maintain clean colonies. Increased health surveillance is costly and may require a significant portion of a facility’s annual budget. Thus, providing comprehensive yet cost-effective health surveillance can be a challenge. Following a nationwide MHV outbreak, this institution revised its sentinel program to provide more consistent, comprehensive, flexible and cost-effective health surveillance. A simplified sampling system, modified from a randomized system which sampled a few cages to a standardized system where all cages are sampled, and unique sentinel cage identification, easily tracks problems to the technician or animal. The specially designed “step-down” quarantine procedure retains animals from noncommercial sources under extensive surveillance before placing them in the main facilities. The flexible testing program, combination of in-house/reference lab testing, makes the sentinel program more comprehensive and cost-effective. This modified program has had many positive results: surveillance consistency has increased to 100% and problems have decreased by 25%; residual MHV infection from the nationwide outbreak was successfully “burned out,” saving valuable transgenic strains; and no subsequent disease outbreaks have occurred. Five years of data shows that of 23,200 tests, only 100 were positives; none had clinically significant effects and all were easily tracked, contained and successfully eliminated. Also, a combination in-house/reference lab testing detected a novel REO virus. Thus, the simplified sentinel system is a successful solution for standardized surveillance to meet the challenge of maintaining healthy rodent colonies at this institution.
derlying pathology caused the barbering, we performed wool quality analysis, feed analysis, multiple skin scrapings, skin biopsy, hematology and serum chemistries, and prophylactic treatment with Dectomax parasiticide. Observing dominant sheep barbering their cohorts, incidence increasing with time in pens, and the lack of detectible underlying medical pathology supports the conclusion the barbering results from boredom. We evaluated additional enrichment to alleviate the behavior. Literature searches did not provide suggestions beyond what we were already doing, including housing with conspecifics. Since we could not find suggestions on how to enrich the sheep, we devised enrichment based on naturalistic behavior. We used hanging “puzzle” feeders (equine hay-nets) to add hay and complexity to the daily ration. We also used “toys” such as hanging basketballs to encourage play and non-barbering activity. In March of 2007 all of our sheep were shorn. This has allowed us to monitor the progress of the enrichment program since all animals had the same length of wool at that time. So far the animals have not demonstrated any barbering behaviors since the addition of hay to their diet; we are continuing to monitor the behavior of the animals as their wool grows back.

P74 A Novel Approach Using Metabolism Cages for Timed Matings
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Timed matings are a necessary part of research protocols that require pups or gestational research. A protocol requiring timed matings was presented; due to the limited time and funds available it was necessary to use equipment already on hand. This equipment included metabolism cages that are normally used to measure input and output of animals. These cages will collect solid material in one receptacle and liquid in another from the animal in the cage. It was theorized that plugs that fall from the rat should become deposited into the solid material receptacle. Virgin Sprague Dawley (CRL:CD[SD]) females were used along with male Sprague Dawley breeders. Vaginal cell samples using a Pasteur pipette and sterile saline were collected in the early afternoon to determine the stage of estrous. Females that exhibited signs of proestrus were placed with a male into the metabolism cages within 2 h of the end of the daily light cycle and removed within 1 h of the beginning of the daily light cycle. The solid waste cups were removed and examined for evidence of plugs. Plugs were easily differentiated from the fecal matter. There were no plugs found retained within the rat. When plugs were present the females were known to have copulated. With the exception of 1 female, all in a breeding cage that produced plugs were later determined to be pregnant. Random mating produced plugs 27.6% of the time. Using smearing, the plug percentage increased to 50%; this difference is statistically significant. Using the metabolism cages and combining it with a simple lab technique proved to be a unique and successful means of producing timed pregnant rats.

P75 Building Community Support for Animal Research, One Person at a Time
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The essential need for animals in research can be difficult for the general public to accept or understand, particularly in light of the efforts of anti-research activists to portray biomedical research as cruel and unnecessary. The strategy of maintaining a “low profile” has failed. For this reason, many facilities have become more proactive in public outreach, often having dedicating personnel who give tours of facilities, handle press releases, and interface in other ways with the community. At the Oregon National Primate Research Center, we supplement these public visits with efforts to build lasting relationships with community members. For example, many of our scientists and veterinarians mentor students and local teachers. The Behavioral Sciences Unit (BSU; the part of the Animal Care staff responsible for overseeing primate enrichment) has taken another approach, establishing long-term relationships with school groups such as the Roots and Shoots program and the Girl Scouts. Because we work directly with the animals, and closely with husbandry and clinical personnel, we can offer an intimate perspective into day-to-day operations. One example of a BSU outreach project involved a Girl Scout who proposed to design and build some play structures for our corral-housed macaques as part of an awards program. To carry out her project, she spent several months observing the macaques, as well as talking with animal care technicians. During her time at the ONPRC, she became a regular part of the rhythms of the day. It soon became evident to her that the technicians really care about the animals in their charge. She became more focused on the animals and facilities, and less on the controversy. Additionally, she was able to present her project to her troop, and other Girl Scout troops in her area, thereby helping to educate her community. While our visitor was not opposed to animal research, she was able to get an appreciation of the dedication and effort that goes into the care and enrichment of our animals. Just as important, we were able to see what we do through a new set of eyes, and we gained an advocate who would take what she learned into the community at large.

P76 Redesigning a Process Using Lean Thinking
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In any animal resource program, adequate documentation of procedures is required to monitor employee performance and to demonstrate to regulatory agencies that a program is compliant. Originally developed for manufacturing processes, lean thinking can easily be applied to processes used in animal care. Our long-standing process for documenting approved variations from standard practices and regulations (termed “exceptions”) became overly complicated as additional layers of review and approval were added. The length of time to approve and post an exception form in the affected animal room had gradually increased to as many as 8 d. Such delays led to dissatisfied internal and external customers (including animal care technicians, investigators, and IACUC staff). Since our parent institution encourages the use of lean thinking to redesign business processes, we applied these principles to streamlining this procedure. A team of 5 staff members mapped and evaluated the steps in the existing exception process for value to the customers using a variety of common questions (Why is this step needed? What value does it add to the process? Who requires this step? If we eliminate the step, what will happen?). We determined the number of steps, the amount of time needed to complete each step, the number of handoffs, and the number of people involved in the process. The original process consisted of 20 steps performed by 9 people. There were 8 handoffs; the time
between steps ranged from a few minutes to 5 to 7 d. The total time from initiation to final approval was 6 to 8 d. We identified 12 steps and 3 handoffs that could be eliminated without adversely affecting the value of the process to the customers. A new process was mapped consisting of 8 steps performed by 6 people. There were 5 handoffs and the time between steps ranged from a few minutes to 4 h. The total time from initiation to final approval was 1 to 2 d. The success of the project demonstrated the value of lean thinking to the animal care staff and has resulted in initiation of several more lean projects.

P77 An Evaluation of the Use of BBL™ CHROMagar™ Orientation Media in an In-house Rat (Rattus norvegicus) Health Monitoring Program

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As a part of the routine health surveillance of conventionally housed rats at our facility, adult female Sprague-Dawley rats from Harlan are exposed to soiled bedding. Standard testing consists of serology, bacteriology, and parasitology. In a labor-intensive process, the nasopharynx (NP) and feces (CE) are always cultured. The purpose of this study was to compare results obtained on a quick isolation chromogenic agar, BBL™ CHROMagar™ Orientation (CM) (Becton-Dickinson, Sparks, MD) system with standard culture techniques to determine if it would be an effective alternative. Known gram-positive and gram-negative organisms were plated on CM to assess chromogenic properties. NP and CE cultures were obtained on sentinel mice at necropsy. All cultures were incubated into TSB (Trypticase Soy Broth), incubated at 37 °C overnight, then plated by a 10 UL sterile loop on CM, MacConkey (MAC) and Blood agar. Cultures were evaluated at 24 h. Isolates of pure colonies on the MAC plates were identified using the API20E® system. Gram-positive organisms were tested using catalase. Tests on peroxidase-negative organisms included bile esculin and 6% TSB. Peroxidase-positive organisms were tested using Staph X®. Sixty-four cultures (32 NP and 32 CE) were plated. CM plates showed 119 organisms isolated, while traditional media isolated 118. Of these totals, CM and traditional methods reported out the same organism 83 times. The amount of growth was comparable for the same organism on both media. Bacillus did not grow on CM. Gram-negative organisms were more frequently missed on CM when cultures grew more than 2 organisms. Additionally, gram-positive organisms warranting subsequent testing are determined initially on the type of hemolysis seen on BAP. This indicator is absent in CM, thus delaying identification by 24 h. CM effectively allows identification of 2 or less organisms within a 24-h period, thus decreasing the time it takes for organism identification. Therefore, CM may be more efficient and provide quicker turn-around time than standard culture methods in culturing normal flora (CE and NP) in an in-house rat health monitoring program.

P78 Introducing Macaques to Novel Housing Systems Mid-Experiment: Proactive Behavioral Management

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Introducing adult macaques to new caging can be fun, but also challenging, especially when attempted in the middle of an experiment. Novel housing systems may cause the animals to be less “cooperative” for animal resource personnel and investigators. Vanderbilt University (VU) recently purchased 2 new housing situations for macaques: Primate Products (PPI) apartment and activity modules and Allentown (ACE) social interaction units. VU houses 3 species of macaques, rhesus (Macaca mulatta), bonnet (M. radiata), and cynomolgus (M. fascicularis), that participate in visual and auditory behavioral research (n = 41; female = 16, male = 25; age range: 4 to 14 y). The primary concern was that the novelty of increased vertical space within the new housing systems would cause the macaques to be less motivated to transition to the restrainer and/or perform experimental tasks mid-experiment. A secondary concern was that the macaques would not voluntarily enter the transfer boxes employed by the animal care staff for routine husbandry procedures. Macaques were previously trained to actively cooperate by voluntarily entering a restrainer for behavioral research and a transfer box for routine husbandry procedures. A cage conditioning plan (CC) was created using positive reinforcement techniques to train the macaques to return to their “home cage” by shaping desired behaviors, such as rewarding movements toward “home cage” when the cue (pointing to “home cage”) was given. Eighty-five percent (35 of the 41) macaques required less than 6 training sessions for the behavior to be under stimulus control (2 to 3 training sessions per week). Further, the animals continued to voluntarily enter their restrainers and transfer boxes, and remained motivated to work. In this instance, a proactive approach to behavioral management helped to minimize the disruption of the move to new cage systems, while promoting psychological well-being of nonhuman primates through positive human interaction (training) and increased cage complexity (vertical space).

P79 Alternative Training Strategies for Veterinarians: Distance Education in Laboratory Animal Medicine

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An urgent need exists to provide entry-level continuing education and applied training for licensed veterinarians who are new to the field of laboratory animal medicine. This population includes veterinarians who are looking to make a career change, who are working part-time in this field, who own private practices and provide consulting veterinary services, or who are working in the field but are unable to return to university for a full-time residency program or graduate studies in this specialty. An unconventional approach is needed to attract these adult learners who might have busy professional lives with limited time for attending traditional didactic classes. Distance education has increased greatly in popularity in recent years, even amongst students who are campus-based. The main advantage of a distance education program is that course participants can study from home or work when it is most convenient for them. A drawback of this type of education is that students miss the opportunity to engage with instructors face-to-face. This can largely be overcome in an online environment by providing
a personalized, welcoming environment with ready access to the course coordinator. A distance certificate program was determined to be particularly well-suited for providing basic information to veterinarians seeking continuing education in laboratory animal medicine. An US-based academic certificate program totalling 160 h of effort is under development to address entry-level training of veterinarians in laboratory animal medicine. The 4-course program of study consists of an initial web-based, self-study course that provides broad-based theoretical information on major themes in laboratory animal medicine tested by short assignments and multiple choice quizzes, followed by 3 skills-based courses held at regional training sites across the United States. Upon successful completion of the program, participants will receive a Certificate in Laboratory Animal Medicine. This program will seek to address an educational and training gap that exists for veterinarians seeking to make a career change to laboratory animal medicine and is being coordinated by a sub-committee of the American College of Laboratory Animal Medicine.

P80 A Common-sense Approach to Processing Cages and Bottles
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Moving autoclaved rodent cages and water bottles to the point of use can expose sterilized surfaces to microbial contamination. Safety is a second concern if the materials are handled while hot, lifted too high, carried too far, or if the batch size is too large and ergonomic factors for each employee are included. Lastly, labor costs go up when materials are handled more often than necessary. We have developed different transfer/transport carts to optimize batch size, minimize the number of times materials are handled, and limit lift heights. The cage transfer carts have a rolling-top carriage that lines up with the floor of the sterilizer, and carriages are loaded with draped stacks of bedded mouse cage bottoms for sterilization. The loaded carriages are rolled onto the cart and transported to the point of use. The drapes are removed by reversing them when the material is placed inside the workbench. They are also used to cover dirty cages for containment during transport back to dirty cage wash. Adapting this process for a single door autoclave added a second large drape to cover the entire load that is decontaminated inside the workbench. They are also used to cover dirty cages for containment during transport back to dirty cage wash. Adapting this process for a single door autoclave added a second large drape to cover the entire load that is decontaminated and removed before entry into the individual animal room. A second transfer cart has been designed for empty cages of clean water bottles that are then filled on a carriage and rolled into the autoclave chamber for sterilizing. Sterile cases of water bottles can roll onto these same carts and be transported directly to the work area. This ergonomic approach to moving materials reduced the number of times each cage is handled by 50%, ensured that no personnel works below an height of 25 in., eliminated lifting 40-lb cases of full water bottles, and maintains sterility of cages and bottles.

P81 Maintaining Longevity in a Triple Transgenic Rat Model of Alzheimer’s Disease
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Rats triple transgenic (Tg) for genes linked to brain beta-amyloid deposition (SD/Tg478/1116/11587) on a Sprague-Dawley/Wistar star background (generated by Cephalon) were enrolled in a research protocol requiring animals to survive up to 26 mo. As the established colony members aged, the rats began getting sick (10% weight loss, porphyrin discharge), or dying at age 15 to 17 mo (males) or 18 to 20 mo (females). To gauge colony health, any rats found dead or in an advanced state of illness were necropsied. Since the expected phenotype did not suggest immunosuppression, rats were initially housed in micro-isolator shoebox cages on regular hardwood chip bedding and offered ad lib rodent chow and non-acidified water. Consistent necropsy findings from these rats were interstitial and tubular changes, indicative of chronic kidney disease. Some rats additionally showed histologic evidence of hypertension, confirmed by blood pressure readings and echocardiograms (Tg, n = 2). Organ cultures yielded growth of commensal organisms and histopathology of the lungs in multiple animals revealed the presence of large numbers of Pneumocystis carinii; both findings indicate immunocompromise. Blood chemistry and urinalysis (Tg, n = 19, non-Tg age-matched controls, n = 9) confirmed renal dysfunction in transgenic rats older than 12 mo. There also appeared to be an increased incidence of various types of tumors in this transgenic group. In an attempt to minimize these health issues, the rats were moved into autoclaved cages with autoclaved bedding and trimethoprim-sulfamethoxazole-treated acidified water. They have been switched to a 14% protein, 3.5% fat diet, and also are now being pair-housed as long as possible, based on animal weight. These changes have had some success in extending the lifespan and improving the health of these valuable transgenic rodents, as some females have survived up to 24 mo; work continues to extend this lifespan even more. This has been an excellent learning opportunity for all levels of our department (husbandry through veterinarians) to identify problems, develop treatment plans, and coordinate their implementation.

P82 The Light:Dark Cycle May Influence Infanticide Rate in a Breeding Colony of C57Bl/6 Mice
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Infanticide is described as the killing of newborn infants. It is commonly observed in laboratory rodent species, with some strains being more prone than others. Many studies have shown that both male and female C57Bl/6 mice exhibit this behavior. C57Bl/6 mice are one of the most commonly used inbred strains. The incidence of infanticide in C57Bl/6 mice may influence the production of other valuable transgenic strains, as they are a common background strain (wild-type) for transgenic production. A new breeding colony of C57Bl/6 mice was set up in a room with a 12:12 light:dark cycle. Due to the low number of surviving pups, the light:dark cycle was changed from 12:12 to 14:10. Breeding records spanning a 10-mo period (with 5 consecutive months per cycle) were analyzed to determine whether the light:dark cycle had an effect on infanticide rate. Data were compiled from a breeding colony in which breeding pairs were continuously added and retired. A total of 363 records were evaluated, including first-time mothers and experienced ones (178 in 12:12 and 185 in 14:10). With the 12:12 cycle, infanticide rate was 49.4%. The average number of surviving pups per litter was 2.4. The rate of infanticide in first litters was 58.1%. With the new 14:10 cycle, infanticide rate decreased to 26.5%. The average number of surviving pups per litter increased to approximately 4 (4.0). The rate of infanticide in first litters was reduced to 29.7%. It appears that under the 14:10 light-
dark cycle, rates of infanticide (overall, and first litter) decreased, while the number of surviving pups per litter increased almost 2-fold. These results suggest an effect of husbandry practices on reproductive success in a breeding colony.

