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Abstracts of Scientific Presentations

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PS1 Evaluation of Different Bedding Substrates on the Reproductive Success of 4 Mouse Strains

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Bedding substrate selection can be a difficult decision for the advisory staff as picking a universal bedding is highly dependent on cost, researcher need, and facility preference. This study examined the effects of bedding substrate on reproductive success to evaluate concerns that phytoestrogens or mitogens in corncob based beddings may interfere with reproductive success in mice. Thirty-six each of ICR, C57BL/6, C3H, and DBA mice were placed into monogamous breeding pairs and divided into 3 treatment groups with an $n = 6$ per group. Each group was placed on one of 3 substrates: ¼' corncob bedding, shredded aspen, or recycled paper. The pairs were maintained continuously for 4 mo or until the third litter of offspring was weaned. Pups per female per day, a commonly used calculation to monitor the production efficiency of commercial mouse colonies, was compared between bedding substrate using an ANOVA. No statistical significance was found between bedding substrate treatment groups (ICR, $P = 0.4679$; C57BL/6, $P = 0.3912$; C3H, $P = 0.6510$; DBA, $P = 0.9545$). No statistical significance was found in the success survivability for each litter, as defined by number of pups weaned divided by number of pups born, between bedding substrate treatment groups when analyzed by ANOVA (ICR, $P = 0.5999$; C57BL/6, $P = 0.9962$; C3H, $P = 0.5574$; DBA, $P = 0.4307$). No statistical significance was found in the number of litters produced by each female between bedding substrate treatment groups (ICR, $P = 0.4490$; C57BL/6, $P = 0.2948$; C3H, $P = 0.3482$; DBA, $P = 0.9229$) when analyzed by ANOVA. This study demonstrated that there were no statistically significant differences in the reproductive efficiency of these 2 strains of mice when housed on these 3 bedding types, suggesting that all are suitable possibilities for the support of mouse breeding colonies.

PS2 Social Media and Laboratory Animal Science: Dos and Don'ts for Laboratory Animal Science Personnel

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Social media is rapidly gaining enormous momentum, and today 47% of online adults use a social networking website. Social media is changing the way we communicate both in our personal and professional lives. Utilization of these sites provides great opportunities for networking but not without the risk of losing privacy. We will address social media, the effect it may have on management and employees, the security of the employee's company, and suggested guidelines for individuals of the laboratory animal community to consider before posting personal information on social networking sites. Another very important factor is the potential exposure of people in the LAS community to animal rights activists. Social media can expand the interaction between you and your employees beyond the work place, but it can also contribute to daily distractions. Managers should consider categorizing their connections into professional and personal connections. Professional connections provide an opportunity to ask and receive valuable input to questions, share opportunities, and discuss work related challenges. For managers wishing to network (whether it be through electronic media or other means) with their staff, an issue to consider might be the perception of preferential treatment towards a particular employee. Perceived inequities could result in resentment and loss of unit morale. People that connect with business associates on social networking sites should be cautious on how their postings are perceived. Because of the difficulty in managing personal and professional connections on one site, one should consider separating such connections, for example, keeping personal connections on a social site, while maintaining business connections through a professional site. Some social networking websites commonly incorporate applications where one is automatically enrolled which share information with third parties such as what websites one may have visited. This presentation is designed for anyone that may be considering the use, or is already using social networking media. It will provide some "dos and don'ts," as well as educate the participant on successful navigation through the social media cyber world.

PS3 Post Approval Monitoring: Lessons Learned

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Institutional Animal Care and Use Committees (IACUC) are charged, under federal laws, regulations, and policies, with providing oversight of all animal activities. In smaller animal care programs, oversight may be accomplished by IACUC members and veterinary staff; however, as the size and complexity of the animal care program increases the oversight burden also increases. One means of accomplishing this oversight is by creating and implementing a program of post approval monitoring. Endorsed in the eighth edition of the *Guide for the Care and Use of Laboratory Animals*, the concept of post approval monitoring after the initial IACUC protocol approval provides a valuable tool to the institution to assure the integrity of the program. Your institution may consider planning and applying a program of post approval monitoring, just as our institution did in the fall of 2004. Over the last 7 y, our post approval monitoring program has adapted to meet the needs of our research community. The post approval monitoring program that initially revealed a relatively low compliance rate and encountered resistance from investigators has evolved into a program that exhibits a high percentage of compliance, acceptance by the research community, and self-reporting of potential noncompliance and adverse events from research staff. Several supplemental programs have been designed and implemented as a result of the post approval monitoring program: required yearly online training for all animal users which emphasizes the main compliance issues from the previous year, an institutional research animal coordinator program, and numerous IACUC policies including adverse events and controlled substances. The program of post approval monitoring has provided our institution with the best insurance of animal wellbeing and welfare and quality research outcomes.

PS4 Use of Review Software for IACUC Protocol Review and Member Training Documentation

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Many Institutional Animal Care and Use Committee (IACUC) protocol management software systems exist. The majority of these systems require customization, significant lead time, and changes to existing protocol templates. Some have minimal to no ability to document IACUC member training. Our facility sought a readily available, cost effective, web-based system that focused only on document review. A review software was selected as a hosted, web-based document review system that tracks changes, comments, and responses made from multiple reviewers and authors. We chose to retain our IACUC protocol form in a word-processing document template. New IACUC protocols are uploaded into the review software for prereview by the attending veterinarian, since the program allows document review by different, sequential layers of reviewers. During reviews, members can insert comments and questions into the document. Once the attending veterinarian's review is complete, the document is opened up for review by all IACUC members. Emails are automatically generated and sent to

IACUC members when a protocol is ready for review. All members can state if the protocol is acceptable for designated member review (DMR) or must go to full committee. Selected protocols are approved by the DMR, plus time stamped and dated. Standard operating procedures, regulatory documents, and other training items can be uploaded. IACUC members receive automated emails inviting them to review the items and document their training. Each user is given a username and password and has access to the review software training modules. It took approximately 1 mo to convert to the review software system. The existing protocol numbering system was retained. The review software offers a convenient, secure, web-based approach to reviewing and approving IACUC documents without the need to purchase software systems that require customization or changes to existing forms. The ability to document IACUC member training is a unique function necessary in today's compliance driven environment.

PS5 Commissioning the Animal Facility for the Animals

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Typical commissioning of an animal facility by the contractor does not comprehensively prove that the new facility is environmentally optimal for animal studies. A study was designed to validate a new mouse facility to ensure health, wellbeing, and productivity were comparable to the existing facility prior to occupancy. The scope of the study pertained to the characterization of environmental conditions in the facility's holding rooms over a 6-wk period and included a simulated HVAC systems failure test. Environmental conditions likely to affect colony health and productivity were monitored in order to characterize macro- and microenvironments as follows: holding room and cage temperature, relative humidity, CO_2, oxygen, ammonia, light, sound, and vibration. Three simulations were performed based on the most likely equipment and power failures: room AHU failure, rack AHU failure and room plus rack AHU failure. Reproductive rates were monitored throughout the study. Specific results varied for the environmental parameters; however, on average they were within expectations. HVAC failure tests provided data on the length of time microenvironment can endure without ventilation before animal health is affected. Most importantly data on productivity rates, mortality rates, and health issues was gathered and analyzed, assuring research investigators of their colonies' future health and breeding success at the new location.

PS6 Animal Room Light at Night-Induced Circadian Rhythm Disruptions in Laboratory Nude Rats Predispose to Type 2 Diabetes

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Environmental light at night (LAN) may account for the increased incidence in cancer, obesity, and diabetes in humans and causes circadian rhythm disruptions in laboratory animals by its ability to induce inhibition of pineal melatonin production. Here, we examined whether animal room dark-phase LAN-inhibition of the nocturnal melatonin signal disrupts central and peripheral clock gene mechanisms regulating animal metabolism, thus predisposing to metabolic syndrome leading to type II diabetes. Adult, female nude rats (n = 36 per group) were maintained for 4 wk on either a control (C) 12L:12D (345 lx; 141.5 µW/cm^2) or experimental (E) 12L:12D-(0.2 lx; 0.08 µW/cm^2) light:dark cycle (lights on at 0600 h) in an AAALAC-accredited facility. Measurements of arterial plasma melatonin, glucose, lactic acid, corticosterone levels, as well as plasma and liver tissue triglyceride (TGA), free fatty acid (FFA), phospholipid (PL), cholesterol ester (CE), and liver cAMP levels, clock (BMAL1, CLOCK, PER1, CRY1) and clock-associated (WEE1 and cMYC) gene mRNA expression were made every 4 h over a 24-h period. Plasma melatonin levels in C were high in the dark phase (108.8 ± 6.5 pg/mL), low (1.0 ± 0.2 pg/mL) in the light phase and low throughout the 24-h period in E. Animal plasma glucose, lactic acid, corticosterone levels were diurnally elevated in E (animals hyperglycemic over 24-h period) compared with C ($P < 0.05$). While diurnal arterial plasma lipid fraction levels (TGA>FFA>PL>CE) were similar for C and E (high, night; low, day), circadian rhythms of liver lipid fractions (TGA>PL >FFA>CE) were disrupted and significantly elevated in Ecompared with C ($P < 0.05$). Liver cAMP levels in C (high, day; low, night) were elevated over the 24-h period in E. Normal diurnal expression of liver clock gene mRNAs in C was disrupted in E. These findings demonstrate that integrated circadian rhythms of animal clock gene expression and metabolism in vivo can be disrupted by light at night via melatonin suppression, predisposing animals to type II diabetes. Improved facility design and adherence to diurnal lighting protocols, as outlined in *The Guide,* are essential to the health and wellbeing of laboratory animals and to the credible outcome of scientific investigations.

PS7 When Undergraduates are Targets: Considering Animal Rights Extremists and Student Safety in the IACUC Crisis Management Plan

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In March 2011, the animal rights extremist group Negotiation Is Over (NIO) announced that it had mounted a campaign of harassment against an undergraduate life science major from Florida Atlantic University, after which the student had "denounced animal testing and [her] involvement in it". Emboldened, NIO encouraged activists to target student "vivisectors-in-training" with "education" and smear campaigns. While IACUC crisis management plans often anticipate threats posed by animal rights extremists to animal care facilities, animals, and research records, they less frequently anticipate harms to undergraduate life science majors. Given the willingness of extremist groups to target these students, crisis management plans should include measures to protect students from harassment and support them when they have been harassed. Proactive crisis management planning should include: (1) education for students, faculty, support staff, and administrators about extremist tactics; (2) development of policies for research and teaching laboratories that protect student privacy and limit the release of materials that can be used out of context by extremists; (3) concrete strategies to mobilize institutional support for student populations and for individual students who have been targeted; and (4) mechanisms by which to involve students and other members of academic communities in informed dialogues about animal welfare regulations and their implementation, and about the ongoing public discourse about scientific animal use. This planning must recognize the current climate for animal researchers at all stages of training, and it should avoid blaming students targeted by extremists for their own victimization. Undergraduate science majors are both potential new researchers and members of a broader public that would benefit from better education about the rationale for animal use, the humane standards animal use must meet, and the activist climate in which researchers find themselves. Thus, appropriate attention to the needs of these students in crisis management planning may positively impact public understanding of animal research.

PS8 Effective Oral Immunization of Egg Laying Chickens Eliminates All Animal Stress from Polyclonal Antibody Production

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Production of polyclonal antibodies is traditionally associated with injections (immunizations) of the animals with antigen, often in combination with a more or less aggressive adjuvant, and subsequent blood sampling regimens through a period of time. The use of chickens for antibody production is attractive because approximately 10 times more antibody can be harvested from chicken egg yolk compared with what can be obtained from serum of a similar sized mammal. Oral immunization through voluntary ingestion of antigen-adjuvant, combined with purification of antibodies from the egg yolk, has the potential of eliminating all animal stress from polyclonal antibody production. We hypothesized that efficient oral immunization methods of chickens could be developed. After testing a series of adjuvants we found that 2 of them (cholera toxin B-subunit and a trademarked brand) were effective in stimulating a peripheral immune response to an antigen in chickens following oral administration by gavage in manually restrained birds. We are presently working on presenting the antigen-adjuvant mixture in attractive worm-like pasta-based structure to make voluntary ingestion quick and efficient. However, an anamnestic memory response was difficult to induce through oral immunizations rendering booster administrations of little effect. In our most recent studies we have documented that oral immunization with antigen (BSA) and the trademarked adjuvant administered not as single doses, but as doses divided over 3 to 5 d effectively introduced immunologic memory, thus paving the way for very effective oral immunization schemes for chickens. Presently we achieve approximately 12% of the antibody concentrations obtained after classic subcutaneous immunization of chickens, and we are confident that this figure can be increased making

this new animal-friendly technology economically attractive.

PS9 Measuring the Benefits of Hands-on Training

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The Responsible Care and Use of Laboratory Animals Training Program was developed at our institution to fulfill a need for hands-on training. We designed the curriculum based on experiential learning and situated cognition theories, both of which have been described as preferred approaches for skill building in the field of laboratory animal science. The original intent of the program was to provide a safe environment for researchers and animal care staff to practice common research techniques under the guidance of instructors who are experts in the field of laboratory animal medicine. Upon observing a trend of consistently positive post session evaluations, we designed an IRB-approved study to explore additional benefits of hands-on training. Using a series of surveys and focus group questions we tested our hypotheses that the training program: 1) improves comfort with rodents used in biomedical research, 2) promotes long term retention of knowledge and 3) encourages collaboration between research and veterinary staff. Likert-style and open-ended survey questions were distributed to participants before and after training sessions using a web-based survey company. Participants were asked to rate confidence and comfort level with rodents, the likelihood that they would approach veterinary staff for information and the degree to which the training sessions were useful. Survey questions also tested curriculum-specific knowledge before and after training. Focus group sessions were used to gain further information about aspects of the training program that helped or hindered the learning process. Results of surveys and focus group sessions suggest that our hands-on training program improves comfort with laboratory rodents, promotes long-term retention of knowledge, and increases the likelihood of contacting the Animal Resource Staff for information. This face-to-face educational format has the potential to build positive working relationships, improve animal welfare, and promote high quality research.

PS10 Stepwise Approach for Creating and Implementing a Hands-on Training Program at an Academic Institution

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Federal regulations require that all personnel involved in the care and use of laboratory animals be provided training. While the basic content of training is mandated, the delivery method is not. Our institution fulfills its legal obligations with a series of computer-based tutorials. While the computer-based format is convenient, it does not support information retention like other adult learning strategies and cannot adequately train students to perform manual skills. Empirical evidence of procedures performed incorrectly coupled with students' requests for hands-on training has driven the effort to create and implement a training approach novel for our institution. Developing and implementing a training program to support over 500 researchers and 340 active animal use protocols is a daunting task. The following stepwise approach was used during the course of 1 y to mitigate this mountainous undertaking. Step 1: Support and feedback were sought from the IACUC using a broad outline of the proposed program. Step 2: The framework of the curriculum was developed during the process of submitting an animal use protocol while trainers were recruited. Step 3: Once the animal use protocol was approved, the curriculum was further refined during the process of applying for funding. Step 4: Once funding was secured, information technology support was sought in order to manage scheduling and data (survey results, course evaluations). Step 5: Details of each training module were worked out during instructor training sessions. Step 6: The training program was advertised and initiated. Step 7: Course evaluations and student surveys are continually used to shape course content. Our current training program supplements required computer-based training with a series of 6 hands-on training sessions provided quarterly. We are not only providing a safe environment in which to practice humane animal use techniques, we are also establishing relationships that will build "social capitol" for our program.

PS11 Development of Hypercapnic and Hypoxic Conditions in Disposable Individually Ventilated Cages Following Removal from Mechanical Ventilation

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Disposable, individually ventilated cage (IVC) systems are becoming increasingly popular for the housing of mice. However, there is a risk of CO_2 levels increasing and O_2 levels decreasing within the cage (hypercapnic and hypoxic conditions) if the electrical supply fails, if the ventilation rack malfunctions, if the IVC is not properly docked into the ventilated rack, or if investigators remove the cages from the rack for an extended period of time. Using a portable multi-gas detector, we investigated how quickly hypercapnic and hypoxic conditions developed within disposable IVC after removal from the ventilation rack. With 5 adult CD1 mice per cage, CO_2 concentrations reached ≥ 5% (50,000 ppm), the maximum limit of our detector, within 4 h. The mice showed clinical signs of hyperventilation characterized by abdominal breathing. CO_2 levels were verified with a manual gas analyzer. Concomitantly with the rise in CO_2 concentrations, O_2 levels dropped to 13.8% by 4 h and 11.7% by 6 h. As a comparison, room atmosphere was measured at ≤ 0.02% CO_2 and 20.9% O_2. Four adult mice per cage had a slightly slower but similar profile, attaining ≥ 5% CO_2 also within 4 h and 12.95% O_2 within 6 h. For 3 or 2 mice/cage, after being off ventilation for 6 h, CO_2 and O_2 values were 4.6% CO_2 and 14.7% O_2 and 3.04% CO_2 and 17.1% O_2, respectively. These results were not unique to the disposable IVC tested; similar results were obtained with a nondisposable brand of IVC. It has been recommended that prolonged exposures of rodents to CO_2 levels above 3% to 5% should be avoided. In humans, breathing rate doubles at 3% CO_2 and quadruples at 5% CO_2. Prolonged exposure to high levels of CO_2 results in headaches, an increase in heart rate and blood pressure, dizziness, and fatigue. With regard to O_2 levels, the OSHA Respiratory Protection Standard considers O_2 levels below 19.5% to be "oxygen-deficient." Concentrations of 14% to 16% O_2 can result in tachypnea, tachycardia, and impaired judgment. We conclude that hypercapnic and hypoxic conditions can quickly develop in disposable IVC if the cages lose ventilation.

PS12 Novel Multiplex Serological Profiling Assay for Rhesus Macaques Measures Viral Pathogens by Protein Microarray

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Protein microarrays are useful for studying biologic responses in a multiplex and high throughput manner. We successfully designed a serological screening tool that detects specific antibodies to viral pathogens in sera of rhesus macaques. We evaluated several types of antigens for use on this platform including recombinant proteins, peptides, and virus-infected cell lysates. Pathogens represented included B virus (Cercopithecine herpesvirus 1), simian T lymphotropic virus (STLV), simian retrovirus type D (SRV/D), simian immunodeficiency virus (SIV), measles (morbillivirus), lymphocryptovirus (LCV), rhesus cytomegalovirus (CMV), simian foamy virus (SFV), and rhesus rhadinovirus (RRV). Viral antigens were arrayed onto ultrathin nitrocellulose-coated glass slides, and serum samples applied onto individual subarrays. Silver-staining (SS) protein detection reagents were used to visualize bound immunoglobulin complexes through the nonenzymatic silver deposition method. Correct identification of positive or negative sera and overall signal-to-noise ratio was evaluated for each of the antigens. The best performing reagents were used to further screen serum samples from several SPF NHP colonies, to validate the assay performance. We found that our expanded assay identifies serological status for a broad spectrum of infectious agents in NPH in a novel and economical multiplex assay with high levels of sensitivity and specificity, and will be a useful tool for easy and rapid monitoring of super clean colonies. To our knowledge, this assay is the first multiplex serological test to include LCV, RRV, and rhesus CMV in addition to the standard SPF agents. This assay also demonstrates the potential of the protein array and SS detection system for a rapid and high throughput tool for screening potential vaccine candidates and the associated antibody response.

PS13 A High Throughput and Robust Genotyping Pipeline at a Research Support Facility

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The Genotyping Laboratory in the Mouse Genetics Department at our institution's Research Support Facility (Rsf) has developed a highly efficient and robust data production pipeline for providing rapid and accurate genotypic analysis of genetic mouse models. Our laboratory extracts genetic material from roughly 350,000 tissue samples per year, and performs nearly 600,000 individual genotyping reactions on those samples covering nearly 600 unique genotyping assays for hundreds of investigators. Here, we present an overview of this pipeline, focusing on the procedures, software, and automation that facilitate such an endeavor. We detail the types of genotypic analysis we offer, such as PCR fragment analysis, 5′-nuclease protection assay, copy number variation (CNV) assay, single nucleotide polymorphism (SNP) assay, and others, including the strengths and challenges presented by each assay class. This pipeline directly assists our Animal Model Production team, and describes the necessary interactions and cross-functional support required for successful model creation. Finally, we present our typical throughput and turn-around time for genotyping services. Generally, tissues samples are consolidated, undergo DNA extraction, are arrayed and PCR-amplified, analyzed, and reported to customers in 5 d or less. This pipeline model can serve as a useful resource for other laboratories wishing to capitalize on process, software, and hardware automation for genetic analysis.

PS14 Detection of *Pneumocystis carinii* in Rat Colonies by PCR and Serology

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Recently, *Pneumocystis carinii* was demonstrated to be the cause of lung lesions formally attributed to "rat respiratory virus." This discovery led to a shift in diagnostic methods from histology to PCR and serology. To determine the detection of *P. carinii* by real-time PCR and IFA we compared results from 158 immunocompetent rats from studies that included 122 contact-exposed CD rats from temporal transmission experiments and 36 SD rats from an enzootically infected barrier-room breeding colony. Although the progression of infection varied by the mode of transmission, we observed that the peak of *P. carinii* organisms as determined by rtPCR coincided with the initiation of seroconversion. These rats were positive for *Pneumocystis* by combined real-time PCR and IFA 96% of the time, which was significantly more frequent than approximately 60% by each of these methods alone ($P < 001$) and 27% by pulmonary histopathology ($P < 001$). To improve efficiency of serosurveillance, we developed a *P. carinii* multiplex fluorometric immunoassay (MFIA) using a recombinant antigen. MFIA and IFA demonstrated excellent correlation for contact transmission experiments ($P = 1$). A 17% prevalence of *P. carinii* antibodies among samples submitted to our laboratory from North America between January and May 2011 ($n = 5950$) was determined using MFIA. This is a 3-fold increase from the previously reported 6% prevalence, determined by pulmonary histopathology. An additional 3% rats were detected as positive by PCR of lung tissue indicating that the prevalence of *P. carinii* could be higher than indicated by the MFIA survey. In contrast to most viral agents, which replicate rapidly and are most often detected 1 to 2 wk postinfection, *P. carinii* grows slowly (reported doubling time = 4.5 d). Therefore, depending on the exposure dose and exposure time, seroconversion may be delayed or absent in bedding sentinels. Therefore, a window of detection by serology may not occur within a standard 12-wk sentinel program and PCR is required for detection during the preseroconversion period. Our results stress the necessity of monitoring for *P. carinii* concomitantly by PCR and serology to improve detection in a routine sentinel program.