P83 Animal Census: Staying Current and Accurate
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Animal census changes daily and maintaining a database that accurately reflects what is physically present in the facilities is a challenge. Facility management software and database applications can help, but when you have more than 100 housing rooms, hundreds of investigators, thousands of protocols and tens of thousands of cages, accurate tracking can be very difficult. As the number of cages at Vanderbilt University (VU) grew from fewer than 12,000 in 2001 to more than 24,000 in 2006, the need to implement process improvements became obvious. Sirius 5.0 (NTM Consulting Services) was already in use to manage census data, but VU required further improvements in gathering, inputting, and maintaining that data. The aim was to develop a comprehensive census management system that would result in timely and accurate data. A dedicated census manager was hired to identify the areas impacting census and establish procedures for capturing and recording data. Using Sirius 5.0 along with Excel, Word, and Outlook (Microsoft Office 2003), VU developed and implemented new procedures to identify and process the animal deliveries as they arrive, activate and deactivate cages on a daily basis, track the movement of cages between rooms, protocols and investigators as they occur, and reconcile the database with physical inventory scans 3 times/month. As a result, VU has been able to upload per diem invoices for the same month’s financial close which enables investigators to better manage their funds. VU is able to generate meaningful reports to support our operations staff and the IACUC. Finally, rejected invoices have decreased from an average of more than 30 per month to almost zero. VU relies heavily on software to help manage the large inventory and constant turnover of cages, but without good procedures no application is going to produce good census data.

P84 Bactericidal Effects of Rodent Cage-wash Practices on the Viability of Listeria monocytogenes
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Our cancer biology group is in collaboration to develop a novel cancer vaccine composed of an attenuated Listeria monocytogenes engineered to express the receptor tyrosine kinase EphA2. L. monocytogenes is a gram-positive bacteria that is being investigated as a potential cancer therapy due to its ability to induce a potent immune response. EphA2 has been shown to be over-expressed in many different human tumors, including melanoma, breast cancer, and prostate cancer. Although the L. monocytogenes strain used in our studies is attenuated, risk of contamination of animal cages and bedding due to potential shedding in the urine or feces is high. Therefore, we chose to follow the ABSL-2 containment and decontamination practices required for wild-type L. monocytogenes. Currently, staff are required to wear appropriate personnel protective equipment (scrubs, Tyvek™ suit, hair cover, dust mask, shoe covers, safety glasses, and gloves). In addition, cages are dumped into a HEPA-filtered dumping station where the waste is bagged and autoclaved prior to disposal. The objective of this study was to evaluate our established cage wash practices, and determine if the high temperatures and mixture of detergents (Quip Lab Products: 1% Acidulate™, pH 1.5 and 1% EnviroClean 100™, pH 12.5) were sufficient to effectively kill L. monocytogenes. Polycarbonate test tubes containing various concentrations of L. monocytogenes (5e5, 1e6, 5e6 [concentration used in experimental protocols], 1e7, and 5e7 CFU) were treated with detergent combinations. The tubes were then heated for 6 min at 74 °C (average temperature achieved in standard 6-min cage wash cycle) followed by plating on tryptic soy agar and overnight incubation to perform a standard colony-forming assay. Results indicated that these conditions effectively killed 100% of the L. monocytogenes even at concentrations 1 log higher than those commonly used in our cancer biology program. The results of this study enabled us to avoid damaging the polycarbonate-based plastic cages by repeated autoclaving and saved time in husbandry procedures.

P85 Effects of Halothane and Isoflurane Anesthesia on Selected Serum Biochemical Parameters in NZW Rabbits
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Anesthetics have several effects on the functioning of some organs. In order to document the changes in select biochemical parameters in response to volatile anesthesia, 20 female New Zealand White rabbits (weight, 3.5 to 4.5 kg) were assigned to 2 treatment groups (n = 10): group H (halothane) and group I (isoflurane). All the rabbits were clinically healthy prior to the study. The induction of the anesthesia was achieved using a face-mask (3.5% halothane and 4.5% isoflurane in oxygen) and were intubated for the maintenance of the anesthesia for 30 min (1.5% halothane and 2.5% isoflurane in oxygen). Blood samples (2 ml) were obtained from the central ear artery before induction, 1, 10, and 30 min and 1, 2, 24, 48 and 72 h after intubation. Serum glucose, ALT, AST, ALP, BUN, and creatinine levels were measured by the Hitachi 747 autoanalyzer. Reflexes, heart and respiratory rates, but not blood pressure, were recorded. Administration of halothane and isoflurane significantly increased serum glucose from 1 to 120 min after intubation when compared with the baseline levels (from 102.1 ± 6.2 to 216.4 ± 17.5 mg/dl at 60 min in group H and from 98.6 ± 8.3 to 177.8 ± 12.3 mg/dl at 30 min in group I). It has been shown that both anesthetics inhibit insulin secretion from the pancreatic islets of Langerhans. Serum ALT was increased at 1 to 60 min and AST increased serum glucose from 1 to 120 min after intubation when compared with the baseline levels (from 102.1 ± 6.2 to 216.4 ± 17.5 mg/dl at 60 min in group H and from 98.6 ± 8.3 to 177.8 ± 12.3 mg/dl at 30 min in group I). Administration of halothane and isoflurane significantly increased serum glucose from 1 to 120 min after intubation when compared with the baseline levels (from 102.1 ± 6.2 to 216.4 ± 17.5 mg/dl at 60 min in group H and from 98.6 ± 8.3 to 177.8 ± 12.3 mg/dl at 30 min in group I).
interpretation of biochemical data and must be carefully used in rabbits with pre-existing liver or renal disease.

**P86 Effect of Psychosocial Stress on Hyperactivity in Cav2.2 Knockout Mice (Mus musculus)**

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Psychosocially stressed animals, such as those in simplified environment with decreased stimulation, show physiologic alteration. Although many behavioral studies have been done with transgenic and knockout mice, the effect of their housing condition has rarely been considered. Cav2.2 is essential for neurotransmitter release. Because Cav2.2 knockout mice display hyperactivity but no other apparent abnormality, they could be useful to study the effect of different housing densities on behavior. We examined the influence of social isolation on the motor activity of Cav2.2 knockout male mice with CBA/JN background. Mice at 3 wk of age were assigned to individual (stress; n = 1/cage; size, 6 × 18 × 12 cm) or group housing (control; n = 5/cage; size, 18 × 30 × 13 cm) in such a way that the amount of floor space allocated to each mouse was the same. We evaluated the effect of 6-wk period of either individual or group housing on the activity displayed in novel or habituated condition. In novel condition, mice were individually introduced into an acrylic box (15 × 10 × 13 cm) for 15 min, during which time motor activity was recorded by VERSAMAX equipment. In habituated condition, control mice were habituated in acrylic box (n = 5/box; size, 30 × 20 × 13 cm), whereas stressed mice were individually habituated in acrylic box of different dimensions (n = 1/box; size, 15 × 10 × 13 cm). The amount of floor space allocated to each mouse was almost the same. They were habituated for 15 h (1830 to 0930), during which time the mice could feed and drink ad libitum. Motor activity was then individually recorded for 15 min in acrylic box (15 × 10 × 13 cm). We tested both novel and habituated condition at identical time periods; that is, we started recording motor activity at 0930 (n = 10/group). Without habituation, homozygous mice showed significantly higher activity than wild-type controls, with no influence of housing condition. When habituated, hyperactivity was seen in individually housed but not group-housed homozygous mice. No differences in body weight gain were found between wild-type and homozygous mice of control and stressed groups. These results indicate that psychosocial conditions influence behavior and that it is important to control for housing condition when phenotypically analyzing mutant mice.

**P87 Development of a Nonhuman Primate Model of Type 1 Diabetes in African Green Monkeys**

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Streptozotocin (STZ), preferentially toxic to pancreatic beta cells, has been used to successfully induce type 1 diabetes mellitus (TIDM) in numerous species, including nonhuman primates (NHP). To our knowledge, while it affords biosafety advantages over more common macaque models, a TIDM model has not been developed in African green monkeys. Fourteen adult male African green monkeys (Chlorocebus aethiops) averaging 7.1 ± 0.3 y of age were entered into a study of STZ-induced TIDM. Nine monkeys (DM) were induced using 55 mg/kg STZ IV (based on published NHP values), while 5 (CTL) animals received an equivalent volume of saline. Pretreatment fasting blood glucose was similar between groups and was checked daily in DM monkeys using a glucometer and conscious tail stick. All DM monkeys were hyperglycemic 2 d post-STZ (mean blood glucose, 203 ± 32 mg/dl), and twice-daily insulin therapy (Novolin 70/30) was initiated to maintain glycemic control. Insulin doses started at 0.5 U/kg/d and were subsequently adjusted according to twice-weekly blood glucose checks. Blood glucose in the DM group measured 161 ± 17, 216 ± 16, 269 ± 23 and 219 ± 29 mg/dl in weeks 1 to 4 post-induction, respectively, while CTL blood glucose remained below 55 mg/dl throughout. Over the same time period, exogenous insulin requirements in DM monkeys averaged 4 ± 1, 7 ± 1, 13 ± 1 and 20 ± 2 IU/d, respectively. After 3 wk, glucose tolerance testing confirmed severely impaired pancreatic beta cell function with DM, as area under the insulin response curve measured 116 ± 22 in DM monkeys compared with 2878 ± 534 in the CTL group (P < 0.01). HbA1c remained unchanged from baseline in CTL (4.3 ± 0.1% vs. 4.4 ± 0.1%) but rose from 4.5 ± 0.1% to 9.0 ± 0.4% in the DM group (P < 0.01 compared with CTL and baseline). Plasma osmolality was increased from baseline (P < 0.05) with associated electrolyte changes; however, renal function, as assessed by BUN:creatinine ratio, was not impaired. Preliminary indication of liver toxicity [alkaline phosphatase, ALT and AST were significantly increased 3 to 8 wk post-STZ (P < 0.05 vs. baseline)] resolved by 12 wk post-STZ. We have demonstrated that TIDM can be safely induced and effectively maintained short-term in African green monkeys; further observation is ongoing to assess the long-term validity of this model.

**P88 Comparison of the Diaphragm and Thoracic Technique for Telemetric Monitoring of Right Ventricular Pressures in Rats**

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Adaptation of the right ventricle (RV) in pulmonary hypertension (PH) is an independent prognostic factor. It is important to monitor RV function over time in order to study RV adaptation during the progression of PH. For this purpose, RV telemetry in rats was developed. The previously described thoracic technique is invasive. We propose that operating through the diaphragm is easier to perform and less invasive. Under aseptic conditions, the telemetry transmitters for RV pressure measurements (DSI, USA) were implanted in male Wistar Unilever rats (HsdCpb: WU, 250 to 350 g; 13 rats for the thoracotomy technique and 9 rats for the diaphragm technique) for a maximum study period of 4 mo. Post-operatively, all rats were observed and weighted daily. When clinically indicated, they received a single injection of buprenorphine (0.1 mg/kg IM). Echocardiography and histology were performed to evaluate detrimental effects on RV and diaphragm or intercostal muscles. With both techniques the animals recovered well: heart rate, respiratory rate and RV pressures normalized within 2 wk. However, with the diaphragm technique, the animals recovered faster (6.4 ± 0.5 compared with 9.5 ± 1.1 d to reach the pre-surgical body weight, P < 0.05) and
a better overall success rate was achieved (78% compared with 62%). Causes of failed procedures were: blood loss (n = 2), ventilation problems (n = 1), and blood clots in the pressure-catheter (n = 4). Echocardiography revealed normal RV dimensions and normal RV function. Upon necropsy, no herniation or wound dehiscence was seen in the diaphragm technique-treated rats. Histology revealed only local fibrosis in RV and diaphragm or intercostal muscles. RV telemetry is a feasible technique to monitor RV pressures over time. Based on our results, we suggest the diaphragm technique as the method of choice.

P89 Protective Effects of Various Saccharides on Frozen Mouse Sperm

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The protective effects of saccharides in the cryopreservation of sperm may vary according to type and concentration. Raffinose, a trisaccharide, is commonly used as the protectant in cryopreservation of mouse sperm. We examined the protective effects of various saccharides on the viability of mouse sperm in order to determine the best type and concentration to use. First, sperm from the caudae epididymides of retired C57BL/6 breeder mice were frozen with monosaccharides (fructose, galactose, glucose, rhamnose, and xylose), disaccharides (lactose, maltose, sucrose, and trehalose), and trisaccharides (melezitose and raffinose) at concentrations ranging from 4 to 33%. Five to seven mice were used for each saccharide, for a total of 63 animals. The sperm was stored in liquid nitrogen for a few days. After thawing, the optimal concentration at which the highest proportion of motile sperm was observed was determined. Next, sperm from mature BALB/c, C3H/He, C57BL/6, and DBA/2 inbred and B6C3F1, B6D2F1, and MCH(ICR) hybrid mice were frozen with the saccharides at the optimal concentrations, thawed, and used for in vitro fertilization (IVF). All embryos were cultured in vitro to observe their development. The highest proportion (20%) of motile sperm was obtained after thawing with 12% disaccharides and 18% trisaccharides. Sperm frozen with monosaccharides showed less or no viability. BALB/c sperm had deformed heads, so the fertility of both frozen (8% to 46%) and unfrozen (21%) sperm was very low. The fertility of all strains, except C57BL/6, showed the best protective effects of maltose (74% to 91%), melezitose (62% to 86%), and raffinose (78% to 95%) when compared with unfrozen sperm (68 to 99%) (P > 0.05), which indicates these are the best protectants for frozen mouse sperm. The embryos derived from frozen sperm could develop to term (93%, 13 of 14 embryos) when transferred into the oviducts of a recipient mouse.

P90 Development of a Cough Model in Guinea Pigs Using Automated Real-time Software-coupled Whole-body Plethysmography: A New Analytical, Non-invasive Technique for Pulmonary Studies

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There is an unmet medical need for the development of drugs for treatment of chronic cough associated with diseases such as asthma and cancer. The guinea pig (GP, Cavia porcellus) is considered the primary rodent species for modeling cough in humans. Unlike other rodent species, guinea pigs routinely cough and can be induced to cough. This report describes the first application of automated real-time whole-body plethysmography in a GP model of cough, in which we used analytical and automated features such as real-time acquisition and analysis of respiratory data, integrated respiratory signal and video collection, inhalation/exposure tower, and real-time integrated temperature and humidity compensation. Studies measured cough using cough detection plethysmography, which provides a non-invasive method to detect and count coughing occurrences in a conscious, unrestrained animal. The standard setup features a microphone located inside the animal chamber and connected to a signal processing module that amplifies and feeds the sound bursts to the acquisition and analysis software. The system is designed to direct any aerosol not inhaled by the animal to a self-contained source attached to the inhalation chamber, to minimize the agent from becoming aerosolized in the environment. This high-efficiency aerosolization technology produces a high-quality respirable aerosol with extremely low residual volume, which virtually ensures no aerosol exposure to the scientists. Guinea pigs (250 to 350 g) were acclimated for 10 min, after which we introduced nebulization of saline followed by exposure of agents (saline or 25 μM capsaicin) to induce cough or local pulmonary inflammatory response. After 10 min exposure to capsaicin, real-time analysis of various physiologic parameters was performed using IOX software (Emka Technologies) and Buxco hardware. Guinea pigs exposed to capsaicin showed increased incidences of cough events throughout the exposure period. Data was collected from 10 independent experiments (n = 4 in each experiment).