PS15 Chemopreventative Effects of Vitamin D in *Helicobacter*-Infected Smad3$^{-/-}$ Mice, a Mouse Model of Inflammation-Associated Colon Cancer

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Both human epidemiologic studies and animal studies suggest that low serum vitamin D levels are associated with an increased risk of colon cancer as well as autoimmune diseases such as inflammatory bowel disease (IBD). Given that the incidence of colon cancer is increased in patients with IBD and that vitamin D is considered both chemopreventative and antiinflammatory, we investigated the hypothesis that increased consumption of dietary vitamin D would reduce inflammation and tumor development in a mouse model of inflammation-associated colon cancer. 129-*Smad3^{tm1Par}*/J (Smad3$^{-/-}$) mice have defective transforming growth factor β-signaling and develop colon cancer in response to an inflammatory insult such as inoculation with *Helicobacter bilis*. Six- to 14-wk-old male and female Smad3$^{-/-}$ mice were fed a purified diet with either maintenance (1 IU vitamin D/g diet) or increased concentrations of vitamin D (5 IU vitamin D/g diet) for 17 wk. One week after diet initiation, mice were inoculated with either broth or *H. bilis* (107 CFU) to trigger inflammation ($n = 13$ to 14 mice per diet per treatment). Fecal PCR was used for verification of *H. bilis*-infection and to ensure that no cross-contamination between infected and uninfected animals occurred. At 16 wk post inoculation, mice were necropsied and tissues were harvested to determine changes in cytokine levels and to assess tumor incidence as well as changes in inflammation and dysplasia. Serum analysis showed that while a vitamin D-fortified diet significantly increased serum 25-hydroxyvitamin D it did not affect serum calcium levels, suggesting that higher levels of dietary vitamin D did not induce toxicity. Histologic analysis revealed a significant reduction in colon tumor incidence in *H. bilis*-infected mice fed a vitamin D-fortified diet compared with the maintenance diet (7% compared with 50%, $P = 0.016$). In addition, a significant decrease in inflammation and dysplasia of the proximal colon was also noted in the same treatment group (mean IBD score 1.9 compared with 5.5, $P = 0.032$). As expected, no tumors developed in broth-treated mice on either diet. These results suggest that increased levels of vitamin D in the diet may be beneficial in preventing inflammation-associated colon cancer development.

PS16 Infrared Body Temperature Measurement of Mice as an Early Predictor of Death in Lipopolysaccharide-Induced Endotoxic Shock

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We used a highly sensitive noncontact infrared thermometer to determine if surface body temperature can be used as an experimental endpoint for a mouse model of endotoxic shock. To induce endotoxic shock, 22 C57BL/6 mice (male and female, 8 to 10 wk old) were injected intraperitoneally with lipopolysaccharide (LPS), at concentrations of 3 to 15 mg/kg of their body weights. The temperature of individual mice were monitored at room temperature (21.2 °C) using a noncontact infrared thermometer with 150-ms response time and a reading spot size of 1 mm. The mice were briefly restrained and the beam of the reader aimed at 6 cm from the xiphoid process of sternum. Their body temperature was monitored prior to and every 60 min for the first 4 h after LPS injections, with the measurement intervals increasing until we reached the 118-h mark. The baseline surface body temperature of the animals was 33 ± 1.1 °C. Of the 22 mice in the study, 17 suffered body temperature drops to 25.0 °C or below, and died within 4 to 91 h of the LPS injection (100%). On the other hand, the 5 animals whose body temperature never dropped to 25.0 °C or below survived. In the survival group, 2 mice had body temperature of 25.7 °C 3 h after the LPS injection, but the temperature gradually increased and both survived. Adoption of a surface body temperature of 25.0°C or below as a humane experimental endpoint would significantly reduce suffering for animals in the terminal stages of the LPS-induced endotoxic shock model.

PS17 High Prevalence of Enterohepatic *Helicobacter* spp. in Aged Macaques with Intestinal Adenocarcinoma

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Intestinal adenocarcinoma is the most frequently diagnosed neoplasm in aged macaques. Fecal samples of 34 (25 to 32 y of age) rhesus monkeys and tumor tissues from a single monkey diagnosed with intestinal adenocarcinoma were analyzed by a combination of PCR and microaerobic culture for the presence of *Helicobacter* species. Nine (8 female and 1 male) of the macaques had a history of successful surgical resection of intestinal tumors. Using *Helicobacter*-species specific primers, C05 and C97, which amplify a portion of 16S rRNA gene, 32 of 34 (94%) fecal samples were tested positive for *Helicobacter* spp. Isolates of *Helicobacter* spp. were obtained from 27 (79%) of the fecal samples by microaerobic culture. Of the 9 rhesus with a history of intestinal tumor resection, 7 of 9 (78%) were *Helicobacter* spp. positive by PCR. An analyses using a combination of restriction fragment length polymorphism (RFLP), 16S rRNA gene sequencing, and BLAST search revealed that the isolates

obtained from these samples represent 2 previously identified *Helicobacter* species—*Helicobacter* spp. MIT 03-7674C and *Helicobacter macacae* MIT 99-10773 (99% sequence homology). *Helicobacter* spp. MIT 03-7674C represents the species identified in 67% (18 of 34 samples) of the culture positive samples, while *Helicobacter macacae* MIT 99-10733 represents 30% (8 of 34) of the positive samples. A single fecal sample and tumor samples from a monkey diagnosed with adenocarcinoma were identified as containing a mixture of the 2 *Helicobacter* species. Studies should be undertaken to ascertain whether *Helicobacter* spp.-associated inflammation promotes intestinal carcinogenesis in macaques.

PS18 Histologic and Molecular Characteristics of Inflammation in a Mouse Model of Intrauterine *Ureaplasma parvum* Infection

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Ureaplasma parvum is a major cause of intrauterine infection in humans. The inflammatory response associated with chorioamnionitis contributes to fetal morbidity and can initiate premature birth. Our objective was to develop a murine model of *U. parvum*-induced chorioamnionitis that resembles the disease observed in humans. We developed a model of *U. parvum* infection in mice to study the patterns of inflammation in fetal and maternal tissues subsequent to intrauterine inoculation with this pathogen. Timed-pregnant C57BL/6 and BALB/c mice were inoculated at gestation day (GD) 14 with either sterile media (sham controls) or 104, 107, or 109 colony forming units (CFU) of *U. parvum*. Pregnant mice were euthanized at GD 15 or GD 17, and maternal and fetal tissues were evaluated by culture and histology. Degree of placental inflammation was also assessed by expression of TLR1, TLR2, TLR4, TLR6, IL1α, IL1β, IL6, TNFα, and Calgranulin A. Regardless of inoculum dose, both mouse strains exhibited 100% colonization of placental tissues. However, fetal infection rates varied with inoculum dose. All tissues from sham inoculated controls were negative for *U. parvum*. Infected placentas displayed varying degrees of leukocytic infiltration around the decidua. However, placentas with higher S100A8 levels and detectable TLR4 on Western blot had more extensive inflammation that extended towards the chorionic plate and into the vitelline membrane. Our results are similar to what is observed in human disease. Therefore, this model will be useful for elucidating the molecular mechanisms of premature birth in response to inflammation.

PS19 Mouse Parvovirus Infection of Immunodeficient C57BL/6 Mice

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Most contemporary mouse colonies have large populations of genetically engineered mice on a C57BL/6 (B6) background. Little is known about the effect of immunodeficiency on MPV infection in B6 mice. We have previously shown that B6 mice shed low levels of MPV for a short time and MPV transmission from B6 mice is inefficient. In this study, we investigated whether immunodeficiency on a B6 background increases the risk for MPV transmission. Groups of 18 (12 females and 6 males) 4-wk-old B-cell deficient B6.129S2-Igh-6tm1cgn/J (Igh) mice, innate immune deficient B6.129S7-Ifngtm1Ts/J (Ifnγ) mice, B- and T-cell deficient B6.129S7-Rag1tm1Mom/J (Rag) mice and B6 mice were inoculated orally with 300 ID50 of MPV-1d. PCR of feces collected at 1 wk post inoculation (wpi) indicated that 83% of B6, 94% of Ifnγ, 94% of Igh, and 44% of Rag mice were shedding MPV. At 4 wpi, 0% of B6 and Ifnγ, 39% of Igh, and 93% of Rag mice were shedding MPV. From 6 to 18 wpi, all Rag mice were shedding MPV and MPV was shed sporadically from Igh mice through 20 wpi. During the first 2 wk of infection, MPV was transmitted to sentinels in 2 of 8 cages of B6 mice. MPV was transmitted from Ifnγ mice, to sentinels in 6 of 8 cages during the first 2 wk of infection and 1 of 8 sentinels during weeks 2 to 4. Igh mice transmitted MPV to sentinels in over 60% of cages between 0 to 4 wpi, 37.5% of cages between 4 to 8 wpi, and less than 15% of cages between 8 to 16 wpi (6 of 8, 5 of 8, 3 of 8, 3 of 8, 1 of 8, 0 of 8, 1 of 7, and 1 of 8 sentinels). MPV was transmitted to all sentinels in contact with Rag mice between 2 to 16 wpi. Once transmission could not be detected from B6, Ifnγ, and Igh mice, breeding pairs were setup to determine whether MPV infection could be transmitted to offspring. Transmission to offspring was detected in only 1 of 6 cages of Igh mice. In conclusion, B6 and Ifnγ mice had no significant differences in MPV shedding or transmission and they pose a low risk for MPV transmission after 2 wk. Igh mice had prolonged transmission which tapered over time, while transmission from Rag mice was initially low but quickly reached 100% and remained high throughout the experiment. Thus, both Igh and Rag mice pose a high risk for MPV transmission.

PS20 The Role of Neonatal Mice in Transmission of Mouse Parvovirus Infection

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To determine whether neonatal mice housed with MPV-infected adult mice increase the risk for MPV transmission, one 4-wk-old female Swiss Webster (SW) index mouse inoculated with 300 ID50 of MPV-1d was added, 1 wk post inoculation (wpi), to 15 cages, each housing a naive breeding pair of SW mice at 20 d post pairing. All breeding and index mice shed MPV at 1 and 2 wpi, but not at 4 wpi and they all became MPV seropositive. Seven litters were left with their parents and the index mouse and 8 litters were fostered at postpartum day 1. No significant difference in transmission via soiled bedding was observed between cages with and without litters, as all 1 wpi sentinels, 1 of 14 2 wpi sentinels, and none of the 4 wpi sentinels became MPV seropositive. Half of the foster dams (4 of 8) seroconverted, while none of the foster pups had MPV DNA in their feces at 3 or 6 wk of age suggesting that some of the pups served as fomites. None of the pups left with their birth mothers had MPV DNA in their feces at 3 or 6 wk of age and pups in 6 of 7 litters were seropositive at 6 wk of age, suggesting that maternal antibodies protected the pups from infection. To further investigate the role of maternal antibodies in protection from MPV infection, 9 dams each with a litter of 14-d-old pups were exposed to an MPV-infected SW index mouse (1 wpi) for 1 wk. We postulated that 14-d-old mice would become infected via ingestion of feces and that 7 d would not be long enough for the dam to produce and transfer a sufficient quantity of MPV antibodies to protect the pups. Most of the dams and litters (8 of 9) were shedding MPV at the time of weaning. All of the pups were seronegative at weaning, 8 of 9 dams were weakly seropositive at 1 wk after exposure, and all of the index mice were strongly MPV seropositive at 2 wpi. In conclusion, pups born to dams that were infected near birth were protected by maternal antibody, despite being exposed to MPV for several days before maternal antibodies were made. Fostering of pups had no benefit and may spread infection as the pups can act as fomites. However, infection of 14-d-old neonatal mice occurred because maternal antibodies were not transferred to the mice prior to when they began to ingest MPV-laden feces.

PS21 Refinement of the Rice Rat (*Oryzomys palustris*) Model of Periodontitis and Zoledronic Acid-Induced Osteonecrosis of the Jaw

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The rice rat (*Oryzomys palustris*) develops periodontitis-like lesions when fed a diet high in sucrose and casein (HSC). In our first study, we advanced previous studies to assess whether this model accurately mimics human periodontitis. For this purpose, 28-d-old rice rats ($n = 15$ per group; 10 males and 5 females) were randomly assigned to standard (Std) or HSC diets. Animals were euthanized after 6, 12, and 18 wk of diet feeding. Mandibles and maxillae were removed and processed for morphometric, histologic, histomorphometric, and microCT analyses. We found slight to moderate periodontal lesions in the mandibles of rats fed the HSC diet for 12 and 18 wk. Morphometric analysis revealed that, when compared to the Std diet, the HSC diet exacerbated horizontal alveolar bone loss area at the lingual, but not the buccal surface, at 12 and 18 wk and significantly increased vertical alveolar bone loss. Remarkably, the HSC diet significantly increased mineralizing surface, mineral apposition rate, bone formation rate, eroded surface and osteoclast number at the interproximal alveolar bone of rats fed for 6 wk, but not in those fed for longer periods. These findings indicate that the HSC diet induced a transient increase in alveolar bone remodeling, which is followed by accelerated horizontal and vertical bone loss characteristic

of moderate periodontitis. In a second study, we used this model to assess whether nitrogen-containing bisphosphonates (NBP) contribute to osteonecrosis of the jaw (ONJ) associated with periodontitis. We hypothesize that ONJ is a 2-stage process: A) risk factors initiate inflammation/lesions in the oral cavity that lead to a supranormal rate of bone necrosis, and B) antiresorptives (for example, NBP) reduce the rate of necrotic bone removal to allow its accumulation in the jaw. To test this, 28-d-old HSC-fed rice rats (n = 15 per group) were injected subcutaneously biweekly with alendronate (ALN, 0 or 15 µg/kg), or intravenously once monthly with 0, 8, or 80 µg/kg of zoledronic acid (ZOL) and euthanized after 6, 12, and 18 wk. Mandibles and maxillae were analyzed for: A) the progression of periodontitis, B) alveolar bone integrity, and C) bone resorption and formation. We found that 80 µg/kg of ZOL rats had a higher incidence and severity of periodontal lesions after 18 wk of treatment than did other groups. In females, but not males, 80 µg/kg of ZOL ameliorated vertical bone loss at 6 and 12 wk but not at 18 wk. In addition, 80 µg/kg of ZOL increased bone volume and BMD at the interdental alveolar bone at 6 and 12 but not at 18 wk. Remarkably, 80 µg/kg of ZOL increased the area of interdental alveolar bone devoid of basic fuchsin stain (necrotic bone) only at 18 wk. All doses of ALN and ZOL decreased mineralizing surface and bone formation rate at 6, 12, and 18 wk, but reduced eroded surface and osteoclast number only at 6 and/or 12 wk. These data indicate that 80 µg/kg of ZOL decreased bone remodeling before the appearance of increased amount of hyperdense interdental alveolar bone in the jaw. This is followed by exacerbation of the inflammatory response at the periodontium, exposed necrotic alveolar bone and/or osteolysis. These features resemble those observed in patients with ONJ.

PS32 A Case of Spontaneous Zinc Responsive Dermatosis in a Female Rhesus Macaque (*Macaca mulatta*)

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A 5-y-old female rhesus developed yellowish adherent dry scaly skin lesions covering her nose, pinna, digits, and base of the tail. Loose scales were also present diffusely over the body along with multifocal red crusts. Pruritus and alopecia were absent. All the macaques in our facility receive a balanced commercial diet supplemented with fresh fruits and vegetables. No animal housed in the same enclosure or facility was found with a similar dermatopathy. Laboratory diagnostic investigations included hematology, clinical chemistry, serology, parasitology, and microbiology. All results were unremarkable except for the isolation of *Staphylococcus aureus* from the affected skin. An antibiotherapy was instituted based on culture and sensitivity results. After 3 wk, no improvement was noticeable. A skin biopsy was done and histopathology revealed chronic hyperplastic dermatitis with minimal multifocal hyperkeratosis and a mild perivascular lymphocytic inflammatory infiltrate. Based on histopathology results and clinical signs, a diagnosis of exfoliative or dry seborrheic dermatitis was made. Underlying causes such as parasites, bacteria, or fungus were ruled out. Zinc deficiency and atopic or contact dermatitis became major differential diagnoses, as well as immunologic and endocrine disorders, adenitis, or paraneoplastic syndrome. A blood sample was collected for zinc analysis. The result showed a low zinc level (66 µg/100 mL) compared to normal plasma zinc concentration (114 ± 16 µg/100mL). Oral zinc supplementation decreased the extent and severity of the lesions as the serum zinc concentration increased up to a normal level. To confirm that the lesions were associated with zinc deficiency, we progressively decreased the dose of zinc supplementation. In just 1 wk, the lesions reoccured. This report suggests that rhesus macaques can present a spontaneous zinc responsive dermatosis without signs of anorexia, alopecia, weight loss, or apathy.

PS33 Diagnosis of Amyloidosis and Differentiation from Chronic Enterocolitis in Rhesus (*Macaca mulatta*) and Pigtail (*Macaca nemestrina*) Macaques

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A 9-y-old female rhesus macaque (*Macaca mulatta*) presented with diarrhea and weight loss. Fecal culture revealed *Shigella* and *Campylobacter* infection. The diarrhea resolved after treatment with enrofloxacin and erythromycin; however, no weight gain was observed. Significant findings on repeat clinical chemistry analysis were persistent hypoalbuminemia (1.6 g/dL, 1.9 g/dL) and elevated ALP (579 U/dL, 691 U/dL). An exploratory laparotomy was elected and histopathology with Congo red staining confirmed diffuse, severe amyloidosis of the liver and intestinal tract. This case led us to examine the incidence and character of amyloidosis in our colony of rhesus and pigtail macaques (*Macaca nemestrina*), and investigate whether we can better differentiate amyloidosis from other gastrointestinal disease by measuring serum amyloid A (SAA). Review of necropsy records suggests a 9% (rhesus) and 15% (pigtail) incidence of amyloidosis in our colonies, with the liver, small intestine, and large intestine most commonly affected. Animal records indicate that diagnosis of amyloidosis is almost always made postmortem; though changes in clinical pathology parameters have been associated with amyloidosis, they are nonspecific and resemble those seen in the frequently comorbid conditions of chronic anorexia and enterocolitis. Further, the current diagnostic gold standard in macaques is histopathology of the affected organ, requiring necropsy or invasive laparotomy in often poor surgical candidates. SAA, an acute phase protein produced in response to inflammation and a precursor to amyloid, has been shown to be elevated in individuals with amyloidosis as compared to those that are clinically normal. We have found that SAA more specifically distinguishes rhesus macaques with amyloid (n = 6) when compared with macaques with chronic enterocolitis (n = 9) and healthy controls (n = 19) (ANOVA with post hoc Kruskal–Wallis test, P = 0.0040). To confirm these findings we used a series of minimally invasive antemortem biopsies using endoscopy of the small intestine, colon, and ultrasound guided trucut needle device of the liver to diagnose amyloidosis. Our data suggests that SAA can provide a powerful noninvasive screening tool to identify animals with amyloid from those with other chronic disease.

PS34 Characterization and Clinical Relevance of Gastric *Helicobacter heilmannii* in Research Macaques

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Helicobacter heilmannii comprises a group of large, spiral bacteria inhabiting the gastric mucosa of humans and a variety of animals, including dogs, cats, pigs, and nonhuman primates. Infected humans, while not commonly recognized, can be diagnosed with gastritis, gastric ulcers, and MALT lymphoma. As the organisms are notoriously difficult to culture, fluorescent in situ hybridization (FISH) has been used to differentiate 5 *H. heilmannii* subtypes in human patients. To investigate the status of macaques used in neuroscience research relative to *H. heilmannii*-like organisms (HHLO), endoscopic or postmortem gastric samples from 12 animals were analyzed by FISH; all-*Helicobacter* 1200 bp PCR and 16S rRNA sequencing were also selectively performed. The results demonstrate the presence of HHLO subtype 1 in 10 of the cases examined (83%); one animal from 2002 was also positive for HHLO subtype 4. Of interest is that HHLO subtype 1 was found to be the one most often identified in humans, by a large margin. Histopathologic examination of the macaque tissues revealed mild to moderate gastritis, albeit in most animals the inflammation was not associated with any clinical signs. In 3 macaques where vomiting or abdominal discomfort did occur, sometimes infrequently, triple therapy with 2 antibiotics and an H2-receptor antagonist was undertaken for 4 wk. Follow-up gastric biopsies from 2 treated animals were *Helicobacter*-negative by PCR, and the gastric mucosal morphology had reverted to within normal limits. In one singly housed animal, successful eradication has been confirmed by PCR for 5 mo after treatment. Two of the 3 treated animals have had no further clinical episodes; however, it is not certain this is directly related to the absence of HHLO. Zoonotic transmission of HHLO should be considered in a research setting where personnel and nonhuman primates are in frequent contact.