P91 Effects of Medetomidine-butorphanol on Heart and Respiratory Rate, Rectal Temperature, and Cortisol Concentrations in NZW Rabbits

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The aim of this investigation was to investigate the effects of the anesthetic combination medetomidine-butorphanol on the depth of anesthesia, heart and respiratory rates, rectal temperature, and serum cortisol concentrations in New Zealand White (NZW) rabbits (Oryctolagus cuniculus). All the rabbits were clinically healthy prior to the study. Ten animals maintained under conventional conditions (12:12 light:dark cycle; 20 to 22 °C; 50% to 55% relative humidity; 10 to 15 air changes/h) were anesthetized with medetomidine (0.5 mg/kg IM) and butorphanol (0.4 mg/kg IM). Blood samples were obtained from central ear artery using 23-gauge needles at 6 time points: before injection (0 min) and at 10, 30, 60, and 120 min and 24 h post-injection of the anesthetics. The depth of anesthesia was monitored by using the pedal withdrawal, ear pinch, and righting reflexes. Serum cortisol concentrations were measured by competitive enzyme immunoassay. An adequate level of surgical anesthesia was observed for about 1 h, with no reaction to pedal and ear pinching. The times required for the loss of the reflexes were 3.5 ± 1.29 min for righting reflex, 9 ± 5.29 min for pedal withdrawal and 9.75 ± 5.25 for ear pinch. Recovery of righting reflex was 65.75 ± 14.79 min after injection of anesthesia, pedal with-drawal reflex at 60.0 ± 5.0 min, and ear pinch at 60 ± 5.0 min. Heart rate was significantly decreased at 10 to 120 min when compared to baseline (time 0) from 244 ± 8.94 at 0
min to 145.6 ± 17.55 beats/min at 60 min, and respiratory rate decreased at 10 to 120 min (from 144 ± 6.32 to 0 min to 44 ± 3.09 breaths/min at 30 min). Both medetomidine, an α-2 adrenergic agonist, and butorphanol, an opioid agonist-antagonist, has been documented to induce bradycardia and bradypnoea in several species. Rectal temperature was significantly decreased at 120 min when compared with the initial levels (from 39.02 ± 0.14 to 37.6 ± 0.31 °C). Although a small increase was observed at 10 min, serum cortisol concentrations did not significantly change after injection of anesthesia when compared with the baseline levels. The combination had little effect on cortisol secretion. Although the respiratory and cardiac depression effects have to be considered for any experimental procedure.

P92 Guinea Pig Adenovirus Infection Does Not Inhibit Cochlear Transfection with Human Adenoviral Vectors in a Model of Hearing Loss

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Guinea pig adenovirus (GAV) infects respiratory epithelium and causes pneumonia. Our colony is maintained within a barrier facility and was specific pathogen-free for GAV; however, routine surveillance detected GAV in sentinel animals. Guinea pigs (Cavia porcellus) are group-housed in open caging, and serologic confirmation of GAV was demonstrated at experimental endpoints for numerous animals housed in this room. These guinea pigs are used as a model for induced hearing loss followed by regeneration of hair cells through transdifferentiation of nonsensory cells within the deaf cochlea using gene therapy with human adenovirus (hAV). Animals are deafened chemically followed by surgical inoculation of hAV directly into the inner ear. We wished to evaluate the effect of natural GAV on the epithelium of the inner ear to determine if infection inhibited the ability of hAV vectors to transfect cells. Adult male pigmented guinea pigs (n = 7), approximately 5 mo of age, were selected for this study because of their chronic exposure to GAV-positive conspecifics. No clinical signs were noted in these animals. Animals were deafened chemically (n = 2; controls), deafened chemically with surgical inoculation of hAV-GFP (Green Fluorescent Protein, n = 3), or were administered hAV-GFP surgically without prior deafening (n = 2). Two weeks after experimentation, animals were necropsied and cochleae were evaluated using fluorescence microscopy to assess GFP expression. GFP expression in hair cells demonstrated that the hAV-GFP vector was able to transfect inner ears in GAV-positive guinea pigs that had been chemically deafened (similar to transfection levels historically seen in GAV-negative animals). All guinea pigs had histological evidence of pleural hemorrhage and interstitial pneumonia, attributable to chronic GAV infection. Serology tests confirmed all animals were positive for antibodies against GAV at the time of necropsy, approximately 7 mo after initial detection of sentinel infection. This is the first study to demonstrate that natural GAV infection does not inhibit transfection with hAV vectors into epithelial cells of the guinea pig inner ear.

P93 Evaluation of Catheter Lock Solution in Different Heparin Concentrations in Sprague-Dawley Rats (Rattus norvegicus)

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Multiple blood samplings for pharmacokinetic and drug discovery studies are effectively and humanely conducted using a surgically implanted vascular catheter. The catheters’ patency is typically maintained by instilling a heparinized dextrose and heparinized glycerol (heparin 500 IU/ml) lock solution within the catheter lumen. We evaluated the rat catheter patency for extended use, and evaluated the effect of different heparin concentrations in maintaining catheter patency. Forty-eight male SD rats (10 wk old; weight, 300 to 325 g) with their femoral artery surgically catheterized (0.025 ID x 0.040 OD, 28 cm long polyurethane tubing with tapered tip) were obtained from an outside vendor. After a standard 24-h PK study (24-h blood sampling via catheter post-drug administration), catheters were primed with heparinized dextrose lock (1000 IU/ml heparin sodium added into 50% dextrose aseptically) in 3 different heparin concentrations (groups 1, 4, and 7, 500 IU/ml; groups 2, 5, and 8, 200IU/ml; groups 3, 6, and 9, 100IU/ml) and rats left untouched until the time of patency check. For groups 1–3 (n = 6/group), we evaluated patency once weekly for 3 wk. For groups 4–6 (n = 6/group), we evaluated patency once after 2 wk; for groups 7–9 (n = 4/group), we evaluated patency after 3 wk. All catheters from groups 1–3 remained patent with either no or gentle saline flush on day 8. The same observation was recorded in groups 4–6 on day 15. All the rat catheters from groups 7–9 maintained good patency after 21 d. We concluded that after a standard rat PK procedure, femoral artery catheter patency may be maintained for up to 3 wk without the need of intermittent flushing. This study suggests rats may be reused on an additional PK study up to 3 wk later, thus achieving one of the 3Rs (reduction) in animal research. Heparin concentration of 100 IU/ml of hep-dex lock solution maintained the catheter patency as effectively as that of 500 IU/ml; therefore, we can minimize the potential problems attributable to heparin side effects in the rat by using 100 IU/ml as the preferred heparin level.

P94 Serological Profiling of Lymphocytic Choriomeningitis Infected Wild-derived Mice

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RIKEN BioResource Center received lymphocytic choriomeningitis virus (LCMV)-infected MAI/Pas mice in 2004. MAI/Pas mice were derived from wild Mus musculus and seemed immunocompetent. Since the mice were reported as LCMV negative prior to import, we rederived them by cesarian section treatment. MAI/Pas progeny was vertically infected with LCMV and the infection was revealed by immunofluorescent assay (IFA) by monitoring foster mothers. In this report, we tested sera from MAI/Pas progeny and BALB/c nu + /+ mice (caged with progeny for 12 d) using IFA and a new multi-channel immunonasay microfluidic chip system. For the latter, purified LCMV antigen was immobilized in microchannels by electrospray deposition...
method. Diluted sera were applied in microchannels, goat anti-mouse immunoglobulins was added after washing, and signals were detected by chemiluminescence system. IFA detected 1:20 diluted M104 antiLCMV monoclonal antibody, whereas 200 times diluted M104 was easily detected by microfluidic chip system. IFA and microfluidic chip system showed that 1 of 3 BALB/c nu/+, 7 of 18 MAI/Pas mice (age between 132 d and 167 d) and none of 7 MAI/Pas mice (69 d old) produced antiLCMV antibodies. Kidneys of MAI/Pas mice were tested for LCMV via RT-PCR, and all of them had LCMV genes. We analyzed immunoglobulin isotype of produced antiLCMV antibodies by microfluidic chip system. Major isotypes were IgG2b or mixed type (IgG1, IgG2a and IgG2b) for MAI/Pas and IgG2a for BALB/c nu/+. As several reports said that isotype IgG2b or mixed type (IgG1, IgG2a and IgG2b) for MAI/Pas and IgG2a for BALB/c nu/+.,. As several reports said that isotype of antiLCMV antibodies of experimentally infected mice was IgG2a (Th1 type regulation), these results suggest vertically LCMV infected MAI/Pas mice had different type of regulation for antiLCMV antibody production.

P95 Use of a Telemetry Mouse Model for the Evaluation of Antihypertensive Agents
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Hypertension is a leading cause of mortality, affecting nearly a billion patients worldwide. There is a growing need for the development of more effective antihypertensive agents. Rat models, such as the spontaneous hypertensive rat (SHR) and Dahl salt sensitive (DSS), have been traditionally used for screening antihypertensive agents. Recent developments in the technology for generating specific gene overexpression as well as gene knockout mice have resulted in widespread use of mice in the area of blood pressure (BP) related research. Despite many advantages, the use of a mouse model for screening novel antihypertensive agents has been very limited. This is largely due to a lack of characterization of those available antihypertensive agents and a lack of reliable BP measurement system under relatively stress-free and conscious conditions in mice. We have studied 3 known classes of antihypertensive agents in conscious, unrestrained mice using a radiotelemetry system. Male C57BL/6J mice (14 wk old) were implanted with a PA-C10 pressure transducer telemetry device in the left common carotid artery. Following 10 d recovery from surgery, mice were treated with losartan, an AT1r antagonist that targets the RAAS; hydralazine, a vasorelaxant with unknown mechanism; and amiloride and furosemide, 2 diuretic agents. Losartan, hydralazine, and amiloride were administered in drinking water. Furosemide was administered by implanting a constant-releasing pellet under the shoulder skin. Before switching to treatment of new agent, mice were given a week-long rest (wash-out period). To maximally use the telemetry mice, transmitters were turned off during the wash-out period and turned on again 1 d prior to the treatment of a new agent. Four to five week-long studies may be conducted in 1 set of telemetry mice. Significant reduction in BP was observed following treatment with all 4 agents, suggesting this model may be sensitive to multiple classes of antihypertensive agents, and may have a broad application for the discovery of novel antihypertensive agents.
ampullae of oviduct were counted using stereo microscopy. As a control, 12-wk-old mature female rats underwent the same hormone treatment for comparison. Twelve rats were used at each dose level of PMSG. For the mature animals, following confirmation of two consecutive 4-d estrus cycles, PMSG was administered in metestrus phase. The PMSG dosages yielding the largest numbers of ovulated ova (mean number ovulated ova) were 200 to 350 IU/kg (30<) for the immature group, and 150 to 300 IU/kg (30<) for the mature group. With the aim of collecting early-stage embryos for culture, following administration of hCG to the 5-wk-old immature and the mature females, they were exposed to mature male animals of the same strain for natural mating. When 2-cell stage embryos of normal appearance, collected from oviducts 50 to 54 h after hCG administration, were cultured in mR1ECM culture fluid for 96 h, the rates of development to the blastocyst stage were more than 75% for the immature group, and more than 85% for the mature group. These results show that selection of the appropriate hormone dosage allows efficient egg collection from immature animals similarly to mature animals, and that the collected embryos developed normally to blastocyst.

**P98 Primary Mouse Hepatocyte Isolation and Culture from Crl:CD1(ICR), A/JCrTac, and C57BL/6NTac Mice and a Comparison of Gene Expression Profiles**

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Primary mouse hepatocyte cultures that retain function are useful tools for biochemical, toxicity, and carcinogenicity studies. With the rapid expansion of inbred and genetically modified mouse models, this system is of great value when studying different genotypes and phenotypes. In addition, these primary cultures can be used to study the cellular response to bacteria in vitro. In this study, we took a comparative approach using 2 inbred and 1 outbred strain of mice. Crl:CD1(ICR) (n = 5), A/JCrTac (n = 6), and C57BL/6NTac (n = 6) 8- to 12-wk-old male mice were used. The liver was perfused using a 2-step perfusion technique. The cells were recovered, counted and viability assessed prior to culture. The hepatocytes were cultured for 7 d and RNA was extracted at day 0, 1, 3, 5, 7. Gene expression profiles for OATP (organic anion transporting polypeptide), ADH (alcohol dehydrogenase), ALB (albumin), and HNF (hepatocyte nuclear factor) were generated using commercial primers and probes. In addition urea production was quantified as an indicator of cell function. The yield of viable cells was greater in the Crl:CD1(ICR) strain (5.2×10^6) than A/JCrTac (4.5×10^6) and C57BL/6NTac (4.8×10^6) strain. The viability was 87%, 91%, and 94% for the Crl:CD1(ICR), A/JCrTac, and C57BL/6NTac strain, respectively. Gene expression profiles indicated that all the tested genes were still expressed in primary hepatocytes, although down regulation of all 4 genes was noted. The most marked expression change was the downregulation of the OATP gene at day 7 in the A/JCrTac strain (2200-fold change) compared to other 2 strains (750-fold change in the C57BL/6NTac strain and 35-fold change in the Crl:CD1(ICR) strain). The HNF gene was down regulated 2-fold with time in all 3 strains. The ADH and ALB gene expression profiles showed down regulation at day 1 with a rebound at day 7. The primary hepatocytes continued to secrete urea into the culture supernatant, decreasing with time. In conclusion, primary mouse hepatocytes retain function and show differences across inbred and outbred strains of mice in terms of yield, viability, and gene expression.

**P99 Comparison of the Pathogenicity of Avian Influenza Virus A (H5N1) in Five Mouse Strains**

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Mice are a useful mammalian model for study of the pathogenesis of avian influenza viruses, specifically the H5N1 virus. To test the hypothesis that the sensitivities of mice with different strain backgrounds respond differently to H5N1 virus, we compared the pathogenicity of H5N1 virus infection in 5 different strains of mice. A total of 150 specific pathogen-free mice, 18 to 22 g (male:female = 1:1), from 2 inbred strains (BALB/c and C57BL/6) and 3 outbred stocks (ICR, NIH Swiss, and KM Swiss), were used. Thirty mice in each strain were subjected to an infected group (20 mice), in which mice were inoculated with 0.1 ml (10-4.87 TCID50) of A/Goose/Guangdong/NH/2003 (H5N1) virus intranasally; the control group (10 mice) received noninfectious allantoic fluid. Experiments were conducted under ABSL-3 conditions. Clinical signs were assessed daily for 14 d post-infection. Necropsy was performed on all the mice that died during the experiment and those euthanized at the end of the study. Tissue samples were either stored at –70 °C for viral isolation or fixed in formalin for pathological analysis. The mortality rate was compared in the 5 strains of infected mice. The results showed that H5N1 virus infection can cause respiratory illness in all 5 strains with severe or minor acute respiratory distress symptom, but with differences in the number of sick or dead mice. Necrotizing interstitial pneumonia was found in all the death cases. The virus was isolated from the lungs of all dead mice of H5N1 infection, but could not be detected in their other organs. The mortality rate was very different in the 5 strains of infected mice: 70% (14/20) in BALB/c, 50% (10/20) in ICR, 40% (8/20) in NIH Swiss, 25% (5/20) in C57BL/6, and 10% (2/20) in KM Swiss mice. Our study indicated that a H5N1 virus isolated from a goose in Guangdong province resulted in the highest lethality for the BALB/c strain of mice when compared with the other 4 mice strains. The clinical symptom and pathological changes of the infected BALB/c mice are similar to those found in humans infected with H5N1 viruses. Thus, BALB/c mice can be used as a suitable animal model for investigation into the pathogenesis of H5N1 virus infection, as well as anti-H5N1 virus study.

**P100 Development of a Microsphere-based Serologic Multiplex Fluorescent Immunoassay to Detect Theiler’s Murine Encephalomyelitis-like Virus in Rats**

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Rat Theilovirus (RTV), also referred to as Theiler’s murine encephalomyelitis-like virus of rats, is a newly characterized rat cardiovirus that has been shown to be related, but genetically distinct from, other viruses in the TMEV group. Serologic assays to detect antibodies to RTV in rats have historically used mouse TMEV strains as antigens, exploiting the antigenic cross-reactivity of these viruses with RTV. In this study, we describe the development of a multiplex fluorescent immunoassay (MFI).
to detect antibodies to RTV in the sera of rats using in vitro propagated RTV as antigen. A receiver operator characteristic (ROC) curve analysis, with a RTV indirect fluorescent assay (IFA) as the reference standard, was performed using sera from uninfected and naturally infected rats to establish 98% sensitivity and 98% specificity thresholds for the RTV MFI. The performance of this assay was compared to a MFI using the GDVII strain of TMEV as antigen. Sera from 4-wk-old male SD rats (n = 10) orally inoculated with 2.5 x 106 PFUs of RTV displayed 5-fold greater average signal intensity as measured with the RTV MFI compared to the GDVII MFI with both assays having comparably negligible reactivity of sera from uninfected control rats (n = 6). Given the greater signal intensity generated on the RTV MFI to RTV-infected rats, this assay has the potential to perform at a higher sensitivity for detecting rats naturally infected with RTV than immunoassays that use GDVII or other TMEV strains as antigen and become a valuable tool in RTV research and disease monitoring.

P101 Continuous Partial Liquid Ventilation with Perfluorocarbon in Mice
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With early detection, lung cancer has an increase in survival rate. Perfluorocarbons such as perfluorobron (or PFB) are unique liquids that improve oxygenation by using partial liquid ventilation (PLV). PLV with PFB has been used clinically in premature infants, adults with acute respiratory distress syndrome as well as in newborn animals. Rodents are increasingly being used as models to study the development and treatment of diseases, including lung disease. In our endeavor to develop novel magnetic resonance imaging (MRI) techniques to non-invasively image the lungs of mice, we have developed a method of intubation that allows for the continuous PLV of mice during an MRI procedure. We chose MRI because of its superior technique of non-invasive tumoral tracking. Mice in particular are challenging to work with because of their small size. The low spin density of hydrogen nuclei and air boundaries make lung imaging difficult. We will report on the construction and implementation of the modified tracheal intubation tube in more detail on the poster itself. Briefly, it is a 20-g, 1.25-in. tube with polyethylene tubing attached both proximally and distally with silastic tube. The controlled infusion of PFB into the mouse lungs can be achieved with a syringe pump. The attachment of the modified infusion tube to the syringe pump allows the imaging parameters of the mouse to remain constant. Due to the evaporation rate of PFB components in lung, adequate dosing or PFB is necessitated through the continual administration. We will show the comparison data of both intermittent tracheal injections as well as continuous administration of PFB through susceptibility on MR images presented on the poster. The ability to use continuous PLV through our modified tracheal tube provided more sensitive diagnostic images. Using 19-F MRI, we can also study the dynamics of PFB evaporation during PLV procedure. In our previous 16 studies using male C57BL6 mice, we have not used blood gas sampling, but in the refinement of this study, we have incorporated blood gas analysis to compliment our findings. Our modified infusion tube is instrumental in enabling more sensitive diagnostic means of detecting, tracking and monitoring diseases of mice through MR imaging.

P102 Time and Dose Exposure Effect on MPV-1 Transmission to Sentinel ICR Mice
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Intermittent serodetection of mouse parvovirus (MPV) infections in animal facilities is frequently observed when soiled bedding sentinel mouse monitoring systems are used. We evaluated induction of seroconversion in naive singly caged weanling ICR mice (n = 10 per bedding dilution group) maintained on fivefold serially diluted contaminated bedding obtained from SCID mice persistently shedding MPV-1e (mean = 1.3 x 107 viral DNA copies/fecal pellet). Soiled bedding from the infected SCID mice was collected, diluted, and redistributed to ICR mouse cages every week to represent chronic exposure to MPV at varying prevalence in a research colony. Sera was collected by mandibular venipuncture every other week for 12 wk and evaluated for reactivity to MPV nonstructural and capsid antigens by multiplex fluorescent immunoassay. Mice were euthanized after seroconversion, and DNA extracted from lymph node and spleen was evaluated by quantitative PCR to estimate lymphoid tissue viral load. Cumulative seroconversion rates for the 7 soiled bedding dilution groups (range = 1.5 to 1.78125) over the 12-wk study period were 100%, 100%, 80%, 20%, 70%, 60%, and 20%, respectively. Most seropositive mice (78%) converted within the first 2 to 3 wk of soiled bedding exposure, correlating to viral exposure when mice were 4 to 7 wk of age. Viral DNA was detected in lymphoid tissues collected from all mice that were seropositive to VP2 capsid antigen (mean = 5.3 x 105 viral DNA copies per 2 ml DNA), while lymphoid tissue viral DNA was not detected in seronegative mice. These data indicate that while seroconversion occurs consistently in young mice exposed to high doses of virus, seroconversion is less consistent in mice repeatedly exposed to lower doses of virus that are equivalent to those shed from MPV-infected mice after an adaptive immune response (<103 viral DNA copies/fecal pellet).

P103 Dietary Ammonium Chloride (NH4Cl) for the Acidification of Mouse Urine
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Some compounds induce urinary bladder tumors in rodents secondary to crystalluria. In carcinogenicity studies performed simultaneously in both rats and mice, a novel therapeutic compound was found to induce bladder tumors in male rats. Given that urinary acidification reduces the formation of calcium and magnesium containing solids including struvite, a method of acidifying the urine of both species was needed to investigate whether tumor formation was related to increased crystalluria rather than a direct pharmacologic effect on the urothelium. This study tested the efficacy of adding 1% ammonium chloride (NH4Cl) to a standard rodent diet in reducing the urine pH of mice (Mus musculus). Male, 9- to 10-wk-old Crl: CD-1(ICR)BR mice were randomly assigned to 2 groups and acclimated to individual housing in wire-bottomed cages with Nylabones for enrichment. A 12:12 light:dark cycle was used,
and water purified by reverse osmosis was provided ad libitum through an automated watering system. Ten mice were given a commercially available rodent diet (Harlan Teklad 8728C), and 10 mice were given the same diet with 1% NH₄Cl added. Freshly voided urine samples were collected 1 h after the start of the light cycle by inducing urination into a Petri dish and measuring urine pH with a pH meter. After 1 wk, the control group’s average urine pH was 7.51 ± 0.32, compared with 6.21 ± 0.31 for the NH₄Cl-fed group. After 2 wk, the average urine pH was 7.78 ± 0.41 for the control group, compared with 6.20 ± 0.30 for the NH₄Cl-fed group. Samples were also collected 8 h after the start of the light cycle on the day of the 2-wk collection. Average urine pH was 7.12 ± 0.28 for the control group and 5.80 ± 0.23 for the NH₄Cl-fed mice. The pH differences between control and NH₄Cl-fed groups and the differences in pH within groups at 1 and 8 h were all statistically significant (P < 0.05). Dietary 1% NH₄Cl is an effective urinary acidifier for mice. When evaluating the pH of mouse urine, care should be taken to compare samples collected at the same time after the start of the light cycle.

P104 Eliminating Autofluorescence from Living Subjects in Small Animal Fluorescence Imaging Using Lifetime Imaging
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The availability of new near-infrared fluorophores and imaging enhancement software has led to a recent upward trend in the optical imaging market, as these systems are more readily affordable and feasible for a broad spectrum of end-users. However, autofluorescence emanating from ingested food still remains to be one of the major setbacks in the area of noninvasive small animal fluorescence imaging, even at near-infrared wavelengths. Although changing the regular animal diet to an alfalfa-free diet can significantly reduce the autofluorescent signal, it is not completely eliminated and cannot be discriminated from fluorophore signals with a similar absorption and emission spectra. In the present study, we used a fluorescence lifetime (decay) technique to filter out the residual autofluorescence from ingested food. Nude mice (nu/nu, female, 10 wk old, n = 8), fed with a regular rodent diet, were anesthetized and placed on a scanning stage, which is warmed to 37 °C. Two ventral, whole body scans, taken once, of each mouse with 635 nm and 670 nm pulsed laser diodes coupled with 670 nm and 700 nm emission filters, respectively. Significant amounts of autofluorescence were detected from the lower abdomen (stomach, gut, intestines, colon) in both scans. The fluorescence lifetime imaging technique was used here to filter out the signal emanating from ingested food and eliminate background autofluorescence from these animals. These results demonstrate the utility of fluorescent lifetime information as a valuable tool for improving image quality and for discriminating between multiple sources of fluorescence with similar absorption/emission spectra.

P105 Intravenous Pharmacokinetics and Pharmacodynamics of a Low Molecular Weight Heparin (Enoxaparin) in Cats: Impact on Anticoagulation
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The relative anticoagulant effects of unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) in feline blood are much weaker in comparison to the anticoagulant results observed in human blood. A previous report had described these differences where UFH and a LMWH (enoxaparin) were found to produce weaker anticoagulant responses as measured by APTT. These results indicated that much higher circulating levels are needed in cats to achieve similar results found in humans. Since pharmacokinetic differences are also observed in different species, additional studies were undertaken to determine the relative pharmacokinetics (PK) and pharmacodynamics (PD) of enoxaparin in cats. Eighteen domestic shorthaired cats (Felis catus) had a baseline blood draw taken and were subsequently administered a dose of 1.25 mg/kg of enoxaparin SC every 6 h for 3 d. On the 3rd day, blood was drawn every hour for 6 h. A final blood draw was taken after the last dose on the 3rd day; plasma was separated and frozen at -70 °C. Anti-Xa activity was determined using a standard amidolytic method and ranged from 2.6 to 6.2 μg/ml at 3 h. The PK parameters were calculated by using PD of the anti-Xa activity of circulating level in μg/ml. Wide variations in the PK parameters were noted among individual cats: The t1/2 range was 1.1 to 2.6 h in comparison to the human t1/2 of the same drug (> 3 h). The clearance rates in cats also varied widely and ranged from 2.0 to 10.0 ml/h/kg and were faster than observed in humans, which are 2.0 to 5.0 ml/h/kg. These results clearly suggest that besides the lower heparinization index, the PK parameters of cats are significantly different in comparison to humans; moreover, wide intra-individual variations among cats were also noted. The faster elimination of enoxaparin in cats and the wide variation of the activity due to the PD effects of the drug suggest the current dosing of enoxaparin in cats is subtherapeutic for specific indications. Therefore the dosage optimization requires periodic monitoring and adjustment to achieve the desirable therapeutic outcome.

P106 Binding of Mouse Mannan-binding Lectins to Murine Bacterial Pathogens
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Mannan-binding lectin (MBL) is an important protein of the innate immune system that binds specific, common oligosaccharide pathogen-associated molecular patterns on the surface of bacteria, fungi, and viruses. It contributes to host defense by microorganism opsonization or direct complement activation by way of the lectin pathway. Humans have 1 MBL in circulation but mice have 2: MBL-A and MBL-C. Because of the difficulty in isolating these proteins, little is known about the functional binding of this protein to potential pathogens in mice. Plasma forms of these proteins have similar carbohydrate binding activity in vitro, but may differ in their ability to bind other microbial targets. In these studies, we compared carbohydrate-dependent binding of mouse plasma MBL-A and MBL-C to mannan-sepharose beads and to intact bacteria isolated as pathogens from mice (Klebsiella oxytoca, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli). Following a 4-h incubation of pooled mouse plasma (commercial source, various strains) with intact bacteria, MBL-A and MBL-C were eluted with N-acetylglucosamine (GlCNac) and identified in nondetecting SDS-PAGE using Western blot analysis and MBL-A or MBL-C specific monoclonal antibodies. Results were repeated at least two separate times for each bacterium. GlCNac eluates of plasma incubated with mannan-sepharose beads, K. oxytoca and...
S. aureus contained similar bands (mainly ~50 kDa) that were immunoreactive with MBL-C antibody. A smaller form of MBL-C (~45 kDa) was also detected bound to P. aeruginosa. Additional replicates (total of 5 assays) were performed with P. aeruginosa to confirm these findings, and all replicates revealed the same ~45 kDa bands. By comparison, immunoreactive MBL-A (a ladder of ~175 kDa and larger bands) was identified in these GlcNAc eluates from mannan-sepharose beads, S. aureus and K. oxytoca but not P. aeruginosa. Neither MBL-A or MBL-C was detected in GlcNAc eluates of plasma incubated with Escherichia coli. These studies demonstrate that mouse MBL-A and MBL-C in plasma have differential binding activities and are not equivalent in their ability to recognize bacterial pathogens.

P108 Development of a Nonhuman Primate Chronic Ambulatory Infusion Model

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Increasing demands on basic research to shorten drug development timelines have prompted the need for the development of a nonhuman primate chronic ambulatory infusion model (NHP CAI). Contract research organizations have typically conducted chronic infusion studies in Phase 1 trials using jacket and tether systems with syringe pumps externally attached to the cage. We developed an infusion model in nonhuman primates that would be used to evaluate the effects of chronic compound administration in early development stages. Our goal was to develop a system that would allow accurate, chronic (24 h or longer) infusion of test material or vehicle and also be ambulatory, allowing manipulation of the animals outside of the home cage without interrupting infusion. Six adult female rhesus monkeys (Macaca mulatta) with jugular vascular access ports (VAPs) were fitted with jackets carrying infusion pumps. Pumps were attached to VAPs via right angle Huber needles and animals were continually infused with saline for up to 7 d. Two studies were conducted (n = 4 animals per study) evaluating stability of chronic Huber needle placement, VAP bacterial contamination, and pump delivery rate accuracy. The Huber needle assembly was found to be durable and remained in place for the study duration. Blood was drawn from VAPs prior to and post-study and evaluated for bacterial contamination. All blood cultures were found to be negative after the 7 d infusion period. With a pump setting of 2 ml/h, our average calculated rate of infusion for 8 animals, over 7 d, was 1.95 ml/h. We can conclude from this data that this NHP CAI model will be durable and uncontaminated and will accurately deliver test material continually for up to 7 d.

P109 Intramuscular Administration of 4-Vinylcyclohexene Diepoxide (VCD) Destroys Primordial Follicles in Adult Sprague Dawley Rats (Rattus norvegicus)

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Rats and mice treated with daily intraperitoneal (IP) injections of 4-vinylcyclohexene diepoxide (VCD) undergo gradual ovarian failure due to destruction of primordial follicles, thus mimicking the perimenopausal transition in women. Here we describe an alternative route of VCD administration (intramuscular [IM]) that might be more suitable to larger models, such as monkeys. Ten adult (age 129 ± 4.8 d) female Sprague Dawley (SD) rats were anesthetized with isoflurane and injected daily (0.08 to 0.1 ml) for 10 to 15 d in the semimembranosus/biceps femoris muscle with VCD doses comparable to previous IP VCD rodent studies, as follows: saline: 10 mg/kg (n = 5); VCD 80 mg/kg (n = 2); VCD 160 mg/kg (n = 3). VCD was dissolved (in a fume hood) in saline at a dilution rate of 1:5 (80 mg/kg) and 1:1 (160 mg/kg). One-way analyses of variance and pair-wise t-tests were used to identify significant treatment group differences in follicle counts and body weight. Animals treated with 160 mg/kg VCD IM lost significant weight (approximately 19%) after 10 to 12 d and were euthanized and necropsied. Severe necrotizing myositis at the injection site, with intraleisonal foreign material (unabsorbed drug vehicle) was the primary finding. Rats treated with 80 mg/kg VCD IM or vehicle (n = 2) completed 15 d of treatment without adverse clinical signs or significant weight loss from baseline (~4% and ~1%, respectively) and a mild, focal fasciitis (vehicle) and a moderate, focal, fasciitis, myositis and steatosis with necrosis (80 mg/kg) was observed at the injection site. No other VCD related lesions were found in the other organs examined. Primordial follicles counted per ovary were reduced significantly (P < 0.01) with both 80 mg/kg (15 d, -80%, vehicle 78.4 ± 11.7; VCD 16 ± 3.0) and 160 mg/kg VCD (10 to 12 d, -90%, vehicle 78.4 ± 11.7; VCD 8.7 ± 2.6). This study demonstrates that 80 mg/kg VCD IM destroys primordial follicles more efficiently (~80%) and in less time (15 d) than IP administration (70% reduction in 30 d in previous studies in Fisher rats) and was statistically indistinguishable from 160 mg/kg IM, which was not tolerated in adult Sprague Dawley rats.