PS35 Cervical Neoplasia in African Baboon (*Papio hamadryas anubis*) Associated with Novel Baboon α-Papillomaviruses

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Genital human α-papillomavirus (αPV) infections are one of the most common sexually transmitted diseases worldwide associated

with virtually all cases of human cervical cancer. Related oncogenic αPV exist in rhesus and cynomolgus macaques. Here we identified 3 novel genital αPV types by PCR in cervical samples from 6 of 18 wild-caught female Kenyan olive baboons (*Papio hamadryas anubis*). Three of the 6 animals had proliferative changes consistent with high (n = 1) or low (n = 2) grade cervical intraepithelial neoplasia (CIN). Histologic features included basal epithelial expansion and cytologic atypia including karyomegaly. Phylogenetically, the baboon papillomaviruses (PhPV1, PhPV2, and PhPV3) were closely related to oncogenic macaque and human αPV. This is the first identification of an αPV in the baboon, and the first association of an αPV with cervical neoplasia in a species other than humans or macaques. Baboons have certain advantages over macaques as reproductive animal models, specifically size, anatomic characteristics, tractability, and decreased risk of zoonotic disease. Cervical αPV infection in baboons may be useful for modeling the pathogenesis, therapeutics, and prophylaxis of genital PV-induced neoplasia. Additionally, this discovery suggests that genital αPV with oncogenic potential may be more widely distributed among primate species than previously thought.

PS36 The Effects of a Prebiotic (Inulin) on Idiopathic Chronic Diarrhea of Rhesus Macaques (*Macaca mulatta*): A Randomized Controlled Trial

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Idiopathic chronic diarrhea (ICD) of rhesus macaques is the cause of 5% to 15% of the loss of the breeding colonies of this species, not related to research. The role of prebiotics in changing the gut microbiota and subsequent clinical changes has been extensively researched in humans and other animal species but there is not enough study to support the effects of prebiotics in nonhuman primates. We studied the effect of an oral prebiotic (inulin) on ICD in rhesus macaques. Thirty-two juvenile male and female subjects with ICD were randomly assigned to inulin or placebo for either 21 (n = 14, G21) or 48 d (n = 18, G48) of treatment. Blood samples and colonic biopsies were collected before and after treatment. Stool consistency was scored daily from 1 (normal) to 4 (liquid diarrhea). Data demonstrated that the prebiotic significantly changed the stool consistency score (SCS) over the 48 d of treatment. Inulin-treated animals showed a mean SCS 0.96 lower than that of the placebo group (P = 0.028). Semiquantitative scoring of histopathologic changes demonstrated significant improvement in crypt pathology (P = 0.038) and overall colonic pathology after 48 d of treatment (P = 0.039). C-reactive protein level was lower in the inulin-treated subjects in G48 (P = 0.075), and colonic tissues in the inulin treatment group (G48) had a lower expression of tumor necrosis factor-α (P = 0.097). Our results indicate that inulin is effective in alleviating the clinical signs of ICD and the associated inflammation in rhesus macaques and suggest that inulin supplementation has potential for improving ICD in rhesus macaques. Further investigation is needed to reveal the specific underlying mechanisms for the prebiotic effect of inulin on ICD of rhesus macaques.

PS37 Meloxicam: An Oral Nonsteroidal Antiinflammatory Drug for *Macaca fascicularis*

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Nonsteroidal antiinflammatory drugs (NSAID) are frequently administered to *Macaca fascicularis* in research settings. Currently, there are no published studies pertaining to the administration of meloxicam to *M. fascicularis*. At this time, the veterinary team at our institution administers meloxicam oral suspension to *M. fascicularis* for analgesia and antiinflammatory properties using current published canine dosages and frequency. The veterinary clinical observations of meloxicam administered to *M. fascicularis* have shown clinical therapeutic efficacy, which includes decreased edema and erythema of surgical incisions and areas affected by trauma. This study evaluated palatability, stability, and bioavailability of meloxicam administered to *M. fascicularis* intravenously and orally. Meloxicam was dosed orally alone as well as mixed within a highly palatable commercial product, raspberry nonwetting naturally flavored gel. The latter is a commercial product available as a hydration supplement. Results showed that both meloxicam for injection and oral suspension were 100% bioavailable in *M. fascicularis*, administered in a single oral or intravenous dose of 0.2 mg/kg. Further investigation showed that oral administration at 0.1 mg/kg of the commercial meloxicam oral suspension combined with raspberry flavored gel was also nearly 100% bioavailable. Maximum plasma concentration after oral administration is 3 h. In conclusion, meloxicam is appropriate for clinical use in *M. fascicularis*. Results showed that this analgesic is 100 % bioavailable when administered intravenously and orally at several dosages. Moreover, meloxicam was shown to be highly bioavailable whether or not the oral suspension was combined within the flavored gel, the latter greatly improving palatability. The clinical significance of our findings, combined with our previous anecdotal conclusions of therapeutic efficacy, suggests a new therapeutic modality for relief of pain in *M. fascicularis* used in biomedical research. Combining meloxicam within a flavored gel for oral administration provides an extremely palatable analgesic formulation for *M. fascicularis*. Further investigation is warranted to evaluate the frequency of administration, duration of effect, and safety with repeated dosing.

PS38 Periocular Abnormalities in an SIV-Infected Rhesus Macaque

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An adult, male, SIV-infected rhesus macaque presented with mild swelling of the right eyelids. Initial examination revealed mild, unilateral palpebral swelling without erythema, discharge, tearing, or ocular abnormalities. After several days, the abnormality progressed to severe palpebral and conjunctival swelling and erythema with no abnormalities of the globe. Initial treatment consisted of eye flush with saline, cephalexin, and buprenorphine. Progression to severe thickening of the conjunctival and eyelid tissue occurred despite treatment. Additional treatments included subconjunctival administration of dexamethasone and penicillin, topical antibiotic and steroid, enrofloxacin, and meloxicam. Progression of the conjunctival and eyelid swelling continued with discoloration of the skin over the eyelids. Two weeks after initial presentation, examination revealed proptosis, positive forced-duction test, loss of retinal reflex, blurring of the margins of the optic disc, lack of venous or arterial congestion, and choroidal folds. Ocular ultrasonography revealed a noncystic retrobulbar mass with distortion of the globe. Differentials included neoplasia, particularly lymphoma, or infection. Enucleation with removal of periocular structures was performed. A friable mass was identified inferior temporal to the globe. Additionally, a wedge biopsy was taken of an enlarged right salivary gland. Four days after enucleation, palpebral and conjunctival swelling and erythema were noted in the left eye. Swelling and erythema progressed. Ultrasound of the left eye revealed a retrobulbar mass. Imaging of the thoracic and abdominal cavity revealed splenomegaly, renomegaly with dilation of the left renal pelvis, and an abnormal soft tissue opacity in the cranial abdomen. Necropsy findings included a mediastinal mass, irregular left kidney with a mass at the caudal pole and dilated medullary cavity, splenomegaly, hepatic mass, and multiple enlarged mesenteric lymph nodes. Histopathology revealed multisystemic lymphoblastic lymphoma of B cell origin without intraocular involvement. This case represents an unusual initial presentation of malignant B cell lymphoma, which occurs commonly in late stage SIV in rhesus macaques.

PS39 Tracheal Adenocarcinoma in an Olive Baboon (*Papio anubis*)

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An adult female baboon (*Papio anubis*) presented for progressive difficulty in endotracheal intubation. Over a 7-y period prior to presentation, she was anesthetized 67 times for use in single-photon emission computed tomography studies, all of which required intubation. Laryngoscopic examination revealed tracheal stenosis. Due to the increased anesthetic risk and lack of alternative use, she was euthanized and a partial necropsy performed, focusing on the larynx, trachea, and associated structures. Gross examination revealed rigidity and functional fusion of the proximal 5 to 6 tracheal rings and narrowing of the lumen. Histology of this region revealed ossification of tracheal rings and fibrosis of overlying tissue. In addition, a 2-cm diameter transmural umbilicated mass was present midway down the cervical trachea on its dorsolateral aspect. Histology of the tracheal mass identified a relatively well-circumscribed transmural adenocarcinoma. The combination of overall histologic pattern, evidence of anaplasia and immunohistochemical staining was consistent with a diagnosis

of adenoid cystic carcinoma. Anterior tracheal stenosis is a reported consequence of repeated intubation in humans and animals. Primary tracheal neoplasms are rare in domestic animals, and, to the authors' knowledge, have not previously been reported in nonhuman primates.

PS40 Improved Clinical Oversight with Development of a Custom Database Application

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Effective management of clinical cases is an important component of a responsible laboratory animal program. Clinical case management involves documentation, reporting, correct assessment, consistent treatment, and regular case observation with appropriate follow up. Due to the significant growth of our animal program in recent years, we recognized that improvements were necessary in our paper-based documentation processes to enhance animal health surveillance. Although several commercially available electronic animal health management options existed, we chose to develop our own application. This strategy was more cost-effective and offered a customized approach to meet the needs of our program. Additionally, we designed this application to interface with our animal protocol management system. When a new health concern is discovered, veterinary personnel enter animal information and select applicable health concern(s) and predetermined treatment regimens to ensure consistent veterinary care throughout all facilities. Researcher information is automatically retrieved from the animal protocol management system by entering the animals' cage card number, minimizing the time required to log a new case and make appropriate communications. Emails are automatically generated to notify research personnel of animal health concerns, treatment options and health status modifications. Since recipients of correspondence generated by the system are based on animal protocol number, research personnel now recognize the importance of keeping protocol information up to date. Other features include generation of custom reports, staff scheduling, room entry order, and service charges. Although implementation of this electronic application required training and was a significant change in the clinical case management process, veterinary personnel readily accepted this application as an improvement and observed time saving effects immediately. Implementation of an electronic clinical case management application has also improved communication and permits consistent veterinary care with enhanced clinical oversight and trend reporting, all of which are critical elements of an effective animal care program.

PS41 Lean Concepts for Cage Processing Operations: A Tool to Improve Overall Efficiency and Accountability

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Cage changing is a regular activity that constitutes a full-time workload for most of the animal care staff in a vivarium. A regular supply of required clean cages for all technicians is critical for efficient and smooth operations. Yet, several facilities experience issues with irregular supply of clean cages leading to disrupted workflow and, eventually, chaotic operations, loss of accountability, and reduced employee morale. Lean concepts were applied by identifying the inventory, bottle necks, and value added operations at cage processing and supply. They aided our facility to not only streamline the process with efficiency but also to improve the overall accountability. In implementing lean concepts, 1) dirty cages to be processed were considered as inventory; 2) the equipment, like tunnel washer, rack washer and sterilizers/autoclaves as bottle necks; 3) and clean cages that are to be supplied as the final product. From that perspective: A) the inventory to be processed (for example, dirty cages left over from yesterday) was questioned, which helped identify and correct the disconnect in staff arrival timings; B) cages were processed only to meet the demand, which required identification of the exact required number for each technician per day during the week; C) supplying a predetermined number of cages for each technician on a daily basis and visual monitoring of the number of cages returned to dirty side with the time of return, enabled the efficiency to be bench-marked at 50 cage changes per hour. Such benchmarking facilitated improvement of efficiency and accountability, resulting in the availability of 2 to 3 h per day from each technician for additional projects/functions. With streamlined operations, fair and transparent work assignments, and a uniform benchmark, improved morale was observed from all employees resulting in reduced workman compensation cases and complaints at human resources department. Thus, applying lean concepts to cage processing and supply can be a tool that could streamline the daily operations and improve overall efficiency and morale of all employees in an animal facility.

PS42 Use of a Full-Time 3Rs Scientist/Alternatives Coordinator to Promote Refinement, Reduction, and Replacement in a Drug Discovery and Development Paradigm

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Animal researchers have an ethical obligation to adopt alternatives whenever possible but have little time to pursue validation studies. Scientific validation of alternatives is essential for adoption by the scientific community. To fill this unmet need and strengthen our institutional commitment to alternatives, we created a full-time position dedicated to 3Rs research and coordination. The primary responsibility of the 3Rs Scientist is to address ethical issues related to animal research by designing, executing, and coordinating the scientific studies necessary to validate alternatives. This helps to support decision making regarding pain and distress alleviation and 3Rs adoption and implementation. Recent and current projects include the assessment of rodent postoperative pain, the assessment of the effects of analgesics on rabbit polyclonal antibody production, the assessment of microsampling to reduce the number of mice used in pharmacokinetic experiments and to allow for collection of plasma concentrations in rodent efficacy testing, and the development and implementation of a training program for rodent use techniques to improve technician proficiency and heighten awareness of potential animal welfare issues. The 3Rs Scientist also leads a number of working groups to optimize collaboration across various research teams and to track forward progress. In addition, the 3Rs Scientist serves as vice-chair of our Global Alternatives Committee; serves as mentor to our CARE program, a group dedicated to caring for animals in the research environment; and serves as a member of the Enrichment Committee. We are one of just 2 companies in the US to create a full-time position dedicated to the 3Rs. This is a unique and effective way to increase the probability of successful implementation of the 3Rs as well as improve communication and sensitivity surrounding animal welfare issues in an industry setting.

PS43 Water Quality Control for a Stand Alone Recirculation Rack with Reverse Osmosis Intake

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Water in nature is rarely pure in the "distilled" sense, meaning that it has no dissolved minerals. Water appropriate to sustain biologic conditions contains essential dissolved salts, buffers, nutrients, and minerals. To create an environment for aquatic species to thrive in an enclosed aquatic system, one must establish and maintain specific water quality parameters found in nature. This is dependent on careful management of environmental needs of the target species; mismanagement of which may lead to illness and death. Periodic testing of water values using strips, reagent titration solutions and handheld probes measures the efficiency of the aquatic system. Efficiency being a simple ratio of energy spent (input) adjusting water parameters compared with amount of tanks with animals being housed in the system (output). Routine testing is dependent on deviation of the range of the set parameters. Ammonia, nitrite, and nitrate indicate if the biologic filter is working efficiently to eliminate the toxic ammonia generated from waste products. Alkalinity, measured by general hardness, carbonate hardness, and the pH range indicating buffering capacity against carbonic acid generated from dissolved CO_2. Correct osmotic standards are verified by testing salinity. Testing for chloramine ensures that the incoming water supply is free of chlorine via carbon filtration. Fluctuations can be successfully adjusted manually by scheduled water changes and/or the addition of salts such as NaCl and $NaHCO_3$. Routine inspection of the UV sterilization is necessary for proper pathogen control. Algae growth is controlled by mechanical filtration and colored (green or blue) tank lids that reduce the red spectrum of light. These methods have been successful in a 60-gal fresh water stand alone recirculating system housing amphibians such as *Xenopus laevis* tadpoles and adults and *Abystoma* species in 44 tanks. Manual testing has proven successful and automated system probes have not been needed with the exception of ambient temperature controls.

PS44 Developing and Teaching an Academic Course in Rodent Biomethodology

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Biomedical researchers often enter their careers with little to no experience using laboratory animals. Since our department offers a number of academic courses and rodent technique workshops targeted toward research staff and scholars, it was a natural extension to create a research methodology course based on those workshops for undergraduate and graduate students. Our goal was to create a curriculum that would produce students with appropriate technical skills using laboratory rodents that would qualify them for entry level biomedical research jobs or provide a solid foundation for graduate research programs. We organized the course to include a progression of topics in lecture and laboratory formats, so that there was a gradual accumulation of increasingly challenging but common skills including handling, dosing, sampling, and surgical technique. We also included demonstrations of sophisticated procedures such as stereotaxic surgery and exposure to special technologies such as imaging modalities. Lectures and specific reading assignments preceded laboratories so that students were prepared in advance. The syllabus was adjusted throughout the quarter to accommodate student abilities and interests, or immediate needs, if already working in a laboratory. At the end of the quarter, we assessed the students with a summary hands-on session and a take-home final examination. We met our course goal successfully based upon students' performances, knowledge, and feedback. We will share our experiences and challenges in developing and teaching this curriculum, as well as the perspectives of our students.

PS45 Training and Development: A Collaborative Approach

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The development of an effective training program is often limited by such factors as financial resources, time, and institutional support. Most training programs are mandated through the IACUC but the logistics of how training is provided, who delivers the training, and how the training is documented can be diverse. Many institutions provide training through their animal care or veterinary care networks; a few institutions have a centralized training core with dedicated trainers who specialize in husbandry, regulatory, or clinical training responsibilities. There are also institutions that do not have the internal resources to provide training and may look to an outside contract group or use the AALAS Learning Library to meet their organizational training needs. Regardless of the approach, how institutions "ensure that all personnel involved with the care and use of animals must be adequately educated, trained, and/or qualified in basic laboratory animal science to help ensure high-quality science and animal wellbeing" is the challenge. The result of a 1) training needs analysis, 2) census of organizational goals with the objective to ensure regulatory compliance, and 3) by evaluating the cost-effectiveness of employing either a centralized or decentralized training program allowed this institution to employ a collaborative, decentralized collegiate approach. Through such an approach, the institution has been able to realign their training program to increase efficiency, identify and use training resources, and improve communication between the IACUC and research community. By working with both the animal care and research groups intimately, surveying and responding to their needs accordingly, formulating voluntary focus groups, encouraging subcommittee participation, and increasing the availability of training coordinators tasked to facilitate both required and recommended training events; regulatory compliance has steadily been improving the institutional animal care and use program.

PS46 Biocontainment Training and Access Process for Animal Care and Research Staff

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In recent years there has been an increase in regulations and oversight for all animal users in containment. The necessity to meet these requirements, maintain institutional compliance, and along with the increase in new biocontainment users has prompted our institution to further expand its biosafety training program. Training is biosafety-level specific, agent and species specific, job specific, and must provide a method of assessing understanding of the provided information. This type of training program provides the necessary training documents required by the institution and regulatory agencies. The program consists of multiple theoretical courses, practicum training, and assessment phases for all in vivo and in vitro researchers and support staff. Upon successful completion of biosafety level 3 and 4 courses, a trainee is required to complete a mentorship period with qualified personnel before independent access to the facility can be granted to the individual, this is a very critical phase of any training program. Access to containment laboratories is a long and time-consuming process that is often overlooked by supervisors. Access should be progressive and not rushed; an issue for facilities building up their staff numbers and for researchers responding to grant obligations. The success of our animal biosafety training program is due to the collaboration between the biosafety, animal care staff, facility directors, principal investigators and by the continuing support provided by institutional management. Our training program including the courses offered continues to grow and evolve based on the needs of our biocontainment users and regulatory changes.

PS47 Prevalence of Murine *Helicobacter* spp. Infection Is Reduced by Restocking Research Colonies with *Helicobacter*-Free Mice

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Naturally acquired persistent, murine *Helicobacter* spp. infections, while generally subclinical in immunocompetent mice, pose a significant risk to murine health and experimental variables in immunodeficient and select inbred strains of mice. According to recent global surveys, the majority of academic and private research mouse colonies harbor multiple *Helicobacter* spp. Decreasing the prevalence of murine *Helicobacter* spp. infections remains a significant hurdle for many institutions. We surveyed the *Helicobacter* infection status of surveillance mice housed in 6 facilities using genus level and species-specific PCR. Several *Helicobacter* spp. were detected in feces of surveillance mice after less than 3 wk of dirty bedding exposure. Our current prevalence data was compared to a similar survey conducted in the same buildings a decade ago. We determined that facilities housing static breeding colonies that did not receive a regular influx of *Helicobacter*-free mice from approved vendors experienced an increase in prevalence of *H. bilis*. In mouse colonies housed in rooms with the lowest percentage of animals restocked from clean vendors, an increase of multiple *Helicobacter* spp. infections was noted. In contrast, in facilities producing embryo transfer (ET) rederived animals and receiving a higher percentage of *Helicobacter*-free mice from approved vendors, a complete eradication of all *Helicobacter* spp. was noted in 44% of the rooms and a significant reduction of *Helicobacter* spp. present was noted in an additional 11% of rooms. This represents the first long-term study demonstrating that using importation of *Helicobacter*-free mice and ET of mice from nonapproved sources resulted in a significant decrease in the prevalence of *Helicobacter* spp. infection.

PS48 Novel Technique for Cardiac Blood Collection in Rodents

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The correct administration of a drug, cell line, gene, or a collection of blood from the left ventricle of the heart can be essential for the success of the experiment. In order to improve the accuracy of intracardiac phlebotomy in rodents, we developed a device to amplify and record the sounds of the heart in order to allow more precise localization of the heart. The device consists of a hybrid ministethoscope made from a unidirectional high-sensitivity microphone connected to an amplifier, a recording device, and headphones. To evaluate the effectiveness of the device we compared the success of terminal cardiac blood collection in anesthetized mice and rats with (n = 5 mice, n = 5 rats) and without (n = 5 mice, n = 5 rats) the use of the amplification device. With the use of the device the phlebotomist was able to accurately locate and draw blood from the heart with the first insertion of the needle 90% of the time (5/5 rats, 4/5 mice). Without the use of the device the success of drawing blood at primary insertion was 30 % (rats 2/5, mice 1/5). In addition to accuracy, the technical time per injection was decreased in average by19.6 s per procedure in mice and 6 s for rats. The device can also be used connected to a computer to chart and record the intensity of heart sounds for evaluation and analysis. This new technique provides an efficient way to improve a valuable experimental procedure

with commonly available technology. It is also compliant with the 3Rs guidance principle for conducting scientific experiment using animals humanely by minimizing the time of exposure and the accuracy of technique. Future studies will include assessment of the device in improving the success intracardiac injections.