P110 Fluorescent Microangiography: A New Technique to Visualize the Cardiac and Renal Microvasculature

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The assessment of coronary and renal vasculature is a key tool in determining the underlying pathophysiology of many diseases such as diabetes and post-myocardial infarct remodeling. Fluorescent microangiography is used to demonstrate that abnormal pulmonary vasculature is a fundamental feature of pulmonary hypertension, but it has not been applied to cardiac and renal microvasculature beds. Thus, we set out to optimize fluorescent microangiography in the cardiac and renal vascular beds in an attempt to develop a quantitative microangiographic technique. Four male, 6-wk-old Rattus norvegicus (Fisher-344 strain) were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg)/xylazine (10 mg/kg), and a mid-line incision was made through the thoracic and abdominal regions. To assess the coronary vasculature, the ascending aorta was cannulated and a ligature applied just distal to the brachiocephalic trunk. A low viscosity perfusate (sodium chloride, heparin) was then used to flush the coronary vasculature, followed by the perfusion of intensely fluorescent polystyrene microspheres (0.2 μm with agarose). A similar protocol was used to study kidney vasculature, with the cannulae placed in the supra-renal aorta and a ligature placed infra-renally, followed by infusion of perfusate then microspheres. Tissues were then obtained and imaged through confocal optical sectioning of 200 μm-thick slices. This technique, coupled with the strikingly enhanced depth of field in projected images allowed a detailed, 3-dimensional view of the cardiac and renal microvasculature, which cannot be obtained through any other imaging technique. In summary, fluorescent microan-
P111 Chemotherapeutic Agent Doxorubicin Induces Cardiac Failure in Males and Mammary Tumors in Females Exposed as Neonates

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Epidemiological data demonstrate that pre-pubertal girls exposed to doxorubicin for cancer therapy are more likely to develop cardiomyopathy later in life. Additionally, there is strong evidence that estrogen is cardioprotective against doxorubicin toxicity in adult rodents. We attempted to model this problem in neonatal prepubertal rats (day 7) in order to develop cardioprotective strategies for children during cancer therapy. In this pilot study, 2 litters of Sprague Dawley rat pups were divided by sex and randomly assigned to 8 treatment groups (n = 3). Rat pups were administered either saline or 1 mg/kg doxorubicin every other week intraperitoneally in a volume of 25 to 50 μl in 1, 2, or 3 doses starting at day 7. Rats were monitored daily. Animals were monthly evaluated by trans-thoracic echocardiography to follow left ventricular systolic function. At 4 mo of age, male rats treated with doxorubicin showed a significant decline in cardiac left ventricular systolic function (FS%, fractional shortening) with a FS% mean ± SD of 51% ± 9% compared with 69% ± 2% in female doxorubicin treated rats (ANOVA P < 0.001). FS% was 70% ± 2% for female controls and 70% ± 2% for male controls. FS% declined to 40% in some male rats before euthanasia and correlated with histopathology. A dose response was observed in the development of cardiac dysfunction. Currently, at 10 mo of age, female rats have a range of 55 to 65 FS%, demonstrating partial protection from doxorubicin cardiotoxicity. The second major finding of this study was unexpected; 80% of the female rats exposed to doxorubicin (at all doses) developed rapidly growing mammary masses by 10 mo of age (70% incidence of fibroadenomas at 2 y in published studies). This is the first report of mammary tumor development after doxorubicin treatment in neonatal rats. These rats are currently being followed after mastectomy for additional neoplastic growths and cardiac dysfunction. Histopathology of the masses revealed mammary fibroadenomas (85%) and adenocarcinomas (15%) in only the doxorubicin-treated female rats. No masses were found in the littermate control (females or males). The authors speculate that doxorubicin acted as a mutagen in the female neonatal mammary tissue.

P112 Evaluation of a Portable Glucometer for Rapid Glucose Measurement in Rats

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The Zucker Diabetic Fatty (ZDF) rats have been extensively used to investigate the mechanisms of diabetes. A number of portable blood glucometers have been developed for diabetic human patients to monitor their blood glucose levels. The Horiba Antsense III is one such device that has been used in human clinics. However, literature describing the use of portable devices in rats is limited. The purpose of this study was to evaluate the analytical accuracy of the Antsense III by comparing test results with a common glucometer (LifeScan One Touch Ultra) and a clinical chemistry analyzer (Beckman Synchron CX4 Pro). Twenty male 13-wk-old ZDF obese and lean rats were used in this study. Obese ZDF male rats become diabetic at 10 wk of age on Purina 5008 diet. Under anesthesia, 3 whole blood samples were collected from the jugular and tail vein from each animal and analyzed with the glucometers. The remaining samples were added to heparin and EDTA tubes and retested with the glucometers. The samples were then centrifuged and the subsequent plasma samples were analyzed with the clinical chemistry analyzer. The study showed that the Antsense III values were 12% higher than the values measured with the clinical analyzer. In contrast, the One Touch Ultra values were 19% lower than the blood glucose levels measured with the clinical analyzer. The average difference between two consecutive tests was only 4 mg/dl (2.4%) when using Antsense III; however, the difference between the tests was 13 mg/dl (7.3%) when using One Touch Ultra. Because of the hyperglycemic status of the obese ZDF rats, 20% to 70% of the blood samples were outside the linear range of One Touch Ultra meter. The results from the Antsense III indicated that heparinized samples resulted in lower blood glucose (6.5%) after extended blood storage (1 h). However, EDTA resulted in minimal effect on blood glucose values. In conclusion, the Antsense III provided better precision and more reproducible results than did One Touch Ultra. The anticoagulant EDTA was associated with the smallest change in blood glucose values, and may be preferred for prolonged blood storage. Given the wider linear range, the Antsense III was found to be a more suitable glucometer for measuring hyperglycemia in the ZDF rat model.

P113 Characterization of Poly I:C-induced Airway Inflammation: Comparison of Methacholine versus Bradykinin in Whole-body Plethysmography

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We have previously demonstrated that whole-body plethysmography (WBP) is a useful, valid method for assessing airway hyperreactivity (AHR) in mice, and that we have successfully used this method to show that ligation of TLR-3 with polyinosinic-polycytidylic acid (poly I:C), a synthetic dsRNA which mimics viral replication, results in impairment of lung function as demonstrated by increased airway hyperresponsiveness to methacholine. Bradykinin is a peptide that is produced by activation of the kinin system and is a bronchoconstrictor in asthmatic but not healthy subjects. Bachar and colleagues demonstrated an increased airway hyperresponsiveness to bradykinin in a mouse tracheal organ culture system, where the trachea is exposed to poly I:C. These data suggest a mechanism for increased airway reactivity in response to TLR3 stimulation. Bradykinin may provide a tool to understand the TLR-3 dependent pathways that contribute to changes in pulmonary function in response to poly I:C. In our previous model, methacholine was used as the bronchoconstrictor and significant hyperresponsiveness resulted. In the present study, we compare poly I:C-induced lung function impairment in response to either bradykinin or methacholine. Female C57BL/6 mice (10 to 12 wk old) and their C57BL/6 TLR3 gene-deleted littermates were dosed intranasally with 100 ug poly I:C daily for 3 consecutive days, followed by evaluation of airway hyperreactivity using...
dose responses of either methacholine (10, 20, and 40 mg/ml) or bradykinin (0.1, 1, and 10 mg/ml). Our results show that poly I:C-treated WT mice have profound increases in AHR as determined by enhanced pause (penh) when challenged with either methacholine or bradykinin and that TLR3 KO mice are partially protected from poly I:C-induced lung function impairment. PBS treated mice did not respond to increasing doses of bradykinin, confirming literature data that shows that unperturbed lungs do not respond to bradykinin. Together this data suggests that bradykinin may be a more useful agonist in dissecting out the role of TLR3 in viral-induced airway inflammation.

P114 Survey of Commercially Available Fish, Frog, and Bird Diets for Phytoestrogen and Zearalanone

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The use of fish, frogs, and birds as animal models for conducting estrogenic, reproductive toxicity and carcinogenicity studies is increasing. Dietary estrogens have been shown to significantly alter hormonal and non-hormonal endpoints in mice and rats. Very little is known about the concentration and role of dietary estrogens in fish, frog, and bird diets. The purpose of this study was to determine the concentration of dietary phytoestrogens and zearalanone in commercially available fish, frog, and bird diets. A total of 27 diets, including 3 frog diets, 10 fish diets, and 14 bird diets, were assayed for the phytoestrogens daidzin, daidzein, genistin, genistein, glycitin, and glycitein using high-performance liquid chromatography (HPLC) by an independent laboratory. In addition, all diets were assayed for the estrogenic mycotoxin zearalanone using HPLC by an independent laboratory. All 27 diets assayed contained daidzein and genistein. In the frog diets, the Frog Brittle contained the highest concentration of total daidzein and genistein, at 408 μg/g diet (ppm). Zeigler Pond Fancier for fish contained 392 μg/g diet total daidzein and genistein. In the bird diets, Zeigler Hand Feeding Formula for all hookbills parrots contained the highest level of daidzein and genistein, at 636 μg/g diet. Only ZuPreem Hand Feeding Formula Plus for parrot chicks (Embrace Plus) contained zearalenone 118.8 μg/kg (ppb). The dietary phytoestrogens daidzein and genistein were detected in all 27 diets which contained soybean meal or soy protein. It was concluded that some diets contain phytoestrogens in concentrations that may cause biological effects and could impact behavioral studies and hormonal endpoints in estrogenic studies using these species. The phytoestrogen content of these diets should be given appropriate consideration when using fish, frogs, and birds as animal models. This is the first report to survey fish, frog, and bird diets for estrogenic compounds.

P115 Survey of Various Types of Rodent Bedding for Lipo polysaccharide (Endotoxin) and Dust Content

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Endotoxin is derived from the cell wall of gram-negative bacteria and is ubiquitous in the environment. Reports indicate that endotoxin in grain dust and animal bedding may induce respiratory symptoms and may alter immunological parameters in mice and humans. This study surveys various types of laboratory rodent bedding for endotoxin, coliforms, and dust content. Duplicate 10-g samples from at least 2 different lots of paper, hardwood, corncob, paper/corncob mix bedding, and cotton Nestlets were assayed for total coliform counts. Endotoxin concentrations in bedding samples were determined using the Kinetic-QCL test kit (Lonza Walkerville, Inc.). Dust content was determined by using a ROTAP shaker. The average concentrations of endotoxin units/g (EU/g) and % dust (%) found in the paper bedding were: ALPHA-dri® 46 EU/g, 0.13%; Harlan Diamond Soft <5.0 EU/g, 0%; certified/irradiated Harlan Diamond Soft 5.8 EU/g, 0.1%; Harlan TEK-FRESH™ 126 EU/g, 0%. The average concentrations found in hardwood bedding were: Beta Chip 3,903 EU/g, 0.15%; Sani-Chips 2,365 EU/g, 0.08%. The average concentrations found in corncob bedding were: Bed-o’cobs (0.25 in.) 1,908 EU/g, 0.08%; Bed-o’cobs (0.125 in.) 2,370 EU/g, 0.16%; Harlan Corn Cob (0.25 in.) 2,795 EU/g, 0.02%; Harlan Corn Cob (0.125 in.) 1,964 EU/g, 0%; ALPHA-dri®/bed-o’cobs (0.25 in.) 1,047 EU/g, 0.03%; Harlan Soft Cobs Enrichment (mix) 1,040 EU/g, 0.01%; Nestlets 44.3 EU/g, 0%. The lowest endotoxin concentrations were detected in the paper beddings, and the highest levels in the hardwood and corncob beddings. Endotoxin levels varied between different lots of corn cob and hardwood bedding. Coliform counts varied from <10 to 12,700/g in beddings containing corncobs. It is uncertain what levels of endotoxin in bedding induce respiratory symptoms in rodents. It was concluded that the levels of endotoxin in hardwood and corn-cob beddings may impact the results of respiratory and immunological studies in rodents. This is the first report to survey the major types of rodent bedding for endotoxin, coliform, and dust content.

P116 Validation of Fentanyl Patch Use in Thoracotomized Sheep (Ovis aries)

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Our institutional veterinary staff is committed to the provision of appropriate dosing and administration of post-operative pain relief in animals that have undergone major surgical procedures. To provide 24-h oversight for post-surgical animals, one must consider personnel shortages, the relatively short duration of commonly used analgesics, and compliance. In larger laboratory animals, specifically sheep, the best-case scenario would typically require injecting a 12-h analgesic (such as buprenorphine) and requiring the technician to administer the medication outside the normal work day. This study examined the application of the fentanyl patches for long-term analgesia and to confirm therapeutic plasma levels of the analgesic. Male and female Dorset-cross sheep (30 to 50 kg, n = 8) with 50 μg/h patches were studied over 5 d. We applied the patches 0-2 h pre-operatively, in the axillary region, wrapped with a light bandage, to sheep undergoing cardiovascular surgery with an open thoracotomy. Plasma fentanyl levels were measured and pain scoring assessed incrementally at 0, 6, 12, 24, 48, 72, 96, and 120 h post-patch placement. Efficacy of the fentanyl patches was confirmed with a comprehensive pain scoring system, coupled with the plasma fentanyl levels. Plasma fentanyl levels attained in the sheep were comparable to therapeutic range of fentanyl demonstrated in humans through 96 h. Control animals (n = 8) received 2 μg/kg buprenorphine and flunixin meglumine 2 mg/kg every 12 h for
2 full post-operative days. Our pain scoring system employed a ranking of normal and abnormal cage side behaviors, responses to the presence of the handler, and reactions to direct palpation of the thoracotomy site. Baseline (pre-surgical) pain scores were $2.31 \pm 1.1$ and $2.67 \pm 1.1$ fentanyl patch and injectable-treated groups, respectively. No animals receiving fentanyl had pain scores requiring administration of additional analgesics, and all consistently had normal physical examinations. These series of experiments provide the laboratory animal community with a relevant and convenient option for providing long-term, continuous analgesia in post-operative sheep.

**P117 Gait Analysis of Mice Emerging from Isoflurane Anesthesia**

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Isoflurane, a halogenated inhalation anesthetic, is routinely used in laboratory animals for numerous procedures including blood sampling, tissue biopsy and surgery. Residual effects of isoflurane on motor function in animals as they recover from anesthesia have not yet been described. Accordingly, we studied posture and gait in mice after brief isoflurane anesthesia. Ventral plane videography was used to image 4 adult female Crl: CD1(ICR) mice walking 25 cm/s on a transparent treadmill belt (DigiGait, Mouse Specifics, Boston, MA) just prior to 5 min of isoflurane (2.5% in oxygen) anesthesia. Gait analysis was again performed at 25 cm/s 1, 15, 30, and 60 min after the animals regained their righting reflex. Gait was supranormal in mice 1 min after recovering from isoflurane anesthesia. Stride length was significantly increased (7.6 ± 0.2 cm compared with 6.3 ± 0.1 cm, $P < 0.05$) 1 min after recovery compared to baseline values; yet stepping frequency was reduced (3.3 ± 0.1 Hz compared with 4.1 ± 0.1 Hz, $P < 0.05$). Stance width variability was lower 1 min after recovery from isoflurane, indicating enhanced postural stability in the mice walking on the treadmill belt. Swing duration was increased by ~ 50% compared to baseline 1 min after recovery (103 ± 3 ms compared with 77 ± 3 ms, $P < 0.05$). Additionally, propulsion duration of the hind limbs was significantly longer 1 min after recovery than at baseline (167 ± 6 ms vs. 128 ± 5 ms, $P < 0.05$). Some supranormal characteristics persisted for approximately 30 min, and all of the gait metrics returned to baseline within approximately 60 min following cessation of anesthesia. The supranormal gait immediately after recovery from isoflurane anesthesia may be related to the known effects of isoflurane on dopamine release in the striatum. These factors should be considered by researchers studying motor function in mice that have been anesthetized

**P118 Spleen Morphology and Morphometry in Wild-caught Cynomolgus Macaques (M. fascicularis): A Comparison between Short- and Long-term Captivity**

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Prolonged stress has been shown to cause a reduction of immune function, evidenced by lowered antibody production and also the development of chronic diseases in some species. We hypothesize that cynomolgus monkeys in short-term captivity have fewer stress-related changes in the spleen when compared to age and gender matched animals captive for greater than 2 y. To identify stress-related changes, splenic lymphoid development was assessed. The spleen was collected from both groups at necropsy, weighed, and routinely processed for histopathologic examination. Histomorphometry of splenic white pulp was measured using computer image software, and lymphoid follicles were counted. The short-term captivity group were female monkeys (n = 24) that had been free-ranging in Indonesia and captive for 2 to 3 mo at the time of necropsy. The long-term captivity group were female (n = 22), wild-caught in Indonesia, held in a laboratory animal facility for more than 2 y and selected from archived cases which had undergone diagnostic necropsies. The short-term captivity group was singly housed; the long-term captivity group were housed in group pens with indoor/outdoor access, indoor group pens, or cages (singly and in pairs). The number of years in captivity differed significantly among the short-term and long-term groups (P < 0.0001) and the mean was 0.24 and 5.11, respectively. Ages differed significantly (P < 0.0034), ranging from 12 to 17 y (mean of 14.53 y) in the short-term group and 12 to 25 y (mean of 17.56 y) in the long-term group. Body weight did not differ by group (P > 0.76). Mean splenic weight was significantly different among the short-term and long-term groups (13.32 g and 7.28 g, respectively; P < 0.0001). Follicular number was slightly higher in the long-term captive group (P > 0.05), though follicular area was markedly significantly larger in the short-term captivity group (P < 0.006). Histologic evaluation revealed lymphoid depletion in the long-term captives. The findings suggest differences in the functional state of the spleen, possibly associated with prolonged captivity.