PS49 Transmission of Mouse Parvovirus by Fomites

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Because mouse parvovirus (MPV) is highly stable in the environment, fomites have been postulated to be a source of endemic infections. The goal of these studies was to determine the risk of transmitting MPV infection by caging/equipment and common husbandry practices. To determine whether MPV can be transmitted during cage changes in a biologic safety cabinet without the use of disinfectants, 14 cages of 4-wk-old female Swiss Webster (SW) mice inoculated orally with 300 ID50 of MPV-1d (4 mice per cage) and 14 cages of naïve SW mice (2 mice per cage) were placed on the rack such that infected cages were adjacent to uninfected cages. At 1, 2, and 4 wk post inoculation (wpi), cages were changed across each row and resulted in a cage change order of infected, uninfected, infected, uninfected, and others. All naïve mice housed adjacent to infected mice were seronegative after all cage changes. To determine the risk of environmental contamination, nestlets affixed to the bottom of shoe covers were used to sample the floor at 1, 2, 4 and 6 wpi during cage changing and were then placed in cages with naïve SW mice to determine if animal room floor contamination could serve as a fomite. None of the mice exposed to floor nestlets became MPV seropositive. To determine whether components of cages housing 4 MPV-infected mice (1 wpi) have sufficient MPV on them to transmit MPV, SW mice were exposed to soiled bedding alone, used cages with all cage components but without soiled bedding, used lixits, used food, used cage bottoms, used wirebars and filtertops, used nestlets or used igloos. With the exception of the lixits, all (14/14) mice exposed to the other cage components were MPV seropositive at 3 wk post exposure. Only 3 of 14 mice exposed to used lixits became MPV seropositive. An additional 14 cages that had housed 4 MPV-inoculated mice for 1 wk were washed, but not autoclaved. Naïve SW mice were housed in the washed cages for 3 wk. None of the sentinel mice housed in the washed cages became MPV seropositive. In conclusion, all cage components can serve as fomites, with the automatic watering system being the least risky, and cage washing alone was sufficient to remove or inactivate MPV on cage components.

PS50 Comparison of the Contact Plates Test with the ATP Test for Evaluating Sanitation Efficacy in the GLP-Level Vivarium

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Industrial pharmaceutical testing companies must follow Good Laboratory Practices (GLP) as part of the requirement to attain FDA approval for introducing new drugs to the market. Any tests used by these companies must be validated for GLP compliance prior to incorporation as standard operating procedures. For over 40 y, the contact plates test has been the "gold standard" for evaluating sanitation efficacy in both GLP- and nonGLP-compliant animal facilities. Although this test is sensitive and reliable, it has some significant limitations: A) results can take from 2 to 7 d, and B) cost. Samples sent to a commercial laboratory for analysis currently average about US$34 to US$38 per test. Even though the cost can be halved by conducting the analysis inhouse, this amount is still high. The adenosine-triphosphate (ATP) test offers a more cost-effective method for evaluating sanitation without loss in accuracy, reliability, or reproducibility. A significant improvement over the contact plates test is that the results are immediate. The ATP test also differs from the contact plates test in its method of detection. The contact plates test detects the presence of live bacteria via colony forming units (CFU) on a culture plate. The ATP test detects the presence of ATP, a ubiquitous component in most organic matter including live or dead bacterial cells. ATP detection occurs through measurement of a light emitting chemical reaction between ATP and luciferin/luciferase reported in relative light units (RLU). The sanitation efficacy experiments described in this report were designed to A) validate the ATP test for a GLP-compliant vivarium, and B) statistically compare the performance of the ATP test with the contact plates test. The findings show that the ATP test has GLP validity, is as reliable and reproducible as the contact plates test, and that it is significantly more cost-effective and sensitive (because it can detect the presence of organic matter even on sterile surfaces). For these reasons, it is recommended that the ATP test be considered as an addition or replacement for the contact plates test for sanitation efficacy testing in both GLP and nonGLP-compliant facilities.

PS51 Behavioral and Physiologic Testing to Determine Shipping Acclimation Time in Sprague–Dawley Rats

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Female Sprague–Dawley rats were tested to determine if daily handling for 1 min accelerates or retards acclimation after a 2-d ground transport. Acclimation was assessed by body weight, blood glucose and corticosterone levels, and exploratory behavior up to 7 d after arrival. Rats lost 8.5% of their body weight during shipment (24.4 ± 32.4 g, $n = 56$) but regained it by day 2 following receipt. Handling had no effect on body weight. Glucose levels did not differ between handled (H) and not-handled (NH) groups or over time (range 91 to 134 mg/dL). Corticosterone averaged 709 ± 325 ng/mL upon arrival and did not differ between H and NH rats at any time point. A significant decrease in corticosterone was observed over time ($P < 0.001$) averaging 380.5 ± 183.7 ng/mL at day 7. Exploratory behavior tests consisting of an open-field arena and elevated Y maze were used to characterize animals along the proactive (exploratory)/reactive (cautious) behavioral syndrome axis to evaluate whether reactive animals adapt at a decreased rate to transportation, housing conditions, or experimental procedures. In an open-field maze test, handled rats traveled further distance on day 7 (52.2 ± 15.5 m H, 39.8 ± 6 m NH), and made more contacts with a novel object on day 7 (12.4 ± 7.4 H, 6.3 ± 6 NH) than NH rats. All animals in the H group entered the open arm of an elevated Y maze by day 4 ($n = 8$) compared to only half of the NH group ($n = 8$). On arrival, 12.5% of rats were characterized as proactive, and by day 4, 100% of the H and 50% of the NH rats were proactive. Daily handling accelerated behavioral acclimation and may have shifted intermediate rats to a proactive behavioral syndrome. Body weight and entries out to the open arm of the elevated Y maze were useful indicators of acclimation, whereas blood glucose was not. These results support a minimum 48-h acclimation period for physiologic acclimation but a longer period for behavioral acclimation.

PS52 Quality Assurance Testing of Autoclaved Rodent Drinking Water

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This water quality study was performed to improve operational efficiency of procedures for immune-compromised rodents at our institution. Previously, we discarded sealed autoclaved water after 7 d and used for no more than 2 d once the bag was opened. This study was designed to evaluate the shelf-life of the autoclaved bottles to determine if the interval of use could be expanded. We selected minimum standards that autoclaved acidified water must remain negative for adenosine triphosphate (ATP) with a pH below 3.0. To verify achievement of these standards, samples were tested using ATP chemiluminescence, a pH/conductivty meter, and autoclave bioindicator strips. Four bottles of autoclaved acidified water and corresponding sipper tubes from an unopened autoclaved crate were tested every 7 d for 4 wk in the first experiment (exp 1) with a total of 4 crates (16 water samples and 16 sipper tubes) tested. In experiment 2 (exp 2), 2 bagged crates of autoclaved acidified water were opened and "in-use" under simulated conditions. From these crates, we tested 2 bottles per crate (4 per testing day) every other day for 19 d (a total of 40 bottles and 40 sipper tubes). Throughout the testing period the water in both experiments maintained a pH of less than 3, and ATP measurements for the water and sipper tubes yielded zero relative light units (RLU). One sipper tube at day 11 post autoclaving in exp 2 tested positive for ATP (16,000 RLU); however, this finding was not significant when compared to the other 55 sipper tubes in both experiments. This study supports that, under normal environmental conditions at our institution, autoclaved acidified water remains suitable for use as drinking water for at least 4 wk post autoclaving. This information can be used to assist with operational efficiency and water quality determinations.

PS53 Automatic Watering: The Importance of Monitoring

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Sphingomonas pauclimobilis, formerly *Pseudomonas paucimobilis*, is a gram-negative bacillus that thrives in both soil and water. This organism has been isolated from human hospital water systems, distilled water, respiratory equipment, and others, and has been reported as a cause of infection for both immune competent and immune comprised individuals. During routine environmental monitoring of our institution's watering systems, *S. pauclimobilis* was cultured from the manifold lines of several individual ventilated caging (IVC) racks. An automatic watering system supplied by a reverse-osmosis (RO) water system serves the IVC racks of this rodent vivarium. These findings led to an increased evaluation of the entire RO water production and distribution system. Water samples were collected from multiple locations on the system. Approximately 2 wk after discovery in the water system, *S. pauclimobilis* was isolated from a peritoneal abscess of a B6.Cg-*Slc11a1*^{r} *Rag1*^{tm1Mom}/Cwi mouse housed in the same vivarium. Oropharyngeal cultures from related mice were obtained, and *S. pauclimobilis* was grown from 2 of 9 samples. Water samples were also collected at various time points throughout the day from the specific IVC rack housing these mice to determine if there was a difference in colony counts before and after the twice daily, automated flushes of the system. While determining the extent of the RO system's contamination, plans were developed to sanitize the entire system via hyper-chlorination. Water samples were obtained prior to and after sanitation. In addition, a schedule was implemented to remove and sanitize all racks and recoil hoses throughout the facility. Investigation into this situation revealed that routine rack and RO system sanitization was not being carried out, leading to biofilm accumulation within the automatic watering system. Rodents drinking from this system were likely exposed to fragments of biofilm on a random, but consistent basis during both daily and weekly rack and system flushes. Such exposure puts rodents at risk for oropharyngeal colonization and subsequent bacteremia, especially those strains that are immune compromised. This presentation will discuss the intricacies of the husbandry-related events associated with this case.

PS54 Implementation of an Enrichment Rack for Singly Housed Male Nonhuman Primates

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It is widely accepted that pair- or group-housed nonhuman primates (NHP), exhibit a lower incidence of atypical behavior as compared to singly housed NHP. In spite of this demonstrated benefit, single housing may be necessary as a result of study design or an individual NHP's lack of tolerance to conspecific housing. We hypothesized that a novel, complex enrichment device we recently designed would reduce atypical behavior in a group of singly housed NHP in our facility. The enrichment racks are made using 1-in. stainless steel square tubing to create 4 ft × 1 in. × 74 in. The base plate of the rack is 1-in.^{2} tubing and is 18 in. wide with 3-in. casters. The racks provide 4 different levels of horizontal bars where manipulanda can be hung. Novelty manipulanda are strategically placed to maximize accessibility and to encourage interactive play between animals housed above and below each other. The manipulanda consists of easily sanitized materials that can withstand industrial rack washer temperatures, avoiding the need to individually sanitize each item manually. The racks are positioned in front of a 4-caged housing system inhabited by 4 singly housed males. Stainless steel chain and padlocks are used to secure the device to the housing system. We currently use 9 racks and provide each caging system in the room with their own enrichment racks. Single housed males in our facility are offered this type of enrichment 1 to 2 times a week for a minimum of 6 h. At the time the racks were initially offered increased discovery and conspecific interactive play was observed. NHP interaction was often exhibited for the duration the enrichment rack was offered. Staff reported positive anticipatory behavior when the racks were given at subsequent periods. The animals appeared to have become more desensitized to nonroutine noises, colors, and shapes. The enrichment racks promoted increased interaction with the animal care staff. This resulted in a positive effect on both the animals and the staff. Finally, the implementation of the enrichment racks have produced an increase in species-specific behaviors, while decreasing unwanted atypical behaviors in singly housed males.

PS55 Impact on Breeding Performances of Mice Housed in Individually Ventilated Cages at 50 Air Changes per Hour

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The 50 air changes per hour (ACH) setting of individually ventilated cages (IVC) in combination with a "bedding-only" change procedure every 2 wk and over a period of 26 wk has been previously shown to affect negatively the production efficiency index (PEI) in C57BL/6 trios when the air is injected from the rear bottom of the cage. This reduction in the PEI is not detectable when the number of air changes per hour is set a 75 and the air is injected from the rear top of the cage. To determine whether the impact on breeding performances in C57BL/6 trios is due to the lower number of air changes per hour, the point of injection or a combination of the 2 conditions we compared the performances of 2 IVC systems. The first is preset at 50 ACH and with the air injected in the cage from the rear bottom, the second normally operating at 75 ACH and with the air injected from the rear top of the cage, was set at 50 ACH in order to work in a comparable quantitative condition in terms of air changes the main difference being the point of air injection. In both systems the PEI was reduced and comparable with our previous findings, indicating that 50 ACH are not suitable when cage cleaning is accomplished only by changing the bedding. In addition, the number of litters cannibalized in the system with the air injected into the cage from the rear bottom was 35% higher and the body weight at weaning of both males and females was significantly lower ($P < 0.0001$). These findings strongly suggest that there is a lack of flexibility when IVC are set at 50ACH. While there appear to be no great differences between the 2 systems in terms of pups weaned per female per week, the impact on body weight at weaning and increased cannibalism of pups in the system where air is injected at animal level certainly require further investigation.

PS56 Variations in the Microenvironment of Individually Ventilated Caging Systems

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Individually ventilated caging systems have become widely used to assist in the support of a superior microenvironment while improving operational efficiencies in the vivarium. However, factors such as noise, vibration, and intracage air movement inherent in IVC systems may cause detrimental and undesired confounders to research. In this study, a multiinstitutional evaluation of IVC systems was implemented to assess animal welfare using physiologic evaluations. C57BL/6NCrl breeder mice were pair-housed after random assignment to one of 3 treatment groups: static microisolation (no vibration/noise or air; "S"), on IVC but not-attached to air (noise/vibration, but no air; "NA"), or attached to IVC (vibration/noise, and air; "A") and allowed to breed for 4 mo. These treatment groups were then replicated with one group at the top of the rack ("T") and another at the bottom of the rack ("B"). Intracage environmental values, reproductive data and some physiologic data were recorded. Here, we present the findings from the intracage environmental analyses. Between the treatment groups, there was no difference in intracage temperature; however, humidity was lower in A as compared to the NA and S ($P < 0.0001$). As expected, light was increased in cages that were located at the top of the racks compared to those located at the bottom of the racks ($P < 0.0001$). Noise was not different between treatments, but was significantly increased at the top of the caging systems ($P = 0.0012$), closer to where the motors for the IVC units were attached. IVC racks showed more powerful vibration on the cage floor than static racks at frequencies <100Hz, while static racks showed more powerful vibration above 100Hz ($P < 0.0001$). Thus, the intracage environment varies depending upon caging system and cage location, and IVC racks may be contributing to unrecognized variability within research mouse populations.

PS57 Inspiring Youth to Pursue Education and Careers in Laboratory Animal Science: Fresh Ideas for Public Outreach

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Have you noticed elementary, junior high, and high school students have very little, if any, concept of laboratory animal science and veterinary care for lab animals? They hear many falsehoods about how terribly lab animals are treated and the negative aspects of animal research, but what do they hear from us? Over the past 5 y our laboratory animal veterinary team has led discussions at several area elementary

schools, talked with various 4-H and FFA groups and given hands-on demonstrations to junior high and high school students regarding lab animal care. We incorporate the AALAS DVD "Accept the Challenge to Care" for older students. For younger groups we use many of the AALAS Kids for Research activities. For all age groups we may use live guinea pigs, young rats, dogs, kittens, chickens, and/or young pigs to demonstrate handling and restraint techniques in these species. We talk about the animals as valuable models for studying many human and animal diseases. Children get to handle and cuddle the animals, listen to their heartbeats and even administer oral preparations. As part of an annual summer camp, we let students build creative mazes out of cardboard and tape for rodent races. At our institution we have several groups of wild animals on campus, from pygmy rabbits to grizzly bears, involved in studies of wildlife diseases and conservation efforts. Students of all ages tour the facilities and hear about various projects from wildlife investigators. They also listen to animal husbandry and veterinary groups discuss care of captive wildlife. Students come away with new interest and a desire to learn more about the field of laboratory animal science.

PS58 Strategies to Achieve an Optimal Housing Program for Nonhuman Primates

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Allowing for social interaction in nonhuman primates can prevent behavioral problems and reduce boredom and stress by catering to the animal's innate social needs. The capacity to express more complex natural behaviors (for example, grooming, foraging, and exercise) is a measure of welfare. Our goal was to address the social and physical needs of nonhuman primates as an integral part of their everyday life without interfering with the ongoing research. The priority was to increase the social interactions through pair or group housing. We faced significant challenges: predominance of sexually mature males in our colony, frequent transfers of animals to behavior testing rooms (daily) or for imaging procedures, numerous individuals on water control for behavioral testing, and surgical interventions requiring individual postoperative care. We will show how we addressed those challenges with a strategy based on a structured environmental enrichment program that included the following key-elements: an innovative housing infrastructure, an increased complexity of the housing environment, the behavioral training by positive reinforcement, the use of castration or vasectomy for increasing social housing capabilities of males, and the use of substrates to allow expression of normal behaviors (for example, foraging). We will also discuss how those strategies were implemented with the involvement of researchers and students, to ensure their buy-in for success.

PS59 Renovation Realities in the Animal Facility: Planning for Success

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As technology changes and facilities become outdated many animal care and use programs are being faced with the challenges surrounding facility renovation. These renovations can be anything from minor touchups and simple updates to major structural overhauls. Often this requires closing of facilities, relocation of animal colonies, decommissioning of cage wash equipment, and a substantial shift in the facility's workflow. The strategies employed to deal with facility renovation will vary greatly depending on the type of facility being renovated, as well as the logistics surrounding the adjustment of day to day operations. This presentation will demonstrate the case study of a vivarium designated for capital improvement and will cover the solutions to challenges encountered in the planning process. No matter what the size of the renovation, there are some key points to consider and techniques to use, to ensure that all stages of the renovation progress as smoothly as possible. Planning, communication, logistics, creativity, scheduling, and flexibility all have their place in the process and often involve different departments and individuals. We will highlight each stage of the planning process, and discuss the challenges faced during any type of renovation. Following these steps can ensure that the facility in question is able to continue to provide excellent customer service, as well as attend to animal welfare and the overall staff and facility needs.

PS60 A Centralized Colony Management Program Facilitates Research Integrity and Improves Animal Welfare

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Communication between research labs and a centralized animal management program can be challenging in a large educational program with a constant influx of new researchers. Rodent breeding colony management can be especially overwhelming to both new and seasoned animal researchers. The most common problems faced in colony management include coordination of weaning and genotyping, overcrowded cages, and unintentional production of surplus animals. These unfortunate issues adversely impact animal welfare, and reduce efficiency of research programs and also animal facilities. Nearly 10 y ago, the Division of Comparative Medicine (DCM) at our institution initiated a centralized rodent colony management program to overcome these difficulties. This program consists of part- or full-time DCM employees that can be hired by research labs on a fee-for-service basis to provide assistance with rodent colony care. DCM colony managers are fully trained in breeding paradigms, weaning, collection of tissue for genotyping, and other technical procedures, and are also familiar with institutional policies. In addition to providing timely routine breeding management, the colony manager also acts as a direct line of communication between DCM and the research lab. They provide trouble-shooting and consulting assistance on an as needed basis. In all of these ways, the colony managers become valued members of the research teams. Since the colony management program has been implemented the number of labs participating has grown significantly. Overwhelmingly positive feedback from researchers and DCM staff attests to the great benefits of this program. The improved communications and more efficient breeding and record keeping result in improved animal welfare and more accurate and reproducible research results.

PS61 The Rodent Refresher: A New Approach to Continuing Education

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Optimizing communication and continuing education with investigators in a large multifaceted rodent program has proven to be both a challenge and a thrill. Based upon years of experience in a diverse academic research program (including more than 30,000 cages of mice and nearly 700 cages of rats), and given the high investigator turnover typical of a large academic institution, we have found unique opportunities for creativity in outreach to the research community. Over the years, our training programs have evolved to include ongoing annual "Rodent Refresher" sessions to help everyone stay current with modern expectations. The presentation, taking the form of a multisubject slide show, includes multiple speakers from diverse parts of the program and is rebuilt every calendar year in an effort to always keep the material fresh and engaging. Furthermore, the presentation—while covering important and serious topics such as euthanasia, rodent colony management, and survival surgery, among many others—is infused with humor and eye-catching graphics, as we strive to elevate potentially dry and tedious material to something more captivating and entertaining. We have moved toward requiring attendance at one Rodent Refresher session per calendar year, with failure to do so resulting in the loss of one's access to the animal facility. The approximately 1-h long sessions are offered multiple times a year, and we make every effort to accommodate everyone (by way of scheduling sessions on various days and times of the week, and providing ample makeup sessions). Initial implementation of the program was something of a challenge, due either to investigator resistance or lack of communication, but we have found that researchers have quickly accepted the importance of these sessions, and compliance has rapidly approached 100%. Feedback has been vastly positive: for example, "the best rodent training I have ever attended!" The rewards extend beyond our own animal care program to the broader scientific community, as graduate students and postdoctoral fellows move on to leadership roles in animal model development in the future.

PS62 Acute Subcutaneous Emphysema in an Adult White Carneau Pigeon (*Columba livia*)

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A 10-y-old male, singly housed white Carneau pigeon (*Columba livia*) presented with abnormal posture. No experimental procedures had yet been performed, as this incident was reported within a 30-d quarantine period. Physical examination revealed mild subcutaneous emphysema in the ventral neck region but was otherwise unremarkable. Using a 20-gauge needle and syringe, approximately 90 mL of air was aspirated from the area. Three days later, an additional 100 mL of air was removed. Four days following initial presentation, the subcutaneous emphysema recurred and increased respiratory effort was observed but improved as a total of 485 mL of air was aspirated throughout the day. Radiographs confirmed severe subcutaneous emphysema with no evidence of air sacculitis, lung abnormalities, or the presence of a foreign body. A pressure wrap was placed around the neck and upper thoracic region, and the bird was fed a commercial hand-feeding formula multiple times per day to compensate for decreased crop expansion. The pressure wrap was continued with temporary removal every other day for assessment and air removal if indicated. Six days after initial placement, the wrap was removed and the subcutaneous emphysema had resolved completely within 24 h. As of 7 mo after the incident, there has been no recurrence or clinical respiratory concern with this bird. The subcutaneous emphysema was likely due to an acute air sac or tracheal rupture that slowly developed during normal free cage movement over several days. However, the underlying etiology is still not known. This case represents a unique presentation of spontaneous subcutaneous emphysema in an adult bird that was successfully managed conservatively and without surgical intervention leading to a complete recovery.