**P119 Oral and Intravenous Dosing in Combination with Automated Blood Sampling for a Complete Bioavailability Profile in Mice**

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Crossover bioavailability studies reduce interanimal variability and minimize the number of animals used. This design requires that each subject be dosed twice and contribute 2 complete sets of blood samples. Until now, this has not been feasible in mice due to their small size. In traditional manual sampling methods, each animal contributes only a few samples. However, automated sampling techniques enable collection of small volumes, allowing more samples to be collected from each mouse. The objective of this study was to obtain complete oral bioavailability profiles in individual mice. Three catheters were surgically implanted in 8 male CD-1 mice (18 to 30 g). A carotid catheter was connected to an automated blood sampler, while a jugular catheter was used for intravenous dosing. A gastric catheter was implanted for intragastric dosing. Animals were anesthetized with isoflurane; sites were shaved and scrubbed with betadine and alcohol. Gastric catheters were implanted in the stomach and locked with saline. The mice were returned to their home cages for 3 to 6 d of recovery. Upon regaining their pre-operative weight, mice were implanted with jugular vein and carotid artery catheters. All catheters were externalized and secured in the scapular region and locked with a heparin: glycerol solution (300 u/ml). We experimented with locking solutions prior to the study; however, some failed to maintain catheter patency. Animals were returned to their home cage for 48 h, and then placed on an automated blood sampler with a
unique movement-responsive caging system. The automated sampler regularly flushed the catheters with heparinized saline to maintain patency. Eighteen blood samples (5 μl/sample) were obtained from each animal. Each mouse was dosed with an intravenous or intragastric bolus of carbamazepine (5 mg/kg). Nine blood samples were collected over 4 h. After a washout period of 6 h, the dosing and sampling procedures were repeated in a crossover design. The mice remained healthy and showed no ill effects from the dosing or sampling procedures throughout the study. This technique allows complete bioavailability profiles to be obtained from individual mice, and also reduces the number of animals needed for the study.

**P120 Use of Proteomic Profiling for Biomarker Discovery in a Murine Food Allergy Model**

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There is a need to develop a reliable animal model to qualitatively and quantitatively assess the allergenicity of foods. The plasma proteome that develops following an allergic reaction is currently unknown for any species. The aim of this project was to assess the plasma proteome for potential protein biomarkers representative of type-1 hypersensitivity reactions caused by food allergy in mice. Ovomucoid (OVM), a protein found in chicken egg white, is a major cause of food allergy in children and was selected as an allergen standard for these studies. Female 8-wk-old BALB/cAnNCrl mice (n = 15) were sensitized by administering ovomucoid (1.0 mg/mouse) and cholera toxin (CT, 10 μg/mouse) adjutant by oral gavage 9 times over 5 wk, while control mice (n = 15) were exposed to a mixture of amino acids (1.0 mg/mouse) with CT (10 μg/mouse). Four d following the last sensitization, all mice were challenged intraperitoneally with ovomucoid (1 mg/mouse) and aluminum hydroxide adjuvant (50 μl, 2%), euthanized by carbon dioxide inhalation 40 min post-challenge, and exsanguinated by cardiac puncture for plasma collection. Clinical signs of anaphylaxis were noted in all OVM-sensitized mice and elevated levels of plasma histamine, OVM-specific IgE and IgG confirmed the development of type-1 hypersensitivity in these mice. Differential protein expression was quantified in albumin-depleted plasma by 2-dimensional difference gel electrophoresis (2D-DIGE). Significant differentially expressed proteins were identified by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Plasma proteins over-expressed in OVM-sensitized mice included haptoglobin (41-fold, P = 0.004), serum amyloid A (19-fold, P = 0.006) and peroxiredoxin-2 (1.9-fold, P = 0.004). Validation of these plasma proteins is required in other animal models with different food allergens to assess their potential as broad-based biomarkers of type-1 hypersensitivity reactions induced by food allergens.

**P121 Effect of Simple Toys on the Rate of Ethanol Consumption in a Rat Model of Voluntary Ethanol Consumption**

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Current housing conditions for laboratory rodents have been optimized to ensure biosecurity, minimize environmental variables, and maximize cost efficiency, providing rats minimal opportunities to participate in species-typical behavior. While pair-housing promotes species-typical behavior, it may not be possible in certain research situations. For such experiments an alternate method of enrichment, such as toys, should be provided. The purpose of this study is to evaluate the effect of simple toys (Gumabone) on ethanol gel consumption in singly housed rats in voluntary ethanol consumption studies. Five male Sprague-Dawley rats (Crl:SD) with average weight of 655 g were adapted to self-administer ethanol using the “jello-shot” procedure. Rats were exposed for 4 d to each of the following 3 treatments: new Gumabone plus ethanol gel access for 1 h daily (treatment 1); new Gumabone, left for 24 h, plus 1 h ethanol gel access daily (treatment 2); and new Gumabone plus ethanol gel access for 24-h (treatment 3). During treatment 1, time spent with the Gumabone was highest on the first 2 d, which altered the rate of ethanol consumption but not the total amount of ethanol consumed. Rats’ interest shifted between ethanol gel and Gumabone throughout the 1-h toy exposure, rather than consuming the majority of the ethanol gel during the first 5 min (basal level consumption). Ethanol consumption rate resembled basal level consumption during the last 2 d. During treatments 2 and 3, rate and amount of ethanol consumption was comparable to basal level consumption. Average alcohol consumption over 4 d was 0.86 ± 0.13 g/kg, 0.99 ± 0.13 g/kg and 5.19 ± 0.37 g/kg without Gumabone for treatment 1, 2, and 3, respectively; and 1.00 ± 0.13 g/kg, 0.620 ± 0.07 g/kg, and 5.55 ± 0.38 g/kg with the Gumabone® for treatment 1, 2, and 3, respectively, which was not found to be statistically significant. Rats chewed/manipulated the toy during all 3 treatments. We conclude that adding toys for rats to chew/ manipulate does not alter ethanol gel consumption. Environmental enrichment techniques should be considered during the research planning stages to avoid behavioral responses which may temporarily interfere with variables of interest.

**P122 Strain Differences in 2410146L05Rik, an Oocyte/Embryospecific Gene in Mice**

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Embryo manipulation is widely used in mice, but strain differences in oocyte/embryo quality are still a serious problem. This study sought the proteins responsible for strain differences in the developmental competence of mouse embryos using 2-dimensional electrophoresis for protein profiling of in vitro matured oocytes from various mouse strains. Cumulus-oocyte complexes were collected from 21-d-old females of C57BL/6CrSlc (B6Cr), DBA/2CrSlc (DBA/2), and their intercross hybrid Slc:B6D2F1 (BDF1) and cultured for 18 h in Waymouth medium supplemented with pyruvate (0.23 mM), antibiotics, polyvinylpyrrolidone (3 mg/ml), and recombinant human follicle stimulating hormone (0.5 IU/ml). Proteins from 200–400 mature oocytes (oocytes with germinal vesicle break down) were separated using 2-dimensional electrophoresis. Some of the protein spots were analyzed using peptide mass fingerprinting. The genomic sequences of candidate proteins in B6Cr and DBA/2 were determined by direct sequencing of the PCR products from B6Cr and DBA/2 genomic DNA, respectively, using primer sets based on C57BL/6J sequence information retrieved from GenBank. The theoretical PI of the deduced amino acid sequences of the proteins was calculated from the electrical charges of the amino acid residues. Using this method, we consistently found 2 protein spots with a similar molecular weight (~18 kDa) that were both expressed in BDF1,

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while only one of them was expressed in B6Cr (pI ~5.7) and only the other was expressed in DBA/2 (pI ~7). Peptide mass fingerprinting suggested that both spots were 2410146L05Rik (164 amino acids long), which is an oocyte/embryo-specific gene. The deduced amino acid sequences showed that residues 118 to 120 and 163 were proline-lysine-serine and glutamate, respectively, in B6Cr and glutamine-arginine-alanine and lysine in DBA/2. The calculated pI was 5.7 and 7.3 for B6Cr and DBA/2, respectively, which fitted the actual spot positions on the 2D-gels well. Although more concrete identification of the protein is necessary and the function of 2410146L05Rik remains unclear, the protein might be involved in the strain differences in the developmental competence of mouse embryos.

P123 Species Variation in Nitric Oxide Levels in Blood: Pharmacological Implications on Animal Models
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Animal models are routinely used to investigate cardiovascular, cerebrovascular, hemodynamic and inflammatory disorders. Nitric oxide (NO) plays an important role in the overall physiological regulation and pathophysiological responses observed in these animal models. More importantly, differences are reported in the level of NO synthase (NOS) in various species. Despite the wide use of these animal models, the baseline NO data are not available. We compared the baseline plasma NO levels of rhesus monkeys (n = 17 [8 female, 9 male], 12 to 15 y old), Beagles (n = 17 [8 female, 9 male], 2 to 4 y old), New Zealand White rabbits (n = 26 [26 male], 6 to 8 mo old), Sprague Dawley rats (n = 29 [29 male], 3 to 6 mo old), and DSH cats (n = 10 [5 female, 5 male], 1 to 3 y old) with humans (n = 37 [18 female, 19 male], 20 to 40 y old). The blood collection from human subjects was under IRB, and animal samples were taken from anesthetized subjects under approved IACUC protocol. Blood was collected at a 1:10 ratio in 3.8% sodium citrate tubes and centrifuged at 3000 g and plasma was separated. NO was measured using a highly sensitive gas phase NO analyzer (Sievers Instruments, Boulder, CO) and a commercially available NO method using the Greiss reaction (R&D Systems, Minneapolis, MN). There was no difference observed in the NO levels between the 2 assay kits. Mean NO levels were human, 16.8 ± 4.9 μM (range, 15 to 34); primate, 2.8 ± 2.4 μM (range, 1 to 10); dog, 9.8 ± 4.6 μM (range, 7 to 21); rabbit, 89.8 ± 11.1 μM (range, 50 to 94); rat, 15.6 ± 5.1 μM (range, 9 to 22); and cat, 7.8 ± 2.5 μM (range, 5 to 12). The rank order of NO levels were human, 16.8 ± 4.9 μM (range, 15 to 34); rat, 15.6 ± 5.1 μM (range, 9 to 22); dog, 9.8 ± 4.6 μM (range, 7 to 21); rabbit, 89.8 ± 11.1 μM (range, 50 to 94); and primate, 2.8 ± 2.4 μM (range, 1 to 10). The calculated pI was 5.7 and 7.3 for B6Cr and DBA/2, respectively, which fitted the actual spot positions on the 2D-gels well. Although more concrete identification of the protein is necessary and the function of 2410146L05Rik remains unclear, the protein might be involved in the strain differences in the developmental competence of mouse embryos.

P125 Prototype Device for Computerized Blood Sampling and Data Collection in Freely Moving Swine
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Collecting biofluid samples or physiological and behavioral data from pigs presents challenges from excessive human intervention and the stress of manual sampling. Our objective was to construct a device capable of protecting external leads and tubing used to facilitate automated sampling, dosing, and collection of physiological and behavioral data in freely moving swine. We constructed an octagonal, 1.2-m diameter pen with solid walls, plastic-coated perforated floor, self-contained feed trough and in-built water supply. The most important feature built into the pen was its clockwise and counter-clockwise rotational capabilities. A pig fitted with a harness was connected via an umbilicus (a stiff, tightly coiled 1 m spring) to a sensor array above the center of the pen which detected twisting of the umbilicus past pre-established settings, causing the pen to rotate counter to the pig movement. The pig moves relative to the pen but remains stationary relative to the exterior space. A computerized instrument module for automated blood sampling was used to test both the pen platform and the ability to draw blood while keeping the sampling tubing untwisted and open. Instrument modules for preserving the blood samples, dosing, collecting physiological and behavioral data could also be added. Twelve pigs (Sus domestica), including 4 Gottingen minipigs and 8 young conventional pigs (15 to 30 kg), were surgically implanted with jugular catheters. Catheters were implanted in the right external jugular vein with the tip in the vena cava. The catheter was tunneled from the level of the spinal cord. Although the RVM is involved in the inhibition or facilitation of nociception, the underlying mechanisms are not understood. Here we examined the role of the neuropeptide substance P and neurokinin-1 (NK-1) receptors located in the RVM on withdrawal responses evoked by mechanical and heat stimuli applied to the rat hindpaw under normal conditions and during hyperalgesia produced by capsaicin. Forty-six male Sprague Dawley rats aged 3 to 4 mo were used. The mechanical withdrawal threshold was obtained using von Frey monofilaments applied to the plantar surface of the hindpaw. Sensitivity to heat was determined by measuring the latency to withdraw from radiant heat applied to the plantar surface. Mechanical hyperalgesia was defined as a decrease in withdrawal threshold and heat hyperalgesia was defined as a decrease in withdrawal latency. Rats were prepared with a chronic cannula into the RVM and either vehicle or the NK-1 receptor antagonist, L-733,060, was injected into the RVM at concentrations of 10, 100, 300, or 3000 nM. Paw withdrawal responses were obtained before and after RVM injection. Capsaicin (10 μg) was then injected into the plantar surface of 1 hindpaw and withdrawal responses were obtained at 5, 30, and 60 min thereafter. Cannula location was verified via histopathology. Injection of L-733,060 did not alter withdrawal responses to mechanical or heat stimuli under normal conditions but reduced both the mechanical and heat hyperalgesia produced by capsaicin in a dose-dependent fashion. These findings suggest that activation of NK-1 receptors in the RVM contributes to the hyperalgesia produced by capsaicin.
the dorsal mid-neck region to the exposed vein, secured by sutures, and the vein ligated cranial to insertion. The pig was fitted with a harness, 2 to 4 d later installed in the devise and tested for periods up to 17 d, with 130 or more blood samples (1 to 2 ml) drawn without further handling of the animals. We observed no twisted or blocked tubing due to failure or malfunction of our device. Plasmas from blood samples were clear and non-hemolysed. We were able to protect tubing connected to a freely moving pig over an extended period of time, allowing for multiple, high-quality, automated blood samples to be taken without human intervention or physical manipulation of the pigs.

**P126 A Comparison of Intravenous Infusion Dosing Techniques and the Effect on Cardiovascular Telemetry Data in Cynomolgus Macaques**

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The study examined 3 different methods of intravenous infusion on their effects on the cardiovascular telemetry data. Four male monkeys (Macaca fascicularis) were surgically implanted with a DSI telemetry transmitter (PCT) and femoral vascular access ports to compare the difference between restrained infusion dosing and ambulatory infusion dosing. The animals had a 14-d recovery period prior to the initiation of the study. All animals were acclimated on 3 occasions to primate restraint chairs and primate jackets. In each instance, telemetry data was collected predose, throughout the dosing period, and for 23 h following the completion of dose administration. In each method, the animals were dosed for 1 h with saline at an infusion rate of 5 ml/kg/h. Method 1 consisted of dosing animals while restrained in primate chairs. Three of the animals had a considerably elevated heart rate while restrained in the chair during dose. Method 2 consisted of dosing animals in cages after manually starting pumps just prior to dose commencement. All animals maintained a high heart rate from dose though 2 h post-dose. Method 3 consisted of dosing animals using remote-start ambulatory infusion pumps animals were placed in jacket with preset pumps prior to baseline data collection. All animals maintained a heart rate consistent with their baseline data from predose through dosing, and until technicians re-entered the room for jacket removal. Since subtle changes in blood pressure, heart rate, and ECG parameters found in the telemetry data may provide evidence of dose effect, it was determined that the remote dosing method provides the best results and optimal cardiovascular data. This is due to minimal human interference at critical time points, which allows the animals’ heart rate to remain close to baseline levels during and immediately after test material administration.