PS63 Postsurgical Pericardial Effusion in 9 Swine: Presentation, Diagnosis, and Treatment

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From January 2010 to May 2011, we had 9 cases of pericardial effusion in domestic pigs. The pigs had a history of recent cardiac surgery, with exposure of the heart after incising the pericardium. The surgical procedures were as follows: open-heart on cardiac bypass lateral thoracotomy with placement of aortic prosthesis (3 animals), off-pump ministernotomy with apical placement of aortic prosthesis (one animal), off-pump ministernotomy with placement of a mitral device (4 animals), and off-pump ministernotomy with myocardial suturing but no implant (one animal). Pigs presented with lethargy, inappetance, cool, dark colored extremities, as well as tachycardia with normal respiratory rate, normal to low temperature and muffled cardiac sounds. Ultrasound showed a small, contracted heart, surrounded by hypoechoic fluid containing floating material. Necropsy revealed a firm, globoid, hemorrhagic fluid filled pericardium, surrounding a small, fibrin covered myocardial surface. Intracardiac findings were unremarkable in most instances. Cytology of the sampled fluid revealed high red cell counts, with moderate leukocytes and no intracellular bacteria. Culture results in four instances were positive for *Staphylococcus*. After reviewing the literature, we found that these cases closely resembled human cases of staphylococcal pericarditis, in which humans presented with pericardial effusion and cardiac tamponade. Following the human model for treatment, we initiated treatment in the 2 most recent animals, and under anesthesia percutaneously placed a 7F pigtail catheter in the pericardial space and drained approximately 1 L of fluid. The pericardial space was flushed and instilled with cefazolin and the catheter sutured in place. After recovery from anesthesia, the pericardium was drained and flushed for 3 d before catheter removal. We have not seen a recurrence of effusion in the animals receiving this treatment. Prevention of future cases is focused on device sterility and cleanliness of test equipment, as well as impeccable surgical technique. No similar diagnosis for swine has been found in the literature, making these the first documented cases of postsurgical pericardial effusion/staph pericarditis in pigs.

PS64 Efficient Endpoint Monitoring of Hepatitis Virus-Infected Hepatocellular Carcinoma-Positive Woodchucks (*Marmota monax*) Using a Handheld Glucometer

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The hepatitis infected (WHV+) eastern woodchuck (*Marmota monax*) is the only large animal model of spontaneous hepatocellular carcinoma (HCC) in the context of chronic hepatitis infection, making it ideally suited for translational studies of novel antineoplastic therapies. Management of woodchucks with HCC during experimental studies can present a challenge because they may develop altered mentation, lethargy, paresis, paralysis or seizures related to hepatic encephalopathy and/or hypoglycemia as sequelae of HCC. Rapid assessment of blood glucose levels can help to differentiate woodchucks with hypoglycemia, which may be treatable in the short term, from woodchucks with hepatic encephalopathy which usually requires euthanasia. Anesthesia, which is contraindicated in woodchucks with emergent neurologic signs, is usually needed for blood collection in this species. We implemented the use of a portable handheld glucometer and tail-prick blood collection method to quickly assess blood glucose levels in neurologic woodchucks without anesthesia. We used this method to routinely monitor blood glucose levels in woodchucks without clinical signs and to quickly assess woodchucks with emergent neurologic signs. To date, 11 WHV+, HCC+ woodchucks have been followed with regular blood glucose monitoring. Of these, 9 completed their experimental protocol with no clinical signs, 2 failed to complete their experimental protocol due to nonneurologic complications (sepsis and gastroenteritis), and one developed paresis that was rapidly diagnosed as being associated with hypoglycemia and was successfully managed with intravenous dextrose allowing the experiment to be completed. Rapid assessment of blood glucose levels in HCC+ woodchucks with emergent neurologic signs using a portable handheld glucometer can identify woodchucks with paraneoplastic hypoglycemia, which may be treated short term with intravenous dextrose to allow completion of critical experimental procedures.

PS65 Characterization of *Mycobacteria marinum*-Related Mortality in a Colony of *Xenopus laevis* and Effective Management Techniques

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A research colony of *Xenopus laevis* that previously maintained a low steady rate (1% to 3%) of *Mycobacteria*-related deaths experienced a significantly increased mortality rate (up to 9%) in 2008. *Mycobacteria*-related deaths were diagnosed via postmortem cytology and acid-fast staining of coelomic fluid and spleen. Occasionally, histopathology with acid-fast staining was also performed. Further identification as *Mycobacteria marium* was confirmed by a PCR restriction enzyme analysis assay. Initially, numerous management changes to tank water, sand, and filters were implemented and stocking density was decreased, which immediately initiated a steady drop in *Mycobacteria*-related deaths back down to 1% to 3%. Within 5 mo of these management changes, this rate again began to climb up to 6%. A monthly cleaning regiment was implemented at this time which involved comprehensive scrubbing of all system components using a highly concentrated sea salt water solution (1 cup/gal). The aggressive cleaning has effectively controlled *Mycobacteria*-related deaths at a low constant rate for the last year. This report characterizes a significant increase in *Xenopus laevis* mortality related to *Mycobacteria marium* infection in a research colony and illustrates effective management techniques employed to control the outbreak.

PS66 Cost-Effective Solutions to Improve Surgical Technique and Asepsis during Rodent Surgery

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Rodent surgical models are an integral part of many biomedical research protocols. Maintaining good aseptic technique during survival surgery is essential for avoiding subclinical infections, providing humane care, and adhering to regulations. Training investigative staff on proper surgical and aseptic techniques can pose numerous challenges. For many investigative staff, these are novel concepts, so providing training on such topics is essential. However, many problems are often encountered in the practical applications of the concepts provided. Some of the most common problems arise before an incision is even made. Staff often have difficulty stabilizing, draping, monitoring, and manipulating small patients while maintaining aseptic technique. These tasks are especially difficult when only a single surgeon is available with no additional assistance for monitoring or technical support. By incorporating training on the innovative use of adhesive drapes rather than standard or no draping material, many of these issues can be alleviated. A clear adhesive drape can be used to affix both the nose cone and patient firmly to the surgical surface. This helps ensure the patient does not inadvertently become disconnected from the gas anesthesia while being manipulated, and keeps the entire body immobile while surgery is being performed. This draping method also allows for re-

flexes to be checked intraoperatively by the surgeon without disrupting the procedure or breaking sterility. By placing a standard digital thermometer under the drape where it can be seen clearly throughout the surgery, this method can also aid in patient temperature monitoring. Using an adhesive drape to wrap a patient has also proven to be an effective method for procedures that require extensive patient manipulation, such as tunneling subcutaneously for wire or catheter placement. Introducing adhesive draping techniques to rodent surgical training will provide useful cost effective methods for full patient drape coverage, assist in maintaining excellent aseptic technique, allow for more patient stability, and improve patient monitoring by allowing full visual access to patients.

PS67 Megaesophagus in PVRL3 Rats

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Megaesophagus, an abnormal enlargement of the esophagus, is rarely reported in rats. Of the 4 available reports, only one describes a case study of a Wistar descendant. Poliovirus receptor-related 3 (PVRL3) is a gene encoding for a protein important in brain morphology and functions such as memory and learning. PVRL3-Cre rats with restricted expression in the CA1 region of the hippocampus were generated by pronuclear injection in a Wistar then crossed with a Wistar rat (Crlj:WI). The offspring were imported into our institution's animal facility and crossed with Wistar rats (Crl:WI). Only males in the F1 generation were kept due to space constraints. Twenty-one of 24 males were positive for the transgene. Two transgene-positive rats presented with dyspnea, low body weights and dehydration at 3 mo of age. At necropsy the rats had prominent gross appearance of megaesophagus involving the thoracic and abdominal portions with evidence of aspirated food. Mild esophageal myofiber necrosis and degeneration, and adventitial and muscularis inflammation were noted. The number of intramural ganglion structures in the esophagus was lower compared to other parts of the gastrointestinal tract, but were consistent with esophagi of control Wistar rats. The rats were serologically negative for rat viruses and *M. pulmonis* by ELISA. Lung cultures revealed no primary infectious agents. The remaining transgenic-positive rats showed varying degrees of dyspnea, piloerection, dehydration, and porphyrin and saliva staining. Contrast radiography showed megaesophagus in all the rats with little or no clearance of contrast media into the stomach after 60 min. The transgenic-negative rats did not have clinical or radiologic signs of megaesophagus. The transgenic-positive rats were placed on a liquid diet: 11 gained weight, and survived for more than 1 mo. The other 8 were euthanized after losing weight and/or showing severe clinical signs. Megaesophagus was confirmed at necropsy. Of the original parents only one female was identified as having megaesophagus. Breeding trials are currently being conducted to elucidate the mode of inheritance as well as possible association of megaesophagus with the transgene.

PS68 Paraneoplastic Syndrome Associated with Severe Multiorgan Failure in a Lewis Rat Model of Mammary Neoplasia

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An 8-wk-old female Lewis rat was reported for lethargy and dehydration of less than 1 d in duration. Upon physical examination the rat demonstrated pallor, weakness, and was dehydrated and thin. There were multiple extensive subcutaneous and extraabdominal masses palpated bilaterally along the ventral sides of the abdomen. The masses extended cranially to the axillary and cervical regions. During the course of physical examination, breathing became labored and the rat became unresponsive. Despite no obvious signs of trauma or aspiration the condition of the animal deteriorated rapidly and euthanasia was performed on site. A full necropsy was performed. This rat was part of a study that characterizes the development of mammary tumors induced by overexpression of Her2/neu. Her2/neu expression is induced in mammary tissue, via an MMTV-LTR promoter, following doxycycline administration. Rats usually develop detectable mammary tumors following doxycycline administration of a minimum of 10 wk in duration. This rat received doxycycline in the water in accordance with the protocol for only 19 d prior to presentation. Mammary tumors were not expected to have developed at this very early experimental time point. Nevertheless, necropsy revealed an advanced intraductal mammary carcinoma, with extensive multiorgan mineralization. Severe multifocal to focally extensive mineralization of the kidneys, heart, gastrointestinal tract, and lungs was detected and a Von Kossa stain confirmed the presence of calcium deposits within these tissues. Thus, a presumptive diagnosis of hypercalcemia of malignancy was made. This paraneoplastic syndrome is rarely reported in animal models in association with tumor formation, but its presence in this young rat likely explains the unexpected clinical presentation and rapid deterioration. This case may have important implications for the development of a model to study the role of Her2/neu in hypercalcemia of malignancy in rats.

PS69 Hematonephrosis, Hemocephalus, and Telangiectasia (Peliosis) with Disseminated Intravascular Coagulation in a Spontaneous Hypertensive Rat

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A naïve, 23-wk-old female SHR rat obtained from a commercial vendor presented with icteric and pale mucus membranes, gross hematuria, and lethargy. Gross necropsy findings included a cystically dilated thin-walled left kidney containing approximately 7 mL of dark, partially digested blood (Hct 16%) with a dark brown fluid filled ipsilateral ureter and urinary bladder, and a jaundiced liver with numerous small, black, fluid-filled spaces. Serum chemistry was notable for significantly increased total, direct, and indirect bilirubin (hepatic jaundice) and hepatic enzymes (ALT, AST, and AP), as well as azotemia. Histologically, there were numerous markedly dilated and tortuous sinusoidal spaces filled with peripheral blood within the liver. There was no normal renal architecture present in the left kidney except for a capsule lined by a transitional epithelium and containing numerous dilated small blood vessels with thin walls. Anomalous small blood vessels were also present in the mesentery. Both lateral ventricles of the brain were filled with blood. Within the glomeruli of right kidney there were numerous intracapillary fibrin thrombi consistent with DIC as a result of altered blood flow within the dilated vessels. Peliosis is a vascular condition characterized by multiple randomly distributed blood-filled cavities in the viscera. The clinical manifestations of peliosis hepatis may vary from being asymptomatic to jaundice, hepatomegaly, liver failure, and hemoperitoneum. The present case demonstrates that the existence of anomalous small blood vessels in a SHR rat may lead to hematonephrosis and peliosis hepatis with severe clinical and pathologic manifestations.

PS70 Spontaneous Glossal Masses in Experimental Rodents

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Two experimental rodents at our institution presented with spontaneous glossal masses. Both animals were euthanized due to a poor prognosis for treatment and inclusion in the scientific studies. The first case was reported in a BALB/c mouse suffering from dehydration, emaciation, and lethargy with the inability to retract its tongue into the oral cavity due to a 5-mm diameter mass at the tip of the tongue. Grossly, the mass was solid, firm, and white on cut section. Histologically, it was composed of a dense population of spindle cells in a haphazard arrangement that invaded and replaced the surrounding normal tissue leading to a diagnosis of fibrosarcoma. The second glossal mass was described in an otherwise healthy high-capacity running rat on routine examination. The growth was solid, dark red, and friable grossly and composed histologically of spindle cells that often formed vascular spaces containing erythrocytes. The neoplastic cells lining these vascular spaces stained positively for CD34 by immunohistochemistry providing the diagnosis of hemangiosarcoma. No metastases were seen grossly or histologically in either case. Few spontaneous masses of the tongue are described in the literature for rodents. One hemangiosarcoma of the tongue with distal metastases has been previously described in the rat, but this is the first known report of a spontaneous glossal mass in the laboratory mouse. This report also underlines the importance of examining the oral cavity of rodents that may suffer from otherwise unexplained weight loss, dehydration, and lethargy clinically. Other differentials for glossal masses could include granuloma or abscess formation.

PS71 Use of Tramadol Compared with Buprenorphine to Refine the Postoperative Care of Surgically Prepared Endometriosis-Telemetred

Rat Models

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The surgically prepared endometriosis and BP/EMG telemetry rat models allow us to explore sensitivity to painful stimuli (vaginal distention) in conscious rats affected by endometriosis. In this model, buprenorphine had been used as analgesic, and 20% to 30% of the animals were removing their staples within 24 h following the surgery, leading to a repair surgery under general anesthesia and strongly correlated with the development of infection. The purpose of this study was to refine the postoperative analgesic regimen of this model to minimize the issue mentioned above. The analgesic effect of tramadol to alleviate postoperative pain was assessed and compared with buprenorphine in 32 female Sprague–Dawley rats undergoing the surgery (abdominal endometriosis lesion and BP/EMG implantation). Sixteen animals received tramadol 5 mg/kg SC and 16 received buprenorphine 0.02 mg/kg SC. Analgesia was administered subcutaneously twice a day on the surgery day (day 0) and on day 1 after surgery, and once a day on days 2 and 3 after surgery. Daily body weight (BW) and other clinical signs such as posture, activity, coat, and aspect of the surgical site were recorded for 6 d after surgery. Blind pain scoring was performed on day 0 and 3 d after surgery 1 h before and after the administration of analgesic and defined as counting the number of pain behavior (such as back arching, writhing, poor gait, fall/stagger, and writhe) over 5 min. Four animals (25%) of the buprenorphine-treated animals removed their staples compared with zero animals from the tramadol-treated group. Tramadol-treated rats lost less weight and regained weight more quickly after the surgery than animals receiving buprenorphine but this trend was not significant. No statistical difference or trend was noted in the pain scoring between the 2 groups. In light of this study, the postoperative analgesic regimen of the endometriosis-telemetred rat model has been modified from buprenorphine to tramadol. This change promotes a significant refinement avoiding a surgical repair due to excessive chewing of the surgical site and reducing the morbidity of this model.

PS72 A Comparison of Central and Peripheral Vein Intravenous Infusion in the Rat

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In experimental pharmacology and toxicology it is preferable to use nonstressed animals to minimize the confounding effects of stress on study results as well as to maximize animal wellbeing. For compounds requiring repeated intravenous administration, it is not always clear which dosing methodology produces the least stress. This study was designed to evaluate stress levels associated with 2 different methods of intravenous dosing in rats: tail vein infusion in restrained animals versus central vein infusion via a surgically implanted catheter. Male rats were dosed intravenously with 0.9% saline for 1 h/d during 5 consecutive days via the tail vein during restraint or via a surgically implanted catheter in the inferior vena cava. Stress-related endpoints were assessed before and after surgical catheter implantation, recovery from surgery, and intravenous dosing. The surgical catheterization procedure resulted in reductions in body weight and food consumption and elevations in serum corticosterone concentration, with return of all values to baseline 4 to 7 d after surgery. Restraint during tail vein infusion was associated with decreased food consumption, elevated heart rate and body temperature, elevated fibrinogen and corticosterone concentrations and ALT/ALP activities, as well as inflammation at the infusion site. Despite an initial period of postsurgical stress, dosing via a central catheter was associated with less dose-administration stress and was considered to be preferable for prolonged and repeated dosing. Confounding factors noted with restraint dosing, such as increases in certain liver enzymes and inflammation at the infusion site, may be a reason to also opt for a central catheter for studies of short duration.

PS73 Validation of the Hypophysectomy Procedure Using Hormone Assays

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The hypophysectomy procedure is performed on a modified stereotaxic apparatus with a transauricular approach. It is technically challenging and poses difficulty when evaluating postoperative animals for surgical success, as the current method for premortem evaluation of surgical success is indirect and may not be sensitive enough to capture all animals that are successfully surgically modified. Physiologic assessments were explored to refine screenings of animals which may reduce animal use. It was hypothesized that hypophysectomized-animals would have decreased levels of anterior (follicle-stimulating hormone (FSH), leutinizing hormone (LH), growth hormone (GH)) and posterior (oxytocin, antidiuretic hormone (ADH)) pituitary hormones levels compared to control animals. Surgery was routinely performed by 3 separate surgeons on individually-identified animals. Blood was collected from 5 control and 20 surgically modified female Sprague–Dawley animals 7 d after surgery via cardiac puncture. It was submitted for analysis of anterior and posterior hormone levels. Statistically significant decreases in FSH, GH, and ADH levels were noted in surgically modified animals compared to controls ($P < 0.05$). Postoperative clinical assessments and daily weight measurements were also collected and analyzed. Lack of pituitary was confirmed at necropsy. In summary, this study demonstrates the use of a sensitive hormone assays that may be used to evaluate surgically modified animals premortem and to reduce the number of animals required for surgery.

PS74 Assessing the Success of Cervical Dislocation as an Euthanasia Method in Mice

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Research investigators choose cervical dislocation (CD) euthanasia of mice when other methods might interfere with the aims of a research project. Others choose it as a method to ensure death after injected or inhaled euthanasia agents. In this study, 4 techniques of cervical or spinal dislocation of isoflurane-anesthetized mice were compared for time to respiratory arrest. Eighty-one anesthetized mice underwent one of 3 CD methods. Seventeen of 81 (approximately 21 %) continued to breathe and were scored as "unsuccessful." Sixty-four never took a breath after dislocation and were scored as "successful." Intentionally creating a midthoracic dislocation in anesthetized mice failed to induce immediate respiratory arrest and death in 100% of 18 mice subjected to that procedure. Sixty of the cervical or spinal dislocation animals were examined postmortem by radiography, palpation, gross dissection and computed tomography. Of the 33 animals with time to respiratory arrest of 0 (successful euthanasia), only 2 were scored by all 4 methods as having a cervical lesion. This may reflect either lack of sensitivity of the diagnostic methods, or conversely, the possibility that injury lower in the spine can result in immediate, permanent respiratory arrest. CD may lead to immediate respiratory arrest when correctly performed, but may carry an unacceptably high failure rate. Postmortem imaging appears to have too low sensitivity and specificity to serve as a quality-assurance measure of euthanasia skill.

PS75 Minimizing the Impact of Tail Biopsy in Preweanling Laboratory Mice: Inhaled Isoflurane Compared with Topical Anesthetics

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Contemporary laboratory animal guidance suggests that tail biopsy of laboratory mice for genotyping can be performed prior to 21 d of age without anesthesia; however, anecdotal evidence indicates that topical anesthetics are often used in mice aged 10 to 21 d for management of discomfort during biopsy. In this study, we sought to determine whether the administration of inhaled or topical anesthetics had a discernable impact on mice receiving a tail biopsy. We evaluated preweanling BALB/cAnNCrl mice ($n = 80$), aged 18 to 21 d, that underwent a 5-mm or sham biopsy and were treated with inhaled isoflurane or with topical anesthetics (a combination of benzocaine, aminobenzoate, and tetracaine or ethyl chloride). Control animals received no anesthetic intervention. Mice were observed at the time of biopsy and during the following hour at 10 and 60 min for behavioral changes, resulting in an acute observation score. Locomotor activity was recorded post biopsy for 120 min and postmortem histologic examination was conducted on tail sections at day 0, 1, and 7 post procedure. Mice that received a 5-mm biopsy had significantly increased acute observation scores at 10 min post biopsy and had significantly decreased locomotor activity, regardless of type of anesthetic used ($P < 0.05$). Application of ethyl chloride significantly

increased acute observation scores at 10 min post biopsy compared with mice that received isoflurane or no anesthesia ($P = 0.01$). Histologic analysis of distal tail tissue indicated that inflammatory changes remained elevated up to 7 d after the 5-mm biopsy, while inflammation in those mice that underwent the sham procedure decreased with time. In sham-biopsied mice, isoflurane resulted in the least amount of inflammatory infiltrate in the distal 5 mm of tail, while ethyl chloride resulted in significantly increased inflammation compared to all other anesthetics ($P < 0.01$). Our experimental paradigm indicated that neither inhaled isoflurane nor topical anesthetics appreciably enhanced wellbeing for mice at preweanling ages over control mice biopsied without any interventional treatment.

PS76 Using Positron Emission Tomography/Computed Tomography Imaging as a Novel Method to Study Fish

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Modern imaging procedures, such as positron emission tomography (PET), are capable of detecting areas of molecular biology detail by using radio-labeled molecular probes with specific uptake rates depending on the tissue involved. PET scanning with the 2-fluoro-2-deoxy-d-glucose (FDG) is used in oncology because tissues with high glucose uptake, such as cancers, are intensely radio-labeled. PET/computed tomography (CT) combines the 2 systems, so images acquired from both devices are combined into one superposed image. Thus, functional imaging obtained by PET can be more precisely correlated with anatomic 3D imaging obtained by CT scanning. These modern imaging techniques have not been used in fish. The objectives of this project were to determine whether these advanced imaging techniques are applicable to a variety of fish species and to demonstrate the multitude of data that can be obtained without the sacrifice of potentially valuable fish. All imaging was accomplished under anesthesia with minimal time spent out of water. Seven different fish species were imaged to cover the physiologic and anatomic differences among fish. Simple fluoroscopy demonstrated that contrast reagent injected intravenously into the caudal vasculature achieved rapid uptake in the bloodstream. CT highlighted the ease and accuracy of organ measurements and analysis, examples include: swim bladder volume and cranium size. The truly novel aspect of the project was the use of combined PET/CT scanning with FDG to assess metabolic activity of specific tissues in fish at high resolution. This technique demonstrated rapid, quantifiable glucose uptake by selective tissues, particularly the brain, kidneys, and liver. These imaging procedures are powerful new techniques that stand to make important contributions to the fields of fish health, physiology, morphometrics, and functional morphology. This project also has future applications to establish fish as a replacement animal for mammals in carcinogenesis studies.

PS77 Jacketed Telemetry: High Quality Data on Toxicology Studies

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Implantable telemetry is the gold standard for the collection of high fidelity cardiovascular data. Manual collection techniques (external ECG, cuff blood pressure) require restrained animals and offer only brief periods (0.5 to 2 min) of data collection. The use of jacketed telemetry systems on general toxicology studies offers a more sensitive method to monitor these end points on general toxicology studies. Jacketed telemetry uses a minimally invasive technique for the placement of the blood pressure telemetry device and surface electrodes for the collection of the electrocardiogram (ECG). These data can be collected from conscious unrestrained animals for periods of 24 h or more. The purpose of this study was to investigate the quality and sensitivity of the jacketed telemetry data in comparison to implantable telemetry data. Ten cynomolgus macaques were instrumented with a blood pressure telemetry device. After a brief recovery period, the animals were placed in jackets and blood pressure, heart rate, and ECG waveforms were collected continuously for up to 10 d. Preliminary assessment confirmed physiologically relevant ECG and blood pressure waveforms. Cutaneous ECG data collected via the jacketed system resulted in lower R wave amplitudes and increased noise when compared to the subcutaneous/implantable lead placement. ECG signal quality was dependent on appropriate lead placement. Blood pressure waveform data were accurate and within the normal physiologic range. There were occasions when the quality of the blood pressure signal was impacted by the position of the animal and interference from the optional respiratory bands. Heart rate and blood pressure parameters were slightly elevated in jacketed animals (140 to 170 beats per minute, mean arterial pressure of 105 to 115 mm Hg) when compared with implantable telemetry results (110 to 140 beats per minute, mean arterial pressure of 75 to 90 mm Hg), but these changes were minor when compared to results from restrained animals. In summary, these data support the use of jacketed telemetry for the collection of blood pressure and ECG on toxicology studies and is a superior option compared to manual collection methods that have been historically used.

PS78 A New Surgical Technique for the Dual Perfusion of Tissue-Isolated Human Cancer Xenografts

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We have developed a new perfusion system and surgical technique for simultaneously perfusing 2 tissue-isolated human cancer xenografts in nude rats using donor blood that preserves a continuous flow. Adult, female, athymic nude rats (Hsd:RH-*Foxn1*^{rnu}) were implanted with MCF7 steroid receptor unresponsive (SR-) human breast tumors and grown as tissue-isolated xenografts ($n = 54$), a technique developed in our laboratory nearly 30 y ago. When tumors reached an estimated weight of 5 to 6 g, animals were prepared for perfusion with human donor blood and arteriovenous measurements between 0600 and 0800 h. The surgical procedure required approximately 20 min to complete for each tumor prior to initiation of perfusion with donor blood, and tumors were perfused for a period of 60 min. At no time during the procedure was blood flow to the tumor tissues interrupted, but remained unchanged as did host animal respiration and core temperature. Results showed that tumor venous blood flow, glucose uptake, lactic acid release, O_2 uptake and CO_2 production, total fatty acid uptake and linoleic acid uptake and conversion to the mitogen 13-hydroxyoctadecadienoic acid, cAMP levels, AKT, MEK, ERK ½, GSK3β activation were all well within the normal physiologic, metabolic, and signaling parameters characteristic of individually-perfused xenografts. This new perfusion system and technique reduced procedure time by over 50%. These findings demonstrate that 2 human tumors may be perfused simultaneously in situ or ex vivo with either rodent or human blood and suggest that the system may also be adapted for use in the dual perfusion of other organs. The advantages of this dual perfusion technique manifests as a significant decrease in animal exposure time to anesthesia and surgical manipulation, and increased efficiency, thereby reducing the numbers of laboratory animals required for scientific investigations.

PS79 A Pharmacokinetic Assessment for Continuous Intravenous Tacrolimus Infusion in Yucatan Miniswine and Domestic Swine

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Domestic swine are a well-established model for cardiac transplant studies. Recently, the Yucatan miniswine has been proposed as an animal model for long-term studies due to its small size and slow growth rate. The purpose of the study was to establish the pharmacokinetic data for continuous, intravenous tacrolimus infusion in Yucatan miniswine and domestic swine. All study animals were surgically implanted with a jugular vein vascular access port and connected to a stationary infusion pump/swivel tether system. In domestic swine ($n = 8$), tacrolimus was administered by continuous, intravenous drug infusion at a dose of 0.30 mg/kg/d for periods up to 30 d. The steady-state mean tacrolimus blood concentration was 33.8 ng/mL. In Yucatan miniswine ($n = 3$), tacrolimus was administered by continuous, intravenous infusion at a dose of 0.15 mg/kg/d for 10 d. After a 3 to 4 d washout period, tacrolimus was administered by continuous, intravenous infusion at 0.30 mg/kg/d for 10 d. Steady-state tacrolimus blood levels were achieved by day 3 for both drug dosages. The Yucatan miniswine dosed at 0.15 mg/kg/d attained a mean concentration of 30.2 ng/mL; whereas animals dosed at 0.30 mg/kg/d achieved a mean concentration of 56.6 ng/mL. Tacrolimus blood concentrations were dose proportional in the Yucatan miniswine. However, at a dose of 0.30 mg/kg/d, tacrolimus blood levels in Yucatan miniswine were nearly twice the concentration observed in domestic swine. Metabolic and physiologic differences between the breeds are probable causes of the observed difference in

drug levels. It is recommended that breed differences be carefully reviewed to avoid high systemic drug levels and potentially deleterious side effects. Also, the more limited requirement for an expensive drug will provide a considerable cost savings for long-term study protocols using the miniswine as an animal model. In summary, the experiment provided invaluable tacrolimus pharmacokinetic information, and will help facilitate the design of future cardiovascular preclinical studies using Yucatan miniswine and requiring an immunosuppressive protocol.

PS80 The Choice of Bedding Substrate, Route of Blood Collection, and Method of Glucose Determination All Affect Fasting Blood Glucose Levels in C57BL/6 Mice

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This study compared 4 bedding substrates (corncob, paper, hardwood chip, and wire-bottomed cage inserts) and 2 routes of blood collection (lateral tail vein and retroorbital sinus) in 6 male C57BL/6NCrl mice. Mice were maintained with ad libitum food and water on each bedding type for 1 wk, with at least 1 wk of rest on hardwood chip bedding in between sample collection. The night before blood sampling, the bedding was changed for fresh and food removed. Mice were anesthetized with isoflurane for all blood sampling. Blood was collected from both anatomic locations on each mouse up to 5 times over a course of 4 mo. Samples collected from the retroorbital sinus were analyzed both by glucometer and by chemistry analyzer machine following collection into serum separator tubes. The blood obtained from the tail vein was analyzed for glucose by hand-held glucometer only. Depending on the bedding provided, each mouse showed variability in the blood glucose values, even when the same bedding type was used at 2 different time points during the study. Significant differences in blood glucose were noted between the 2 collection routes on each testing day when analyzed by the hand-held glucometer. Variations in glucose levels were also observed when blood obtained from the retroorbital sinus was analyzed using the hand-held glucometer compared with the machine; these values were not significant across the group, but for some individual mice it approached a 20% difference or more at any one time point. These results stress the importance not only of consistency in the choice of bedding substrate for any experiment in which fasting glucose values may be important, but also indicating this information as well as the route of blood collection and method of analysis in any published material generated from such work.

PS81 Comparative Analysis of Blood Sampling Techniques in the Rat

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Blood sampling is one of the most common procedures performed on laboratory animals as part of scientific research. As an ongoing commitment, our institution has identified a need to improve the current blood sampling method in rats from both a sample quality and an animal welfare perspective. The aim of the study was to investigate and evaluate sampling from the jugular vein as a new blood sampling technique and compare it to the alternative methods available to establish the most appropriate method. A 22-d study was conducted comparing the lateral caudal vein, the jugular vein, the sublingual vein, and the orbital sinus as sites of blood sampling whereby animals were bled on several occasions. Throughout the study the following parameters were evaluated: clinical signs, body weight, localized damage at the site of sampling was examined visually, food and water consumption, indirect ophthalmoscopy, haematology, and clinical chemistry. All blood samples were visually assessed for haemolysis and clotting. On completion of the last blood sampling all animals were subjected to a macroscopic examination. The most pertinent findings noted included localized damage of the tail, increase in food consumption, body weight, and water consumption in animals sampled from the lateral caudal vein. Animals sampled from the orbital sinus had lens opacities findings. The jugular vein route was the only in-life sampling method that produced no clotted EDTA or trisodium citrate samples in week 4, whereas the other sampling methods were all associated with the presence of clotted samples in week 4. In particular, the orbital sinus route was associated with the largest number of clotted EDTA and trisodium citrate samples. When compared to alternative methods on welfare grounds, sampling from the jugular vein using our jugular bleeding technique does not require the animals to be heated or anaesthetized. One major benefit is that blood can be taken within 1 min of the animal being dosed as a result of the manual restraining, consequently reducing the stress which could potentially affect the physiologic state of the animal and variables attributed to the blood parameters.

PS82 Floppy Lamb Syndrome: An Inherited Lower Motor Neuron Disease in Open-Faced Romney Lambs

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A naturally occurring genetic condition of a lower motor neuron (LMN) disease in a small flock of related open-faced New Zealand Romney sheep was discovered after a sire-to-half-sister mating resulted in the first case of the disorder. Subsequent matings of this sire to his daughters resulted in more affected lambs. The clinical signs were observed after the lambs were about 10 d old. Over several days the lambs deteriorated to the point that they would be unable to get up without help. The lambs continued to suckle but became more "floppy" and showed marked medial strabismus within 1 wk of becoming recumbent. In the initial case the lamb's copper and selenium status were normal, ruling out copper deficiency and white muscle disease. Further mating of the ram to his daughters has shown that some of the stillborn and weak lambs had histopathologic lesions consistent with LMN disease. In several instances, lambs that were unable to get up after birth but were assisted for 24 to 48 h became ambulatory only to develop the "floppy lamb" condition at 10 to 14 d of age. Histologically, the lesions found in the spinal cord and brain stem showed degeneration and loss of neurons in the ventral horns. Wallerian degeneration of motor neurons and atrophy of muscles was apparent in lambs euthanized at 2 to 3 wk of age. The ocular motor neuron contained spheroids and degenerating neurons. The disorder is an autosomal recessive trait. Initially extensive literature reviews suggested that the condition was similar to spinal muscular atrophy in children; however, the genetic aberration that causes the floppy condition in lambs is now know to be an unrelated missense mutation. Investigation is underway to determine if similar conditions in humans have the same missense mutation. Genetic testing is currently underway to identify carrier animals.

PS83 Current Advances in Live Cell Imaging and Proteomic Techniques Facilitate Ex Vivo Screening and Profiling of Neurotoxic Compounds

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Rapid screening and early detection methods are needed for the assessment of the risks associated with compounds that may affect the nervous system; in contrast with in vivo studies that require prolonged experimentation and large number of animals. We asked whether live cell imaging and emerging proteomic techniques, that is, matrix-assisted laser desorption ionization time-of-flight/tandem mass spectrometry, may help profile compounds with neurotoxic properties such as the neurotoxic active metabolites of ethylbenzene-derivatives. Rat primary hippocampal neurons (7 d in vitro) were treated with 0.25 mM of diacethylbenzene (DAB) isomers in HBSS imaging medium containing 0.3% DMSO (vehicle). Time-lapse recordings of moving mitochondria stained with green-fluorescent mitochondrial stain were taken every 10 min for up to 60 min. Transport events were measured using the kymograph function of an image analysis software. Both 1,2-DAB and 1,3-DAB induced early acceleration of mitochondrial transport; the average initial rate of moving mitochondria was 2.2 times higher (that is, 0.24 µm/s) for 1,2-DAB treated samples ($P = 0.02$) and 1.6 times higher (that is, 0.17 µm/s for 1,3-DAB ($P = 0.06$) relative to vehicle control. However, the rate decreased significantly over time (that is, 11 % per 10 min) only in cultures treated with 1,2-DAB ($P < 0.001$). Changes in number of moving mitochondria followed a similar pattern. In addition, it was discovered the neurotoxic properties of 1,2,4-triacethylbenzene (1,24-TAB), the putative metabolite of 1,3,4-triethylbenzene. Ex vivo proteomic profiling of rat serum immunodepleted from its abundant proteins revealed a 1,2-DAB-specific pattern of protein modifications including a reduction in the expression of neuron-specific enolase. Current technologies should allow further promotion of the 3Rs principles with a reductionist approach in the use of animals for neurotoxicity studies.

PS84 Daily Monitoring of Body Weight: Relevance to the Neurotoxicity of γ-Diketones, Active Metabolite of Neurotoxic Solvents

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Hydrocarbon solvents commonly used in industries such as n-hexane, and its aromatic solvent cousin 1,2-diacetylbenzene (1,2-DAB), cause loss in body weight and neuropathies via an unknown mechanisms. We have shown that their respective γ-diketone metabolites 2,5-HD (2,5-hexanedione) and 1,2-DAB (1,2-diacetylbenzene) induce a breakdown of brain structural proteins α2 spectrin (Spna2) through the activation of calpain-mediated proteolysis. The experiment was conducted to determine whether Spna2 mutant mice, which lack the calpain-sensitive domain of Spna2, were resistant to 1,2-DAB neurotoxicity and less affected in their body weight (BW) relative to their wildtype littermates. The study was approved by the IACUC. Mice from each genotype were treated by either control (2% acetone in 0.9% saline), $n = 3$ per genotype; or 1,2-DAB (35 mg/kg/d), $n = 7$ per genotype; intraperitoneally, once daily, 5 d/wk for 3 wk. Changes in BW were analyzed by ANOVA using a split plot design and the mixed effect models procedure of a statistical analysis software. BW (least square means ± the standard error of the mean) were 25.7 ± 0.8 and 25.1 ± 0.5 g in wildtype mice for control- and 1,2-DAB treatment, respectively, compared with 25.9 ± 0.8 and 23.8 ± 0.5 in Spna2 mutants for control and 1,2-DAB, respectively. Both genotypes equally display neuropathological changes. Time and treatment × time, but not genotype and genotype × treatment, affected BW ($P \leq 0.05$). In sum, BW of Spna2 mutants were more equally affected than wildtype mice by the toxic effect of 1,2-DAB, suggesting that 1,2-DAB toxicity, and possibly that of 2,5-HD, are not mediated solely by the calpain-mediated cleavage of structural proteins such as Spna2. Neurotoxic 1,2-DAB, like its aliphatic γ-diketone cousin 2,5-HD, leads to daily body weight loss, of which the mechanisms still need to be elucidated. This study also underscores the role of daily monitoring of body weight even in neuropathic phenotypes such as those induced by γ-diketones.

PS85 Cellular Targets of Linamarin and Cyanate: Relevance to the Pathogenesis of Cassava-Associated Motor System Degeneration

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Cassava is a staple food for more than 500 million people dwelling in the tropical and subtropical regions. Its main cyanogenic content, that is, linamarin (α-hydroxyisobutyronitrile-β-D-glucopyranoside), together with a poor dietary intake in sulfur amino acids (SAA), have been incriminated in the pathogenesis of an irreversible motor system degeneration called konzo. However, the exact mechanisms of this neurodegenerative condition have remained largely unknown. We used state-of-the-art proteomic methodologies notably 2D differential in gel electrophoresis (2D-DIGE) and matrix-assisted laser desorption ionization time-of-flight/tandem mass spectrometry (MALDI-TOF/MS-MS) to elucidate cellular targets of linamarin, or its SAA-dependent neurotoxin cyanate metabolite, in the nervous system tissues of young adult rats. Animals ($n = 2$ per treatment group) were treated with 50 to 200 mg/kg linamarin, or 200 mg/kg sodium cyanate (NaOCN), or vehicle (saline); and fed either a normal amino acid- or SAA-deficient diet for up to 2 wk. A total of 33 proteins were consistently modified across treatment groups. A functional cluster analysis of these modifications performed using a web-accessible annotation bioinformatics tool showed that targets of linamarin and NaOCN mostly include proteins involved in maintaining the physical integrity of the cytoskeleton (for example, neurofilament proteins), controlling redox/folding mechanisms (for example, peroxiredoxin 6/protein disulfide isomerase), and regulating vesicular trafficking (for example, dynamin 1) ($P < 0.02$ for every term in the annotation clusters, modified Fischer exact test). Several proteins appeared to be subjected to posttranslational modifications that have yet to be identified. This study has generated useful information for further hypothesis-driven research on the biomarkers and mechanisms of konzo.

PS86 1,2-Diacetylbenzene Induces Lou Gehrig Disease-Like Proximal Giant Neurofilamentous Axonopathy in αII-Spectrin Calpain-Caspase Resistant Mutant Mice

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1,2-diacetylbenzene (1,2-DAB) was used to probe molecular mechanisms of proximal giant neurofilamentous axonopathy (PGNA), a pathologic hallmark of Lou Gehrig disease (amyotrophic lateral sclerosis). Analysis of the spinal cord proteome of rodents treated with 1,2-DAB showed reduction in the abundance of αII-spectrin (Spna2), a structural protein that plays a key role in the maintenance of axonal integrity. Protein immunoblotting suggests that this reduction is due to the activation of calpain-mediated proteolysis. The importance of such activation relative to the Spna2-breakdown in the pathogenesis of PGNA has not been investigated. In this study, we used Spna2 mutant mice to elucidate the role of calpain-mediated cleavage of Spna2 in 1,2-DAB experimentally induced PGNA. Homozygous mutant mice lacking the calpain/caspase sensitive domain of Spna2, hypothetically resistant to 1,2-DAB, and their wildtype littermates were treated with 1,2-DAB, 35 mg/kg/d, or equivalent amount of vehicle (saline containing 2% acetone), for 3 wk. As expected, calpain-specific Spna2 breakdown products were not detected in mutant mice. Intriguingly, treatment with 1,2-DAB reduced the expression of caspase-specific Spna2-breakdown products. 1,2-DAB induced motor weakness and PGNA in elongated motor axons irrespective of the genotype. While these findings suggest that calpain-mediated cleavage of Spna2 is a downstream event in the pathogenesis of 1,2-DAB-PGNA, the impact of γ-diketone-like compounds such as 1,2-DAB on calpain- versus caspase-mediated events appears to be a striking event. Our study offers an excellent conceptual framework that can be used to elucidate the role of calpain-caspase cross-talk, including that of the global protease degradomic, in models of motor neuron degeneration.

PS87 Ancillary Care Improves Outcome in a Mouse Model of Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a devastating neurodegenerative disease that is the leading inherited cause of infant and early childhood mortality. Currently there is no effective treatment for SMA and prospective genetic testing is not routinely performed. Human patients afflicted with the most severe form of the disease are born with generalized muscle weakness and usually die from respiratory failure before two years of age. A genetically engineered mouse model of SMA recapitulates key features of the natural history of the human disease. Untreated SMA mice die by 2 wk of age necessitating therapeutics testing in litters of nursing mice. Neonatal SMA mice on a drug treatment study were noted to be outcompeted for access to nursing by their healthy littermates, raising the possibility that mortality resulted from secondary dehydration and starvation as well as muscle weakness inherent to the disease. To optimize therapeutic efficacy, a subsequent drug study was undertaken in which affected mice received supportive care, including hand feeding and subcutaneous crystalloid fluids from 8 d of age, in addition to being dosed with the therapeutic being tested. Treated mice receiving ancillary care showed a dramatic 170% improvement in survival compared to treated, but unsupported, affected mice. Mice continued to gain weight after the therapeutic was discontinued, and sustained long-term improvement in muscle strength as evidenced by their ability to ambulate and perform during behavioral tests. Some long-lived mice developed ischemic necrosis of their extremities similar to pathology seen in some severely affected human patients. Collaboration by scientific and veterinary personnel led to the development of a model of SMA therapy that more closely mirrors the clinical care of

human patients, and allows study of additional aspects of SMA disease pathogenesis furthering progress towards a cure for this devastating neurodegenerative disease.