**P127 Klebsiella oxytoca in C57BL/6j Mice: Modes of Infection, Persistence, and Transmission**

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This study was undertaken to learn more about the acquisition, persistence and transfer of Klebsiella oxytoca infection in mice and to observe any pathogenicity if it occurs. Female C57BL/6j mice (8 wk old) were exposed to a recent isolate of K. oxytoca by 3 methods: direct oral gavage of 10^9 colony forming units (CFU); consumption of contaminated food pellets for 5 d (pellets were prepared by covering them for 1 h with a fresh solution of bacteria, followed by drying in a 37 °C incubator; each pellet contained approximately 10^6 CFU); and by housing the animals for 5 d in cages containing K. oxytoca-contaminated bedding (2 x 10^8 CFU of freshly grown bacteria was sprayed onto clean bedding and allowed to dry before the introduction of mice). Fifteen mice were used in each group, including a group of uninfected mice as negative controls. Mice were tested for the presence of K. oxytoca by culturing freshly collected fecal samples and typing the bacteria that were found. Sampling was performed at 2 d post-infection (PI) and then weekly thereafter for 10 wk. At 2 d PI, all the mice that were fed contaminated feed pellets were infected with K. oxytoca, as determined by its presence in the feces, and the infection persisted in all these mice throughout the course of the study (10 wk). Fifty-three percent of mice infected by gavage showed the presence of bacteria in their feces at 2 d PI; however, only 1 mouse remained infected at 10 wk. Thirty-nine percent of mice exposed to contaminated bedding were infected at 2 d PI; all mice were completely free of K. oxytoca in their feces at 3 wk PI. These results indicate that exposure to contaminated food pellets was the most effective means of infecting the mice, both in terms of the percentage of animals that acquired the infection and its persistence in the mice. Additionally, when mice harboring K. oxytoca acquired in this manner were housed in the same cage with uninfected mice, the infection was transferred to 87% of the previously uninfected mice (14 of 16) within 1 wk. Throughout the study, no signs of pathology or disease were observed, nor were any lesions detected at necropsy in any of the experimentally infected or control mice.

**P128 Effect of a Disturbed Light-Dark Cycle on CCl4-induced Toxicity Test Using F344/N Rats**

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Environmental factors such as temperature, humidity, noise, and light could affect laboratory animals and influence toxicity study results. This study investigated how a disturbed light-dark cycle could affect carbon tetrachloride (CCl4)-induced toxicity in F344/N female rats. Rats were divided into 2 groups and exposed to 200 Lux light for 12 h (n = 50; normal group) or 24 h (n = 50; disturbed group). Each group was treated with either a low-dose CCl4 (150 ml/kg), high-dose CCl4 (450 ml/kg daily), or vehicle (corn oil) for 28 d. CCl4, a well-known liver toxicant, induced liver toxicity in F344/N female rats as demonstrated by histopathologic findings such as fatty changes, necrosis, and elevation of ALT and AST. However, CCl4-induced ballooning degeneration and necrosis in rat liver was different in the normal light group when compared to the disturbed group. High-dose CCl4 treatment in the disturbed group brought about a significant decrease of both mild and moderate ballooning degeneration compared with the normal group. Both low and high doses of CCl4 in the disturbed group showed significantly fewer instances of CCl4-induced necrosis compared with the normal group. The disturbed cycle altered hormone levels, increasing corticosterone level (1020 ng/ml normal group compared with 1409 ng/ml disturbed group; P < 0.005) and decreasing melatonin level (150 pg/ml normal group compared with 62 pg/ml disturbed group; P < 0.005). The disturbed cycle also increased body weight and food intake significantly. These increases were not affected by CCl4 treatment; the disturbed group and the normal group showed similar increases in body weight and food intake after CCl4 exposure, though water intake decreased significantly (20%) in the disturbed group.
results of hematology and serum chemistry which control and CCl₄-treated group showed by disturbed lighting had not the similar patterns. Our results demonstrate that disturbed lighting in a laboratory toxicity experiment causes physiological changes that can affect the results of toxicity tests.

P129 Effects of Environmental Enrichment and Paradoxical Sleep Deprivation on Open-field Behavior of Amphetamine-treated Mice

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Environmental enrichment or paradoxical sleep deprivation (PSD) has been shown to modify responses elicited by drugs of abuse. The aims were to examine the effects of environmental enrichment and PSD, conducted separately or in association, on open-field behavior elicited by amphetamine (AMP) in mice. Male C57BL/6 mice were randomly assigned to live in either an enriched environmental condition (EC) or a standard environmental condition (SC) for 12 mo after weaning (n = 10/group). EC mice were group-housed in standard cages containing rough, non-chewable plastic objects, denominated "toys," such as a ball, a tunnel, a house, a space station, and a passageway. These objects were rearranged daily, with new objects being introduced each week. SC mice were also group-housed in cages identical to the EC cages, but these were not exposed to the objects. Then, some of the EC and SC mice were sleep-deprived for 48 h, while other animals of both EC and SC groups were maintained in their home cages. PSD was induced by placing the animals inside a water tank containing platforms (3.5 cm in diameter) surrounded by water up to 1 cm beneath the surface. The animals were capable of moving inside the tank by jumping from 1 platform to the other. When the animal entered the paradoxical phase of sleep, it fell into the water due to muscle atonia, and woke up. Immediately after PSD or home-cage maintenance, the animals received an IP injection of saline (2.5 mg/kg AMP or 5.0 mg/kg AMP) and, 15 min later, their open-field behavior was quantified. The combination of environmental enrichment and PSD enhanced the stimulant effect of AMP on total and peripheral open-field locomotor activity, but this potentiation had the same magnitude of that observed when these manipulations were conducted separately. Moreover, while PSD, environmental enrichment, and their combination did not modify central locomotion, rearing, and immobility, their combination potentiated the inhibitory effects of AMP on grooming behavior. The present findings demonstrate that some, but not all, of the behavioral effects induced by AMP can be similarly and specifically potentiated by both environmental enrichment and PSD in C57BL/6 mice.

P130 Bedding Types and Their Effect on Pulmonary Inflammatory Response in Sprague Dawley Rats

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Chronic pulmonary obstructive disease is a crippling disease which strikes 1 in 4 Americans over the age of 45. It is currently the 4th leading cause of US deaths each year. In vivo models of acute airway inflammation, such as lipopolysaccharide (LPS)-induced lung neutrophilia in the rat, can evaluate anti-inflammatory therapies for the management of COPD. This study was initiated to investigate two concerns: abnormally high percentage of resident neutrophils measured in bronchoalveolar lavage (BAL) fluid in control animals which had been exposed to aerosolized saline and to evaluate the possibility of refractory response to LPS exposure. Based on previous studies identifying potential contaminants in cob bedding, we chose to examine whether bedding type played a role in increasing resident neutrophil percentages in our rats. Two groups of male Sprague Dawley (CRL: SD) rats were used (n = 16/group), 1 group-housed on paper bedding (Alpha Dri, Shepherd Specialty Papers), the other on cob bedding (Bed o’Cobs, The Andersons Industrial Products Group). After 2 wk, rats were challenged with aerosol saline or LPS (0.1 mg/ml to 5 mg/ml saline solution) for 15 to 30 min. Four hours post-challenge, the rats were euthanized and bronchoalveolar lavaged. BAL cell suspensions were analyzed by flow cytometer for total cells and neutrophil populations. Animals housed on paper and cob bedding challenged with saline had 46% and 37% resident neutrophil population, respectively. LPS-exposed animals housed on paper and cob bedding elicited approximately a 20-fold increase in neutrophils compared to related control animals which received saline. Despite the high percentage of neutrophils in the saline groups, this is still a significant difference between groups to accurately evaluate novel anti-inflammatory therapies. The findings provide evidence that cob bedding may not play a significant role in either resident neutrophil increase or refractory response to LPS. More studies are needed to determine if a longer contact period (<2 wk) to bedding would effect a pulmonary response. Factors such as endotoxin contaminated nebulizing fluid or other environmental exposure unrelated to bedding may also contribute to the cell variability and should be considered.

P131 The Role of Dietary Iron in Liver Lesions and Adverse Health Effects in Bolivian Squirrel Monkeys (Saimiri boliviensis bouliviensis): Results of a Two-year Study

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A previous study in common marmosets concluded that iron levels in commercial primate diets may cause liver lesions and adverse health effects in neotropical primates. Objectives of the current study were to determine if the iron concentration in a commercial primate diet meeting established standards has adverse health effects or causes liver lesions in squirrel monkeys. Forty-six animals (5 male, 35 female, 6 infant) were fed diet containing 409 ppm iron (“conventional”). Forty-two animals (3 male, 34 female, 5 infant) were fed the same diet with 170 ppm iron (“low”). Formulation was verified by analysis. Established social groups were used, heterogeneous for age. Diet was fed for 2 y for data capture from 2 breeding cycles in this seasonally breeding species. There was no vitamin supplementation; fruits and vegetables were the same for both groups. Water was deionized to American Society of Testing & Manufacturing Type II standards. Percutaneous needle liver biopsies (for histology and tissue iron; isoflurane and buprenorphine were given for anesthesia and analgesia), serum chemistries, hematology, and physical examinations were performed at start and end of study. Liver iron at the start of the study was 2774 ± 198 μg/g (mean ± SEM) for the conventional group and 3116 ± 386 μg/g for the low group, and 1838 ± 139 μg/g and 2498 ± 559 μg/g, respectively, at the end of the study. Serum iron at the start of the study was 155 ± 5 μg/dl for the conventional group and 141 ± 9 μg/dl for the low group, and 161 ± 5 μg/dl and 148 ± 9 μg/dl,
respectively, at the end of the study. Starting hematocrits were 38 ± 1 for the conventional group and 39 ± 1 for the low group; at the end of the study, they were 41 ± 1 and 39 ± 1, respectively. There were no significant statistical differences between diets for these indices or mortality. Granular hemosiderin pigment was not detected in hepatocytes from either group. Hemosiderin, when observed, was primarily found in Kupffer cells in sinusoids. There were no relevant differences between groups for clinical incidents, reproductive outcomes, serum chemistry, or necropsy. Results suggest that deleterious effects reported previously in marmosets are not seen in squirrel monkeys.

P132 Development of New Rapid Multiplex Microfluidic Chip System for Research Animal Serology Monitoring

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A new high-speed multi-channel immunoassay microfluidic chip system, which can simultaneously detect multi-pathogen infection of mice in very small amount of serum, has been developed. The microfluidic chip has 16 microchannels (1μl/microchannel). Extremely small amount (several ng/microchannel) of proteins were exactly and uniformly immobilized in channels with a fine porous structure by the electrospray deposition (ESD) method. For serodiagnosis of lymphocytic choriomeningitis virus (LCMV) infection of laboratory mouse, a microfluidic chip with a purified LCMV antigen immobilized in the microchannels has been prepared. The flow chart was constructed on the basis of the indirect enzyme-linked immunosorbent assay (ELISA) with an automatic fluid-flow control apparatus and a high sensitive chemiluminescence detection apparatus. The whole assay was accomplished within 40 min with 3 μl of diluted serum. Using the microfluidic chips we could detect anti-LCMV antibody, which was diluted up to a thousand times from the original solution, with a high correlativity (R2 = 0.996) and reproducibility. Immobilization of LCMV antigens by the ESD method shows approximately 10 times more sensitive in the immunoreactions than by spotting. A validation study with a large number of previously characterized positive and negative LCMV infected serum samples, with running side-by-side by ELISA, and indirect immunofluorescence assay (IFA), resulted in an excellent agreement between the microfluidic chips and the IFA. We have further developed the multiplex microfluidic chip, which immobilized 9 antigens of mouse pathogens in a microchannel, such as Sendai virus, mouse hepatitis virus, Mycoplasma pulmonis, and Clostridium piliforme. The chips performed simultaneous monitoring within 20 min with 1 μl of serum without any cross-contamination. In conclusion, our microfluidic chip system provides an automatic, high-sensitive and rapid diagnostic serology monitoring system of multi-pathogens for laboratory animal with only 1 drop of serum.

P133 Increased Dietary Linoleic Acid Intake Stimulates Growth and Metabolism of Human Breast Cancer in Nude Rats

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In laboratory animals the dietary consumption of linoleic acid (LA), the most abundant n-6 fatty acid in rodent chows and the human western diet, stimulates the growth of rodent tumors and human prostate and breast cancers. In hepatoma 7288CTC and human prostate and breast tumor xenografts grown in nude rats, 13-hydroxyoctadecadienoic acid (13-HODE), the lipoxigenase product that augments EGF mitogenesis, is the mitogen responsible for LA-dependent tumor growth. Here we tested the hypothesis that increased dietary intake of LA, using a 20% corn oil (CO) compared to a 5% CO (control) semipurified diet, would elevate plasma levels of LA and stimulate the growth of estrogen receptor negative (ER-) MCF-7 human breast tumors (poorly differentiated, grade 3, ductal breast carcinoma). Two groups (n = 5/group) of randomized female (200g), nude rats (Hsd:RH foxn1+/-) were fed either a 5% (I) or 20% (II) CO diet and water ad libitum. After 5 wk, animals were implanted subcutaneously with MCF-7 human breast tumors, which grew as “tissue-isolated” xenografts. Latency-to-onset of palpable tumor mass and tumor growth rates for groups I and II measured, respectively, 14 and 11 d, and 0.20 ± 0.03 and 0.45 ± 0.08 g/d. At 5 g estimated weight, tumor arteriovenous blood samples were collected and analyzed for LA-uptake and 13-HODE release; tumors were measured for DNA [3H]thymidine incorporation, cAMP, mitogen-activated protein/extracellular signal-regulated kinase (MEK), extracellular signal-regulated kinase 1/2 (ERK 1/2), and protein kinase B (Akt) activity (by Western blot analysis). Tumor cAMP levels, LA uptake and 13-HODE release were significantly elevated over 25%, 70% and 200%, respectively, in group II compared to group I (P < 0.05). Breast tumor [3H]thymidine incorporation and DNA content was significantly increased (P < 0.05) for group II (59.1 ± 4.1 dpms/μg DNA and 3.9 ± 0.3 mg/g) compared to group I (43.1 ± 2.9 dpms/μg DNA and 28 ± 0.2 mg/g). As expected, activation of MEK, ERK 1/2 and Akt occurred in all tumors. Understanding the mechanism of action of LA-dependent (ER-) MCF-7 human breast cancer growth in the laboratory rat will strengthen our current efforts to reduce LA consumption and increase cancer prevention in humans.

P134 A Model for Metastatic Progression Using a Controlled Surgical Method

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Metastases are malignant cells that separate from primary tumors and infiltrate other tissues via local invasion, lymphatic flow, or blood flow. These satellite cells that form new tumors are the primary cause of death in most cancer patients. Because the lymphatic system is a primary route for metastatic trafficking, the objective of this research was to develop a model to better understand metastases and tumor growth within sentinel nodes. C57BL/6NCrl mice (n = 30) were used in the study. B16F10 murine melanoma cells were used because they are known to form metastases in C57BL/6 mice. Mice were equally divided into 3 groups and inoculated intranodally with 5uL of B16F10 cells or PBS while under anesthesia. B16F10 inoculations consisted of 1 x 105 or 1 x 106 cells per injection. A 2- to 3-mm incision was made adjacent to the inguinal lymph node. After isolating the inguinal lymph node, a short-bevel (0.55 mm), 30-gauge needle on a Hamilton syringe was used to deliver cells into the center of the lymph node. The skin incision was closed using tissue glue and animals were monitored during recovery. At days 3 and 7, animals were euthanized (n = 5/group/d). Treated and naïve lymph nodes were visually inspected, collected, and weighed. Lungs, liver, and kidneys were visually inspected for metastases. At days 3 and 7, animals dosed with 1 x 105 cells
had nodal tumor burdens of 0.010 ± 0.015 g and 0.034 ± 0.010 g, respectively. Animals dosed with 1 x 10^6 cells had nodal tumor burdens of 0.015 ± 0.019 g and 0.139 ± 0.096 g, respectively. No weight increase was noted in the PBS control group. Successful isolation and inoculation of the inguinal lymph node produced a lymphatic cancer model which can be used for studying sentinel lymph node tumor growth and metastases.