PS88 Chronology of Behavioral Symptoms and Neuropathologic Sequellae in R6/2 Huntington Disease Transgenic Mice

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Genetic murine models play an important role in the study of human neurologic disorders by providing accurate and experimentally accessible systems to study pathogenesis and to test potential therapeutic treatments. One of the most widely employed models of Huntington disease (HD) is the R6/2 transgenic mouse. To characterize this model further, we have performed behavioral and neuropathologic analyses that provide a foundation for the use of R6/2 mice in preclinical therapeutic trials. Behavioral analyses of the R6/2 mouse reveal age-related impairments in dystonic movements, motor performance, grip strength, and body weight that progressively worsen until death. Significant neuropathologic sequellae, identified as increasing marked reductions in brain weight, are present from 30 d, whereas decreased brain volume is present from 60 d and decreased neostriatal volume and striatal neuron area, with a concomitant reduction in striatal neuron number, are present at 90 d of age. Huntington positive aggregates are present at postnatal day 1 and increase in number and size with age. Our findings suggest that the R6/2 HD model exhibits a progressive HD-like behavioral and neuropathologic phenotype that more closely corresponds to human HD than previously believed, providing further assurance that the R6/2 mouse is an appropriate model for testing potential therapies for HD.

PS89 Animal Model of Sporadic Alzheimer Disease: The Surgically Modified Samaritan FAB Rat

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The Samaritan FAB model animal is designed to chemically-induce the onset and progression of Alzheimer disease in a Long–Evans rat via the slow release of ferrous sulfate heptahydrate, L-Buthionine-(S,R)-sulfoximine, and Beta-amyloid peptide. Disease development is rapid (approximately 4 wk), which is an advantage over other models of this disease. Further, the animals are a model of sporadic Alzheimer, which represents approximately 95% of human cases. Animals demonstrate memory impairment, histologic lesions, and increased levels of hyperphosphorylated Tau protein in the cerebrospinal fluid. This animal model refines techniques used to study sporadic Alzheimer disease and replaces other models that do not represent human disease as closely.

PS90 Routine Cage Autoclaving: Revised Logistics Permanently Reduce the Cost and CO_2 Footprint

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Cage autoclaving is a time- and energy-consuming process in the daily activities of an animal facility. Small improvements in this process can provide significant benefits in terms of energy consumption, environmental impact and reducing a potential bottleneck in the routine activities of an animal facility. This presentation describes how improvements in cage autoclaving logistics in a mouse facility produced significant paybacks in energy consumption, environmental impact, and labor. The facility holds around 5000 IVC cages, which are integrally changed every 2 wk. Cages are transported as complete set-ups to the washing area where they are disassembled, emptied, and then washed in a rack washer. Assembled cages are autoclaved, complete with bedding and feed, in a large (4620 L) steam autoclave. The autoclave capacity per cycle was 160 complete cages using standard racks (supplied by the autoclave manufacturer). We calculated that steam-sterilizing a cage required approximately 1.88 kg of steam (produced by 0.13 m^3 of methane), 2.0 L of water, and 0.06 KWh of electricity. Since the facility sterilizes around 2500 cages per week, the autoclave consumed approximately 243,750 kg of steam (produced by 17,063 m^3 of methane), 260,000 L of water, and 8125 KWh of electricity and was responsible for the production of 38,513 kg of CO_2 every year. Redesigned cage autoclave trolleys were introduced with the aim of reducing energy consumption and the environmental impact of autoclave process and to increase productivity. The new logistics allowed an increase from 160 to 200 (25% increase) in the number of cages sterilized per cycle and significantly reduced the quantity of steam, water, and electricity required to steam sterilize a cage. The improved process also avoided producing 6793 kg of CO_2 as direct emission and an additional 878 kg of CO_2 for the emission associated to the electricity production getting a 7671 kg of CO_2 per year. These energy savings translated into recurring annual cost savings of around US$3311 or 66 cents per cage. We compare the economic and environmental costs of the 2 processes and demonstrate how low cost modifications to autoclaving processes can produce benefits for the staff, organization, and environment.

PS91 The Effect of Cage Enrichment on Fluctuating Asymmetry and Fecal Corticosterone of Group-Housed Laboratory Mice

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Fluctuating asymmetry (FA) represents small, random deviations from symmetry in bilaterally symmetrical anatomic characteristics, is highly correlated with the amount of stress during development, and thus, is considered as a potential welfare indicator. In the present study, female BALB/c mice were group housed (3 to 4 mice per cage) from 3 to 13 wk of age in standard or enriched cages provided with kraft paper tubes. Body weight gain of each mouse was monitored, and no significant difference between the standard and the enriched groups was found. Three recommended traits in living mice, the width of the carpal bone, the width of the joint between the third metatarsal bone and the digital bone on the hind paw, and the length of the incisor tooth at the top from the gum, were measured at 4, 7, 10, and 13 wk of age for assessing FA. Two traits based on fleshed bones, the length of mandibula bone between the dens incisive and the processus angularis, and the length of femur, were measured at the end of experiment. FA based on the data from living mice decreased over the 9 wk in enriched cages, while for the standard housed mice, FA were fluctuating or increasing. Significant lower FA values were also detected in the 2 skeleton traits of enriched mice. The levels of corticosterone in fecal samples were examined at the end of experiment, and mice in cages with paper tubes have lower levels of corticosterone when compared with those without enrichment ($t = 4.715$, $P < 0.05$). The lower corticosterone levels were consistent with the FA measurements in mice housed in enriched cages. These indicate that housing mice in group with enrichment is much more valid to decrease the environmental stress and improve welfare. Our findings also suggest that welfare can be markedly improved by simple cage enrichment using economical materials.

PS92 Modern Facility Management and the Continuity of Research Work: Balancing the Scientific Outcomes against the Engineering Function

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The Research Support Facility at our institution is a high-throughput facility committed to the production and phenotyping of over 200 new mouse lines each year to support our large-scale mutagenesis and active Faculty programs. To maintain this high level of commitment we have introduced standardization of approaches throughout the facility for all husbandry and breeding procedures to enable optimum performance. This standardization is maintained through to the large scale phenotyping and other research programs to reduce any effect variation may have on the results of the experiments. However, other factors are well known to affect breeding strategies and experimental parameters. These factors are principally those linked with the animals' environment including the disruption resulting from extraneous noise or vibrations penetrating the facility. These disruptions can often be attributed to the maintenance and engineering components of managing a facility. To ensure the smooth running of our facility a program has been introduced to educate the engineering staff with presentations and facility inductions among other strategies. This ensures the engineering and their subcontracted staff can appreciate the repercussions of delayed responses to critical alarms or carrying out unannounced work. Furthermore large and small scale works have the potential to cause unwanted disturbance such as noise and the impact can be highly damaging to the research we are facilitating. Inhouse procedures have been developed so that both researchers and engineers can have input into arranging the most appropriate time for any servicing and maintenance work to take

place. This has resulted in improved working relationships between the groups, a greater understanding of each other's roles, better control of our engineer's actions, and no noticeable effect to our ongoing work. Here we discuss the development of this process and the benefits all parties gain from a structured education and management program.

PS93 Cage Processing in Laboratory Animal Facilities

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The method of cage processing—bedding disposal, washing and autoclaving of cages and racks, refilling of the cages with bedding, transport, and storage—has a huge influence on laboratory animal facilities operation, efficiency, costs, and hygiene level. However, until now there have been almost no common performance standards agreed; no definition of proper cage processing procedures and in particular how to evaluate the performance of the washing machines. Against this background some veterinarians, users of laboratory animal facilities, hygiene experts, and suppliers of equipment (such as cages, washers, sterilizers, bedding handling systems, and chemical agents) in 2005 set up a working-group with the aim to create a guideline, describing in a booklet the main elements of proper cage processing as well as appropriate test procedures for cleaning performance. Operational aspects were to be covered as well as logistical ones; site-requirements would be shown as well as reference to frequently made errors and ways to easily avoid them. The first edition of the guidelines was published in 2006. A second release with detailed test instructions followed in 2008, and the latest edition was published in 2010, now, for the first time, also in English (see www.felasa.eu/announcements/working-group-report-on-cage-processing-published). The booklet was designed not only for the planning of new facilities, but also for the operation and the optimization of existing ones. In addition, the booklet will assist machine-producers to design their products according to a given standard and will also allow users to test machine performance onsite after installation. Our purpose is to explain the structure of the guideline, to review its key aspects, and to give the audience a chance to check what may be applicable to their own facility.

PS94 A Comparison of Manual and Automated Cage Changing Processes for Mice Housed in Individually Ventilated Cages

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The daily routine of an animal technician involves a high percentage of repetitive movements associated with the changing of cages housing laboratory animals. These movements of the upper limbs can lead to repetitive strain injury and development of detrimental muscular conditions over the length of a technician's career. Automation and robotic engineering has been used in a wide range of professions including the laboratory animal field to reduce or replace the manual involvement of staff and this technology is now being extended to the operation of cage front tasks including the changing of individually ventilated cages. New developments in automation are available which aim to transform the repetitive cage opening and lid handling operations into automatic processes and thereby provide benefits in comparison to traditional manual approaches. We compare such automation design with the established manual method of cage changing at our institution in order to evaluate if specific benefits in ergonomics, efficiency, and sterility are conferred. The outcome demonstrates that automation has clear and measurable benefits in reducing exposure to repetitive upper limb movements, improving the consistency and pace of work resulting in technicians feeling less fatigued, and improving the sterile working practices. The observations made during use of the equipment provides valuable understanding of cage changing husbandry tasks and can be used to review and refine current practices for the benefit of both animals and personnel.

PS95 The Naked Truth: Breeding Performance in Outbred and Inbred Strains of Nude Mice with and Without Nesting Material

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In laboratories, mice are housed at ambient temperatures between 20 to 24 °C, which is below their lower critical temperature of 30 °C, but comfortable for human workers. Thus, mice are thermally stressed, which can compromise many aspects of physiology from metabolism to pup growth. These effects may be exacerbated in the breeding of nude mice. We hypothesized that nesting material would allow nude mice to behaviorally thermoregulate, reducing heat loss to the environment. We predict this reduction will improve feed conversion as well as breeding performance. We housed naïve Crl:NU-*Foxn1*^{nu} and CAnN.Cg-*Foxn1*^{nu}/Crl breeding trios (2 haired females:1 nude male; 30 cages per strain) in shoebox cages at approximately 21 °C either with or without 8 g of nesting material for 6 mo within an isolator. Nest quality was scored weekly using a previously published standard scale. Feed was weighed when added and weighed back at the end of the experiment. At weekly cage changes fresh nesting treatment was provided. Reproductive observations were made 3 times a week and pups were weighed and sexed at weaning (21 to 28 d). Analyses used GLM with post hoc contrasts. Nesting material significantly increased the number of pups weaned per cage ($F_{1,55} = 12.44$; $P < 0.001$) by nearly 10 pups on average. The amount of feed needed to produce 1 g of weaned pup was almost halved when mice were provided nesting material ($F_{1,55} = 8.5$; $P = 0.005$). However, the total feed consumed by both treatments was not significantly different ($F_{1,53} = 1.58$; $P = 0.21$). The breeding index (pups weaned/female/week) was significantly higher when nesting material was provided ($F_{1,55} = 10.15$; $P = 0.002$). Thus, nests lessen the thermal impact of standardized cool temperatures on nude mice. However, the energy (using feed consumption as a proxy) conserved by nesting material is not simply freed up from heat generation but reallocated to improved breeding performance. Together these data show that good welfare is good business and good science.

PS96 Material Compatibility Testing and Installation of a Method to Decontaminate the Interior Chambers of a Transmission Electron Microscope using Chlorine Dioxide Gas

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Cryoelectron microscopy has an advantage of studying biologic samples preserved in their near native state owing to an extremely high cooling rate used for specimen preparation. While that is critical for revealing their architecture, for infectious agents being studied in electron microscope it leads to potential danger for researchers' health since loss of a specimen or its accidental warm up inside of the microscope would render the agent to become infectious and thus dangerous for the investigator. Therefore the internal chambers of the microscope have to be decontaminated prior to service or maintenance of the equipment. We developed an electron microscope decontamination method using chlorine dioxide gas, which is a safe and effective agent to prevent health hazards for research and service personnel that come in contact with the instrument. A series of tests with biologic sporicidal indicators proved that chlorine dioxide gas penetrated into all compartments of the microscope to effectively decontaminate the apparatus. At the same time the method has been proved not to be damaging to the system, allowing multiple decontamination cycles to be performed without deterioration of the microscope performance. This method was adapted and successfully used with a cryoelectron microscope in a BSL3 containment facility.

PS97 IACUC-Specified Health Endpoint Threshold Limit Monitoring in Rodents: Using Available Technologies to Facilitate, Maintain, and Oversee Compliance

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IACUC protocols specify health endpoint threshold limits, such as body weight loss, clinical score, or maximum tumor volume to ensure that research animals are maintained as humanely as possible while on study. IACUC and Animal Welfare Protocols specify both the frequency and assessment approach as to how animal health should be monitored. A common problem is that stated objectives do not necessarily translate to actual performance. The drive to improve animal welfare has spawned innovative technological solutions that save time, resources, and effort maintaining compliance while also facilitating IACUC oversight. Electronic spreadsheet macros and study management applications can greatly facilitate health endpoint monitoring compliance by facilitating the collection of individual scores, such as fur, respiration,

and ambulation scores, which contribute to the automatic determination of an animal's total body score. Compliance with the protocol-specified frequency of assessment or measurements can be monitored by automating the scheduling of health assessments or measurements. Changes from baseline can be automatically determined by calculating relative-to-initial values for health-related parameters and the change from the most recent measurement for health-related parameters. Other advantages are the ability to define IACUC-specified, facility-wide, default threshold limits for absolute values or relative values and an alert process in real-time when health-related parameters are outside of specified threshold limit range(s). Specific actions as required by the study design or animal welfare body are sent (for example, schedule more frequent monitoring, consult veterinary staff, euthanize, and/or necropsy). Threshold levels may be associated with color-coded alerts for a data point, such as yellow, orange, and red for ascending threshold limit levels, respectively. By enabling the establishment of one or more threshold limits investigators and technical staff can more readily and easily identify animals that should be administered palliative care or euthanasia. This in turn facilitates IACUC and/or regulatory compliance as well as oversight.

PS98 Effects of Extended Cage Change Interval on Levels of Intracage Ammonia and Nasal Histology

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We measured daily intracage ammonia levels and subjectively assessed the welfare of CD1 mice each week in cages that remained unchanged for 28 d. Mice were housed on a ventilated rack using disposable polyethylene terepthalate cages that contained corncob bedding. We tested the following housing groups: 1 mouse per cage (male and female groups), 3 mice per cage (male and female groups), 5 mice per cage (male and female groups), and breeder pairs with litters. At the conclusion of the study, the respiratory tracts were examined for pathology. We measured significantly elevated intracage ammonia levels in cages containing 5 males, which increased to 140 ppm on day 12 and averaged 95 ppm for the duration of the study. In contrast, cages containing 5 females averaged only 32 ppm. Breeder cages also had higher levels of ammonia, averaging 63 ppm for the duration of the study. Cages containing 3 mice had mean ammonia levels between 19 and 29 ppm for males and females, respectively. Ammonia levels in cages containing only one mouse were less than 10 ppm for the duration of the study. Inhalation of intracage ammonia concentrations that were greater than 52 ppm for at least 2 wk caused rhinitis and degenerative lesions in the nasal passages, but the lungs had no lesions. Mice with rhinitis inhaled an average of 181 ppm ammonia for 18 d, while mice with necrosis of the olfactory epithelium inhaled an average of 93 ppm for 16 d. Histology scores in mice with rhinitis, necrosis of the olfactory epithelium and degeneration of the respiratory epithelium were significantly elevated when compared to groups of mice with either no lesions or no exposure to ammonia. Using this IVC caging system, we concluded that we can reasonably extend the cage change-out interval to at least 28 d in cages housing one mouse. Cages containing 3 mice of either sex should be changed biweekly, and cages containing 5 mice or breeder pairs should be changed once per week, at a minimum.

PS99 Regulation of Laboratory Animal Care and Use: An International Perspective

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Throughout the World, countries approach regulation of animal care and use for scientific purposes in a number of different ways. Such differences include variations in regulation structure ranging from reliance entirely on statutory instruments and secondary legislation, to using a combination of this and guidance from voluntary accreditation schemes, such as AAALAC, or other advisory bodies. Other differences include the degree to which administration is centralized, with a large amount of government oversight in some cases (such as in the UK) to almost complete responsibility devolvement to the institution through a local ethics committee. Guidance may also be largely prescriptive or majority performance based with associated pros and cons to each system. So what makes good animal protection legislation? With the increase in international collaboration in scientific fields and globalization of commercial companies, are there some necessary common features? The author would like to present a personal perspective on these questions based on her experiences working within the Australian, European, and US systems. A model of animal regulation is suggested which provides some clear legal definitions and outlines statutory responsibilities, is easy and transparent for the general public to understand, is based on sound animal welfare science and in this rapidly changing discipline, is flexible enough to allow changes to become incorporated in a timely and efficient fashion. The decentralized system offers much to commend itself with local experts involved in decision-making but some central oversight is likely to provide uniformity across institutions and aids in ensuring adequate resources are provided by the institution to ensure animal health and welfare. Given that issues of law are inextricably woven into all roles within the animal research facility, nonlawyers should not be scared to take a critical look at its structure and format since we are by virtue of our roles in the best position to comment on its value and ability to be implemented.

PS100 Elimination of Animal Facility Dark-Phase Light Contamination and Its Impact on Circadian Regulation of Rodent Physiology, Human Tumor Growth, and Metabolism

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Light at night (LAN), via its ability to suppress nocturnal circadian pineal melatonin production, has been associated with an increased risk of cancer in humans and results in chronobiologic rhythm disruptions that impact the health and wellbeing of laboratory animals. During the relocation of our laboratory animal research program to new facilities we made simple improvements in facility design that included modification of door sills/seals and installation of exterior light-tight curtains. This resulted in the complete elimination of dark-phase LAN contamination and the restoration of normal circadian rhythms of metabolism, allowing us to proceed with our cancer research investigations. In conjunction with our recent GLAS-supported human breast cancer studies, we tested whether, over a 24-h day, dark-phase light contamination disrupts temporal coordination of human tumor-host metabolism and signal transduction networks. Female nude rats (170 g; $n = 6$ per group) bearing "tissue-isolated" human MCF-7 steroid receptor unresponsive (SR-) cancer xenografts were maintained on either a control 12L:12D (300 lx; 123.0 μW/cm^2; group 1) or experimental 12L:12D-0.2 lx (0.08μW/cm^2; group 2) lighting regimen (lights on 0600). When tumors reached an estimated weight of 5 to 6 g, animals were prepared for arteriovenous measurements over 6 circadian timepoints in a 24-h period (beginning at 0400). Results showed plasma melatonin levels in group 1 were high in dark phase (108.8 ± 6.5 pg/mL), and low in light phase, with group 2 animals diurnally low (1.0 ± 0.2 pg/mL). Diurnal tumor glucose uptake, lactic acid release, O_2 uptake, and CO_2 production, and cAMP levels, LA-uptake, 13-HODE release, and DNA [3H]thymidine incorporation were elevated significantly ($P < 0.001$) in light phase (group 1) and in group 2 by over 80%, 400%, 50%, 70%, 140%, 700%, 1000%, and 900%, respectively, compared to nighttime (group 1). Tumor AKT, MEK, ERK1/2 activation was elevated for groups 1 (light phase) and 2. Animal room lighting and adherence to proper lighting protocols, as outlined in the *Guide*, are essential to the health and wellbeing of laboratory animals and the nature and outcome of scientific investigations.

PS101 Development and Management of an Animal Model for the Study of Bone Repair

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Bone pathologies affect a growing number of people. Traditional therapeutic approaches make use of bone transplants or prosthesis implants but many studies on alternative and more efficient therapies have developed, particularly in diseases with significant loss of substance or in subjects with production deficit of the bone callus. Biomedical engineering has long been trying to produce new biomimetic materials (biologic or synthetic scaffold) that, associated or not with cellular therapy, are capable of stimulating bone repair. These innovative therapeutic methods need to be validated in animal models for efficacy and safety. An animal experimental model has been implemented in our Institute to study the development of the bone repair process in vivo by

using microCT scanner and dedicated quantitative analysis software. A semicircular lesion, 4 to 5 mm in diameter at the diaphysis level, with removal of the lateral cortex and medullary canal involvement was made in both femurs of a rat, with the contralateral cortex remaining intact. Scaffolds or other devices may be introduced in the lesion site aimed at intervening on the repair process. The animals are kept in single cages, and receive postoperative follow-up using microCT imaging, for a maximum observation period of 3 mo before bone segments are taken for histologic characterization. The experimental model conforms to the international guidelines and the 3Rs; although it simulates the bone pathology with loss of substance, the lesion is well tolerated and in vivo imaging allows multiple longitudinal monitoring of the entire repair process on the same animal. Finally, the information provided by microCT imaging allows indirect measurement of the mechanical bone properties, thus preventing the use of destructive mechanical trials, and strongly reducing the number of animals used.