P135 Nude Rat Models of Hepatocellular Carcinoma: Translational Advantage?
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Hepatocellular carcinoma (HCC) is the 5th most common cancer worldwide, with incidence doubling over the last 25 y. Availability and scope of preclinical models is limited due to poor growth rates of human HCC cell lines in the nude mouse. We are developing 3 variations of a rat HCC model that will allow modeling in the subcutaneous, primary, or metastatic compartment. Most fully characterized and described herein is the subcutaneous model. Nude (NIH: mu), male and female rats (6 to 8 wk old) were used for these experiments. Rat number allows for tumor “take rate” and is adjusted to provide a final number of 6 to 8 per group. HepG2 human hepatocarcinoma cells from the American Type Culture Collection were RAP tested, expanded under standard cell culture conditions and implanted by subcutaneous injection (5 x 10^6 cells per site). Endpoints included: bi-weekly body weight and tumor size (caliper method); blood collection (2-wk intervals by orbital bleed) for clinical chemistry and biomarkers; tumor collection following euthanasia by CO2 overdose. Tumor take rate in this model was approximately 30%, with tumors first measurable 2 wk post-inoculation. The human specific biomarkers, alpha-feto protein (AFP) and human albumin (hALB) were measured in serum by ELISA. Both AFP and hALB were accurate indicators of tumor, negative in non-tumor-bearing animals and positive in serum at the same time as tumor was apparent by caliper measurement. There is a strong correlation between AFP and excised tumor weight (r^2 = 0.79). Tumor burden caused a significant reduction in body weight (22.5 %) and terminal hind-limb muscle mass (17.6 % in gastrocnemius), indicating development of cancer cachexia. This rat HCC carcinoma model produces AFP, a biomarker with direct translation to the clinic where it is used for diagnosis and disease progression monitoring and the additional clinically observed characteristic of cancer cachexia. This model can be applied according to experimental goals for efficacy, safety and pharmacokinetic research on novel therapeutics targeting hepatocellular carcinoma.

P136 Establishment of an MCF-7/Adr Xenograft Model in the SCID Beige Mouse
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The MCF-7 cell line was derived from the pleural effusion of a patient with human breast adenocarcinoma. An adriamycin-resistant clone, MCF-7/Adr, was developed and provided by Kenneth Cowan of the National Cancer Institute (Bethesda, MD). Presently, published data exists on the growth kinetics of MCF-7/Adr xenografts using either tumor fragments, or tumor cells in athymic nude (Crl:NU/NU-Foxn1(nu) ) mice. The purpose of this study was to develop a more robust MCF-7/Adr xenograft model using SCID Beige (CB17/Jcr:Prkdcscid/Il2rgtm1Sj/dver/Crl) mice, which are completely immunodeficient. Orthotopic implants are thought to grow faster than subcutaneous xenografts, and it is thought that an area with higher vasculature produces faster-growing tumors. For this reason, 3 cell implant locations were compared: intra-mammary fat pad (orthotropic), ventral midline (subcutaneous), and dorsal flank (subcutaneous). MCF-7/Adr cells were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were harvested in log-phase growth and resuspended in conditioned media (CM). Fifteen 8-wk-old female SCID Beige mice were used for the study. For orthotopic xenograft implants, mice were anesthetized with 90/10 mg/kg ketamine/xylazine, and 2.5 x 10^6 cells in 0.05 ml CM were injected into the right axillary mammary fat pad. For subcutaneous xenografts, cells were resuspended to 2.5 x 10^7 cells/ml in a 1:1 mixture of CM and Matrigel. Each animal was implanted with 5 x 10^6 cells in 0.2 ml at either the ventral abdominal midline, or the dorsal right flank (n = 5 per group). Tumors were measured twice weekly beginning on day 4 post-implantation, and animals euthanized on day 71. Based on final tumor volumes, orthotopic tumors had a significantly slower tumor growth rate compared to ventral and dorsal subcutaneous tumors (P = 0.0012 and P = 0.0003, respectively, unpaired student’s 2-tailed t-test). Ventral subcutaneous tumors had a significantly faster tumor growth rate than dorsal subcutaneous tumors (P = 0.0008). In conclusion, MCF-7/Adr cells implanted subcutaneously at the ventral midline produced more robust tumors than the orthotopic or dorsal flank sites, providing a feasible animal model for this cell line.

P137 Novel Serum-based Immunodiagnostic Assays for Human Colon Cancer Detection
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We aim to develop immunodiagnostic assays for the detection of cancers in humans to complement/improve current screening methods. We first generated mouse polyclonal antibodies, then monoclonal antibodies (mAbs) against biomarkers in patient serum with clinical applications in human colorectal cancer (CRC) diagnosis and disease management. CRC is a malignancy accounting for 10% of all cancer mortality. There are several recommended screening methods, yet only 44% of US adults over 50 undergo CRC screening. Colonoscopy, the current gold standard, is invasive, while the non-invasive fecal occult blood test has limited sensitivity. An easy-to-use immunodiagnostic assay would be safer and gain patient preference. We previously developed and validated CRC biomarkers in patient serum using polyclonal antibodies with high sensitivity and specificity for all stages of disease. We generated and screened 24 mAbs on clinical samples using our proprietary multiplex protein array technology, an immunoaassay linked to a data acquisition and infrared imaging system. The same matrix of serum samples (8 CRC, 8 controls) was simultaneously interrogated by a given mAb, and antigen-mAb reaction was visualized with a fluorescence dye-linked secondary antibody. Spot intensities were analyzed. Sensitivity and specificity were determined from receiver operating curves using 194 serum samples from 68 cases and 126 controls (including other cancer patients and normal individuals). In this population, mAb1 and mAb2 yielded 80% sensitivity at 90% specificity, and 75% sensitivity at 80% specificity in the comparison CRC versus non-CRC. mAb specifically stained colon cancer patient tissues by immunohistochemistry.
with membrane, nuclear, or cytoplasmic localization. Western blot analysis using DLD-1 colon adenocarcinoma cell line total protein extract showed that the selected mAb recognize single protein bands, further validating the CRC specificity of these biomarkers. We have also developed an ELISA-based prototype immunodiagnostic assay for CRC detection, and are conducting a large validation study using mAb against CRC serum biomarkers and the corresponding prototype assay. Our approach represents an effective translation of biomarker discovery to the patient.

P138 Application of Bid Driven by Alpha-fetoprotein in Destroying Hepatocellular Carcinoma Cells in Vitro and in Vivo
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One of the common problems in cancer gene therapy is the lack of specificity or selectivity. This problem is particularly troublesome when it comes to use of pro-apoptotic molecule, such as Bid/tBid, as a genetic mediation to destroy tumor cells because Bid may target cells without selection, and thus damage both normal and tumor cells, liver and non-liver cells. Approximately 80% of HCC specifically expresses alpha-fetoprotein (AFP), making it a good molecular sign to guide a tumoricidal agent exclusively to the AFP-producing cells. Thus, in order to overcome this problem, we have decided to use AFP to guide Bid/tBid towards HCC cells only. A 980-bp AFP enhancer/promoter was cloned and fused with Bid/tBid. AFPBid/tBid was then cloned into a recombinant adenovirus to generate Ad/AFPBid, which contained a tBid gene driven by AFP promoter. Our result found that HCC cells (Hep3B and PLC/PRF/5) infected with Ad/AFPBid showed a significant decrease in cell viability. The decrease in cell viability by Ad/AFPBid resulted from apoptosis of HCC cells, evident by enhanced activity of caspases and increased release of cytochrome C. In vivo experiment was performed by the intratumor injection of Ad/AFPBid in 6-wk-old male athymic BALB/C nu/nu mice inoculated with Hep3B (1 x 106). When tumors reached between 100 and 200 mm³, mice were treated with Ad/AFPBid. Two control groups (cell culture medium and Ad/AFPLacZ) were used. The size of tumors was measured weekly after the treatment until week 8 when they were killed. Ad/AFPBid injection inhibited tumor growth and the difference was increasing until end of the experiment (week 8) when the size of tumors became obviously significant inhibition was achieved at week 3 after treatment of Ad/AFPtBid in 6-wk-old male athymic BALB/C nu/nu mice. These nude mice were used to minimize the xenogenic rejection and eliminated the interference of hair pigment, so we could obtain better visualization of luminescent cells. The prostate cancer cells were genetically modified to express luciferase, therefore when the luciferin was injected into the mice these cells emitted the luminescent light immediately, which could be detected using a Xenogene IVIS imaging system. The intensity of the luminescence is correlated to the number of cells, which reflected the size of the tumor. The mice were then injected IP with the WISP-1 antibody, control antibody or saline (10 mice per group, total 30 mice). After 2 wk the size of the tumors was evaluated every week for 6 wk using the Xenogen IVIS imaging system. This system is designed to hook up to gas anesthesia that enabled us to anesthetize the mice through a nose cone while the image was captured by a digital camera. Our study revealed that the prostate cancer cells metastasized to the long bones, and the craniofacial region. Antibody treatment reduced growth of the tumor in the long bones but not the craniofacial region. This new technique was key to the success of this study and could be used for different experiments that need to track cells through out the body.

P140 High-definition Oscillometry: A New Method for Non-invasive Blood Pressure Measurements in Conscious and Sedated Common Marmosets
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The common marmoset (Callithrix jacchus) has been used in a wide range of toxicological research and as a model for human disease and physiology, including cardiovascular research. However, due to small body size and high heart rate, the generation of data on non-invasive blood pressure measurements in unsedated marmosets was somehow arbitrary; therefore the non-invasive, accurate determination of blood pressure in this species represents a challenge. A former in-house study rejected normal oscillometry as numerous recordings failed or were not reproducible (mean artery blood pressure (MAP) ranged between 26 and 171 mmHg). The high-definition oscillometry (HDO) technique allows visible, real-time control of each measurement on a screen by the use of blood pressure amplitude scans with up to 16.000 Hz within 10 to 15 s. HDO was used in 18 marmosets to determine the systolic (SYS) and diastolic (DIA) blood pressure, MAP, and the pulse/min. In conscious animals, these parameters were determined between 2 to 4 times, depending on the animal’s compliance. Ten min after intramuscular injection of ketamine (50 mg/kg), diazepam (0.5 mg/kg), and glycopyrrolate (0.008 mg/kg), the above-mentioned parameters were determined 7 times per individual. Results in conscious marmosets: mean SYS: 142.3 mmHg (standard deviation [SD] 13.2); mean DIA: 64.3 mmHg (SD 7.6); mean MAP: 91.6 mmHg (SD 8.3); pulse/min: 390.7 (SD 41.9). The intraindividual SD ranged as follows: SYS: SD 2 to 13 (mean 7); DIA: SD 1 to 16 (mean 4.2), mean MAP: SD 1 to 15 (mean 4.2). After a first increase in pulse rate directly after induction of anaesthesia, a decrease of SYS, DIA, and pulse rate was determined in regard to unsedated common marmosets. Results in sedated marmosets: mean SYS: 93.9 mmHg (SD 12.4); mean DIA: 46.3 mmHg (SD 6.9); mean MAP: 63.4 mmHg (SD 8.4); pulse/min: 340.2 (SD 26.0). The intraindividual SD under sedation was even decreased. The MAPs in conscious marmosets determined in this study correspond to those gained by implant-
able telemetry (MAP 95 mmHg, SD 9) or by indwelling arterial catheters (MAP 107 mmHg, SD 16). Our results demonstrate the feasibility of non-invasive blood pressure measurements by HDO technique in common marmosets, whether conscious or sedated.

P141 Lesions Induced by UVB Irradiation in a Mouse (Mus musculus) Model of Xeroderma Pigmentosum

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Xeroderma pigmentosum (XP) is a rare human genetic disease caused by defective DNA repair and associated with increased risk of ultraviolet (UV)-induced skin cancers and ocular abnormalities. B6;129S7-<sup>sup<sup>2</sup>sup/cam1Brd</sup> (XPC<sup>-/-</sup>) mice exhibit a phenotype similar to the human XP condition. XPC<sup>-/-</sup> mice were cross-bred with ataxia-telangiectasia mutated and rad3-related (ATR) kinase transgenic mice to create double mutant animals (XPC<sup>-/-</sup>/ATRtg or XPC<sup>-/-</sup>/ATRwt). Eighty-six mice (49 males and 37 females, 13 to 34 wk old at the start of a carcinogenesis study) were exposed to UVB irradiation 5 times/wk at a previously published dose of 250 mJ/cm<sup>2</sup>. After 8 treatments, 39 of 86 mice (45%) exhibited mild to severe cutaneous erythema, suggestive of sunburn. UVB treatments were stopped temporarily, and mice were treated with oral ibuprofen for 3 wk until lesions healed. Mice were then exposed to escalating UVB doses of 25 to 200 mJ/cm<sup>2</sup> (titrated to erythema) 3 times/wk for 21 wk. Subsequent clinical signs of UVB damage varied in severity and included hyperemic and swollen pinnae and digits, erythemic and ulcerated cutaneous lesions, epidermal tumors, and ocular pathology, with effects most apparent in albino mice. Histopathology demonstrated ocular lesions, including phthisis bulbii with hemorrhage, retinal detachment, early cataract, anterior uveitis, synchieae, severe keratitis with neovascularization and ulceration, and corneal squamous cell carcinoma (SCC). Tumors in haired skin included spindle-cell variant and classic SCC. Petrolatum was applied to eyes to block further UVB damage during continued treatment, and mice were again treated with oral ibuprofen. After 19 wk of UVB exposure, 10 of 86 mice (12%) developed anemia, melena, frank bleeding from cutaneous lesions, or acute death. Clotting parameters were normal, and anemia and death were attributed to hemorrhage typical of SCC lesions. Melena was likely due to grooming of hemorrhagic skin lesions. In summary, measures should be taken to protect against UVB-induced ocular lesions in XPC mice, and topical petrolatum is an inexpensive protectant. Adjusting the UVB dose may help prevent cutaneous erythema, and mice that develop SCC need to be monitored for anemia and bleeding.

P142 Generation of Embryonic Stem Cell-derived Mice Using Tetraploid Blastocyst Microinjection: A Description of Technique Optimization

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The production of genetically modified mice is essential to biomedical research. Tetraploid blastocyst microinjection (TBM) generates completely embryonic stem (ES) cell-derived mice. TBM uses tetraploid blastocysts injected with diploid ES cells. Tetraploid cells ultimately contribute only to extraembryonic tissues. As a result, only ES-derived cells are present in the embryo, eliminating the need for costly backcrossing. Here we report successful modifications of the standard TBM protocols. The use of an existing in-house Crl:CD1(ICR) outbred mouse breeding colony as inexpensive pathogen-controlled tetraploid blastocyst donors allow rapid coat color identification and higher ES mouse birth rates compared with the standard B6D2F1/Tac strain. Furthermore, the technique described herein used conventional micromanipulators and hand injection as opposed to the more costly Piezo drill described previously. The electrofusion rate from Crl:CD1(ICR) embryos was 89% (n = 1213); while the rate from B6D2F1/Tac was 91% (n = 662). The ES cell mice birth rate (total live and dead pups delivered per number of transferred embryos) was 8% (n = 10/259) in B6D2F1/Tac mice. Sixty percent of pups in the Crl:CD1(ICR) group were born alive; 70% of these pups survived to adulthood and were fertile. In the B6D2F1/Tac blastocyst donor group, 62.5% of pups were born alive; 75% of these survived to adulthood and were fertile. Thus, all surviving pups came from natural births, demonstrating that cesarian delivery is unnecessary. In summary, this report describes the refinement of TBM, including the use of standard micromanipulators and hand injection, the use of Crl:CD1(ICR) embryo donors, and the success of natural birth. These modifications make the technique feasible for most facilities already equipped to perform standard transgenic manipulation.