PS102 Zebrafish as Model System for Studying the Transcription Factor/miRNA Regulative Network in Brain and Heart Development

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This presentation provides evidence of novel microRNA-dependent signaling pathways involved in heart and brain development in zebrafish. In recent years, zebrafish has emerged as a premier organism to model and analyze complex cellular interactions in vivo and genetic mechanisms of embryonic development. In our institute the zebrafish model was set up for identification and characterization of key regulators of brain and cardiac development. Our interest is focused on microRNA acting as ''rheostats'' and ''switches'' to modulate multiple facets of cardiac and neural development, function, and disease. Because of its outstanding suitability for imaging and transgenesis approaches, the developing zebrafish is set to become a leading vertebrate model for studies of brain circuitry, synaptic plasticity and behavior. The ability of zebrafish to survive during embryogenesis independent of the functional cardiovascular system is a fundamental feature that allows careful analysis of cardiac defects without the confounding context of a dying embryo. High throughput sequencing allowed miRNA enriched by brain tissues and miRNA modulated by TBX5—a TF that plays a critical role in cardiovascular development—were identified in fish embryos. The functional role of the identified miRNA was investigated using the forward and reverse genetics approach: miRNA were up- and downregulated by microinjecting miRNA mimics or antago-miRNA in 1cell-stage embryos and the phenotypes derived by miRNA disruption analyzed. In situ hybridization of miRNA and transcripts crucial for brain or cardiac development allowed to follow the consequences of miRNA dysregulation at the molecular level. Several transgenic lines with reporter genes under the control of organ/tissue-specific promoters have been used to analyze the morphology of mutant phenotype by confocal microscopy.

PS103 Communal Nesting in Mice: Strain-Dependent Metabolic and Neurobehavioral Effects

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Laboratory mice preferentially rear their young in communal nests. In this study, 129x1/SvJ, DBA/2J, and C57BL/6J mice reared under communal nesting (CN) conditions (3 dams and their litters sharing one nest) were compared with mice of the same strain raised in a single nest (SN) in body weight from birth into adulthood, adult behavior in a battery of tests and the expression of BDNF, NGF, and GR genes in the adult mouse hippocampus and prefrontal cortex (PFC). In experiment 1 of this study all pregnant dams were housed individually until after parturition to allow for synchronization of CN birth date ranges. In experiment 2, CN dams were housed in groups of 3 pregnant mice beginning prior to parturition. Averaged across all strains, CN mice were significantly heavier (22%) than SN at weaning but CN benefited DBA/2J most (31% heavier than SN) and 129/J (9%) least, perhaps related to the heavier birth weight of 129/J mice. There were no significant differences in adult male behavior as a result of juvenile rearing condition in either experimental group when tested in the elevated plus maze, holeboard, or Lashley maze. In experiment 2, male CN C57BL/6J mice were significantly more likely than SN C57BL/6J mice to remain near another mouse when tested in a social approach test. Communal nesting increased GR expression in a strain-, sex-, and region-specific manner while effects of CN on BDNF were similarly affected as well as being bidirectional in their influence. In conclusion, being reared in a communal nest resulted in higher juvenile growth rates in 3 inbred mouse strains. CN rearing did not affect adult male behavior except in strain-specific social preference contexts and had variable, strain dependent effects on the expression of specific neurotropins in the brain. Variations in periparturient housing conditions of CN dams and/or litter age ranges may also affect offspring behavior and neurophysiology.

PS104 Comparison of Anticoagulant Affects of Heparin and Low Molecular Weight Heparin in 9 Species

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The anticoagulant drug unfractionated heparin (UFH) is commonly used in the management of thromboembolism in both human and veterinary patients. Earlier studies have concluded that there is a marked difference in heparinization responses among various species; however, current dosing is based on human indication (1 mg/kg). It was hypothesized that the current dosing of these drugs may be subtherapeutic. To address this, 5 mL of whole blood was drawn from clinically healthy subjects from 9 different species: human, nonhuman primate (*Macaca mulatta*), pig (*Sus scrofa*), sheep (*Ovis aries*), dog (*Canis familiaris*), cat (*Felis catus*), horse (*Equus caballus*), rabbit (*Oryctolagus cuniculus*), and cattle (*Bos taurus*) with a total $n = 7$ from each species. Blood was drawn using a 21-gauge needle and immediately transferred to a 3.2% sodium citrate tube at a ratio of 1 part citrate to 9 parts whole blood. Blood was spun to obtain plasma. Pooled plasma was formed for each species and supplemented with UFH (concentration grade of 0.5 U/mL to 0 U/mL). A total of 4 coagulation tests were performed: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT 5 U/mL), and common pathway (a clot-based assay). In the aPTT test human plasma (243.5 s) demonstrated the strongest response to the drug while the dog (20.0 s) was the weakest. Results of the thrombin time were concentration dependent with the pig and horse demonstrating the strongest response and human having the weakest. The feline (300 s) demonstrated the strongest response in the clot-based assay with the primate (96.3 s) and sheep (93.7 s) having the weakest response. These results demonstrate that current dosing regimens may not be ideal for most domestic animals. If using the accepted dosing, careful monitoring may be indicated to assure the animal is receiving clinical levels of the drug.

PS105 The Development of Diethylnitrosamine-Induced Carcinogenesis in Mouse Models is Gender and Genetic Strain Dependent

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Hepatocellular carcinoma (HCC) is the most common liver cancer occurring mainly in men with an extraordinarily poor clinical outcome. To better understand the HCC's pathophysiology and to develop new strategies of HCC treatments, we need reliable animal models. Using chemical carcinogen diethylnitrosamine (DEN), which causes a high incidence of HCC in male mice, we induced HCC in 6 different mice lines/strains. The aim was to study whether the mouse genetic strain background, transgenic modification, immune statue, or gender affected tumor development. We studied 3 inbred strains: C3Hf/Sed, FVB/N, and C57BL/6j; 2 transgenic mice lines: VEGFP-GFP/C3H and VEGFP-GFP/FVB; and 1 immunodeficient strain: NCr/Sed nude mice, outbred with Swiss background. We used mainly males, except in C3Hf/Sed strain, where both male and female mice were used. We studied 16 to 25 mice at 6 wk old in each group. The DEN-induced group received 2 doses of DEN, a 100 mg/kg IP, initial injection and a second dose 30 d later. The control group received 0.9% sodium chloride in a same volume intraperitoneally, and at the same time points. All mice were necropsied at 10 mo after initial injection. Tumors and organs with gross lesions were submitted for histologic examination. Results show that DEN-induced HCC only in male C3Hf/Sed mice with an incidence of 81%, but no HCC in C3Hf/Sed females, nor any of the other 5 mouse lines/strains studied. Unexpectedly, DEN induced a remarkably high incidence of multiple lung alveolar-bronchiolar adenocarcinomas in FVB/N mice (100%) and in both the transgenic lines: VEGFP-GFP/C3H (94%) and VEGFP-GFP/FVB (100%). There was no lung adenocarcinoma observed

in control groups; also no HCC or lung tumor detected in nude mice. Our results show that DEN plays an important role as an initiating chemical carcinogen for HCC, but interestingly, also lung adenocarcinomas, in a clearly gender- and genetic strain-dependent manner.

PS106 The Use of Sedation Prior to Euthanasia with CO_2 Does Not Reduce Behavioral or Physiologic Markers of Pain and Stress in Mice

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CO_2 administration is the most commonly used method of euthanasia of mice in research, yet questions remain regarding whether CO_2 euthanasia is associated with pain and stress. This study aims to characterize the level of pain and stress induced in mice during CO_2 euthanasia, and to determine if premedication with acepromazine or midazolam, or anesthetic induction with isoflurane, alters these levels during CO_2 euthanasia. Female CD1 mice (8 to 11 wk old) were assigned to one of 6 euthanasia groups (n = 10 per group): (control) CO_2 only at a flow rate that displaces 20% of the cage volume per minute (V/min); premedication with acepromazine (5 mg/kg), midazolam (5 mg/kg), or saline followed by 20% V/min CO_2; induction with 5% isoflurane followed by > 100% V/min CO_2; or 100% V/min CO_2 only. Behavioral measures of stress included ultrasonic sound recordings and video recordings examined and scored (0 to 3 scale) post hoc by a blinded observer for level of agitation, dyspnea, and any indication of pain. Physiologic parameters of stress were assessed by measuring plasma adrenocorticotropic hormone and corticosterone levels immediately after euthanasia. Finally, we assessed the acute neuromolecular marker of pain and stress, c-fos, by quantitative PCR. The use of premedication with acepromazine or midazolam did not significantly alter behavioral indicators of stress but did significantly ($P < 0.05$) induce a 3- to 7-fold higher level of c-fos expression in the brain compared to 20% V/min CO_2 alone. Furthermore, the use of isoflurane induction prior to CO_2 euthanasia significantly ($P < 0.05$) increased stress in the mice based on behavioral measures and a 6-fold increase in c-fos expression. These data strongly indicate that in comparison to the other modalities analyzed in this study, 20% V/min CO_2 alone is a humane, rapid euthanasia method that is not associated with measurable pain or stress in mice.

PS107 The Effects of Environmental Enrichment Devices Used in Rodent Cages on the Time of Vaginal Opening in Immature CD1 Mice

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Environmental enrichment devices (EED) provide laboratory animals with sensory/motor stimulation and physical exercise, encourage species-specific behavior, and compensate for lack of social interaction in singly housed animals. However, EED may impact experimental outcome. To address this potential caveat, various EED were surveyed to determine their effect on the time of vaginal opening (VO) in mice. Crl:CD1 dams with standardized litters, 10 female pups born the same day, were fed phytoestrogen-free diet until the pups were weaned at postnatal day 15. All mice were randomly assigned to control or treatment groups (5 mice per cage), fed phytoestrogen-free diet, housed in polysulfone cages with paper bedding, and provided reverse osmosis/deionized water in glass bottles unless otherwise stated. Group 1 received assorted paper or maple wood nesting material; group 2 received new plastic EED; group 3 received a separate set of plastic EED that were boiled for 5 h to maximize leaching of bisphenol A (BPA) or other estrogenic chemicals. Group 3 also received the previously boiled water in glass bottles. Water samples were collected after 0, 1, 3, and 5 h of boiling and assayed for BPA via HPLC-MS/MS. Mice were monitored daily for VO and weighed at weaning, at weekly intervals and at time of VO. The concentrations of BPA (ppb) leaching into the drinking water of group 3 after 5 h of boiling were: polycarbonate mouse igloo (6.1 ± 0.2), polycarbonate rat hut (3.0 ± 0.1), PETE-BPA-free recyclable hut for mice (0.1), polyurethane bones (<0.1), and nylon bones (0.1). No significant differences in the mean time of VO between mice in the control group and in groups 1 through 3 were noted, despite some of the EED containing BPA. It was concluded that the EED evaluated do not release sufficient levels of estrogenic compounds to significantly advance the time of VO in immature CD1 mice.

PS108 The Effects of Bisphenol A on the Timing of Vaginal Opening in CD1 Mice Fed a High- or Low-Phytoestrogen Diet

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Bisphenol A (BPA) is a ubiquitous estrogenic compound found in the environment, plastic cages, plastic bottles, and food can liners. The BPA dose required in drinking water to advance the time of vaginal opening (VO) in CD1 mice is unclear. This report evaluates the effects of drinking water spiked with concentrations ranging between 5 and 2000 µg BPA/Kg BW/d on the timing of VO in mice. Standardized litters of 10 Crl:CD1 female pups, born the same day, were weaned at postnatal day (PND) 15, and randomly assigned to cages (5 mice per cage) and to groups (15 mice per group). Mice were fed either a diet high in phytoestrogens (608 µg diadzein & genistein/g diet; p-low) or a low phytoestrogen diet (<20 µg/g diet; p-high), and observed daily for VO. Body weights were recorded at weaning, weekly, and at time of VO. Urine was collected at PND 30. The levels of BPA in spiked water and phytoestrogens in the urine were determined using HPLC-MS/MS and HPLC-MS, respectively. The mean age at VO was significantly advanced ($P < 0.001$) in mice fed the p-high diet (PND 22.4 ± 0.3) compared with mice fed the p-low diet (PND 27.1±0.3). The mean levels of phytoestrogens (µg/mL): S-equol (145.2), daidzein (147.2) and genistein (43.5) in the urine were higher in mice fed the p-high diet compared with negligible levels (<2.0 µg/mL) in mice fed the p-low diet. No significant differences were observed in mean time of VO between the control and BPA exposed mice fed the p-high or the p-low diet. We concluded that the age at VO was significantly advanced in the control mice fed the p-high diet compared with the p-low diet. The doses of BPA used in this study did not significantly advance the age at VO in mice fed either diet and receiving the same doses of BPA.

PS109 Refinement of Gastrointestinal Procedures in Rat Models for Obesity and Diabetes Studies

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Rat models for diabetes and obesity studies are useful for the discovery of surgical and therapeutic interventions applicable to these human diseases. Surgical models include procedures such as Roux-en-Y gastric bypass, ileal transposition, duodenal-jejunal bypass, gastric liners, and vertical sleeve gastrectomy. We evaluated current surgical practices and identified areas of improvement of these surgical procedures in rat models of obesity and diabetes. Areas of improvement included specific pre- and postsurgical fasting periods, incision closure, specific suture materials, physiologic perioperative monitoring, anesthesia, pre- and postsurgical drugs, and ancillary perioperative enhancements. The presurgical fast period was decreased from 24 h to 2 to 4 h. The postsurgical fasting period was decreased from 24 to 72 h to a maximum of 20 h. The amount of suture material used was decreased by using smaller diameter suture and a continuous suture patterns. The number of postsurgical dehiscence was decreased from 50% to 10% by using a subcuticular closure pattern instead of surgical clips for skin closure. The average dose of isoflurane was decreased from 3.0% to 1.5% and oxygen flow from 1 to 0.5 L/min through the use of physiologic monitoring, which resulted in quicker recovery and a more stable respiratory rate. The use of a homoeothermic blanket, water heating pad, elevated ambient temperature, and warmed saline to decrease tissue dehydration normalized the rats' body temperature and contributed to faster recovery as well as a survival rate of approximately 90%.

PS110 Rhesus Macaque (*Macaca mulatta*) Pair-Housing Assessment Using a Quantitative Data Collection System

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A comprehensive behavioral assessment program has been established at our institution to monitor behavior and evaluate the treatment of behavioral problems in laboratory-housed nonhuman primates. Behavioral data are collected using a comprehensive ethogram, and data for individual animals are summarized and graphed using an automated system. While primarily used to evaluate normal and abnormal behavior in individual animals, we are currently expanding the system for use in monitoring the social interaction of animals and the assessment of ongoing pair compatibility following introductions. By integrating the pair's behavioral data with information about environmental factors such as changes in personnel, experimental treatment protocols, and enrichment applications, our system allows for visualization of the dynamic nature of relationship of pairs of monkeys. In our pilot study with 2 pairs of adult female rhesus macaques, we found that this system allowed us to identify key behavioral patterns and trends. We ran nonparametric Friedman tests to determine whether the percent duration of each behavior differed across levels of socialization (that is, single, visual access, panel access, and full pair). Affiliative noncontact behavior with the partner was highest during the visual access phase (χ^2 (3) = 8.1, P = 0.04) and there was an increase in affiliative behavior (with and without contact) once full pairing occurred (χ^2 (3) = 11.1, P = 0.01). Predictably, social behaviors involving contact were significantly higher when fully paired (χ^2 (3) = 12.0, P = 0.007). Nonsignificant initial increases in anxiety, abnormal behavior, and aggressive behavior were measured during visual access. These behaviors then declined, demonstrating briefly the challenging nature of the introduction process and the dynamics of the behaviors involved in adapting to changes in social housing.

PS111 Monitoring and Supporting Respiratory Function during Laparoscopic Procedures

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Employing a laparoscopic approach to common abdominal surgeries can reduce postsurgical pain and complications and accelerate healing and return to function in multiple species. In comparison to an open laparoscopic approach, abdominal insufflation necessary for visualization during laparoscopic procedures can further compromise respiratory and cardiovascular function. In addition, venous return is often further reduced when animals are tilted on their back to aid in visualization of abdominal organs. In an SPF colony of cats undergoing assisted reproductive techniques (transabdominal oocyte aspiration and intrauterine embryo transfers), a consistent and noticeable drop in respiratory and cardiovascular function was observed in procedures requiring prolonged abdominal insufflation on top of the respiratory depression in frequency and tidal volume observed with general anesthesia. Progressively decreasing oxygen saturation and increasing exhaled carbon dioxide concentrations were detected by pulse oximetric and capnographic monitoring after the initiation of abdominal insufflation. Cardiovascular function was also compromised as decreases in systolic and mean arterial blood pressure were observed. To support animals undergoing lengthy laparoscopic procedures, positive pressure ventilation (PPV) was instituted. Increasing both frequency and tidal volume of delivered gasses improved cardiovascular and respiratory parameters; however, the ability to effectively intervene became refractory over time. In addition, discontinuing PPV after purging of anesthetic gases and allowing patients to undergo periods of transient hypercania were required to stimulate spontaneous breathing. This transitional period required constant monitoring and varied between patients. In conclusion, having the ability to provide or proactively implement PPV is a prudent measure to support physiologic parameters when performing protracted laparoscopic procedures. Even with PPV, patients can further benefit when the length of laparoscopic procedures is minimized through refinement.

Poster Sessions

P1 Investigation into a Fire Caused by Improper Surgical Preparation and Surgical Instrumentation Use in a Protocol-Related Rodent Surgery

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An approximately 4-mo-old naïve male Sprague–Dawley rat on a protocol for rotator cuff tendon injury and repair was anesthetized with isoflurane and surgically prepped and draped for a routine rotator cuff injury surgery. During the procedure, the surgeons noticed a radiant heat emanating from the surgical drape covering the animal shortly after incising the skin over the shoulder. Upon removing the surgical draping, several areas of singed fur and an active fire were present around the muzzle of the animal and in the plastic tubing of the anesthetic circuit. The fire was extinguished promptly by mechanical means and the animal was euthanized immediately prior to recovery from anesthesia. Both the IACUC and veterinarians were notified immediately. The fire had caused extensive damage to the tubing, rebreathing bag, and other parts of the anesthetic circuit. Necropsy was performed to determine the extent of injury to the animal and tissues were submitted for histopathology. Histopathologic examination revealed findings consistent with superficial and partial thickness burns and lesions in the lungs were suggestive of smoke inhalation. Review of the incident with the lab members revealed that a copious amount of alcohol-based solution was used to prepare the surgical site and application of electrocautery for hemostasis was the initial trigger for the fire. Excessive alcohol-based solution and an oxygen rich environment led to the fire spreading and causing the damage to the anesthetic circuit. The fire was a hazard for the personnel as well, and subsequent review of procedures including surgical preparation and electrocautery use was undertaken to ensure the safety of the personnel and animals in the future.

P2 Unilateral Cholesterol Granuloma in a Male C57BL/6 Mouse in a Colony with a High Incidence of Perineal Swellings

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The incidence of perineal swellings among male C57BL/6 mice greater than 8 mo of age was anecdotally reported to exceed 43% in a breeding colony with parathyroid hormone-related protein (Pthrp) genetic mutations. Elevated incidences of bulbourethral (Cowper) gland abnormalities ranging from 6.7% to 83.8% have previously been reported in several strains of mice. At the time of clinical presentation, mice appeared clinically normal with the exception of uni- or bilateral perineal swellings. Affected males had markedly diminished reproductive performance and had not sired litters since the onset of clinical signs. Low fecundity rates have been similarly documented in CFW/R1 male mice with perineal swellings. To further characterize a suspected bulbourethral gland cyst or abscessation of a local structure and possible cause of low fertility, a mouse was submitted for pathologic evaluation. Gross examination revealed bilateral dilation of the saccular portion of the bulbourethral gland measuring 1.4 × 0.95 × 0.8 cm on the right and 0.95 × 0.8 × 0.7 cm on the left. The left sac was clear and mostly transparent while the right sac was dark red and appeared to contain whitish-tan to black luminal structures approximately 0.15 cm in diameter. Microscopically, the right contained multifocal luminal cholesterol granulomas associated with hemorrhage and hemosiderin accumulation. While cystic dilation of the bulbourethral glands is a common and reported lesion in some strains, the unilateral presence of intraluminal cholesterol granulomas and hemorrhage has not previously been reported in this species or this anatomic location to our knowledge. In the veterinary literature, case reports are limited to horses with cholesterol granulomas in fourth and/or lateral ventricles of the brain and one report of a cat with a uterine cholesterol granuloma. Human literature cites that cholesterol granulomas most often occur in the temporal bone. Cholesterol granulomas are foreign-body reactions to the presence of cholesterol crystals. In this case with prominent hemorrhage in the ipsilateral gland, the formation of cholesterol crystals is likely associated with lipid components of red blood cell membranes.

P3 Characterization of Enteropathogenic *Escherichia coli* Isolates from an Outbreak of Diarrhea in a Dutch Belted Rabbit Colony

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Colibacillosis is a common disease of weanling rabbits that produces profuse, watery diarrhea along with anorexia, dehydration, and

lethargy. *Escherichia coli* causes disease in a highly strain-dependent manner, making precise characterization an important component of successful diagnosis and treatment. In this study, culture and PCR-based tests were used in tandem to characterize the agents responsible for repeated diarrhea outbreaks in recently shipped weanling Dutch Belted rabbits. Because successful empirical treatment was accomplished using enrofloxacin at 7 to 10 mg/kg IM daily for 10 d, a bacterial etiology was suspected. Fecal samples from 20 rabbits with diarrhea were cultured for 24 h in tryptic soy broth and streaked on blood and MacConkey agar plates for aerobic and anaerobic culture and were also inoculated into thioglycolate broth for anaerobic culture. Bacteria of several genera were cultured, including *E. coli*, *Bacillus*, *Pseudomonas*, *Proteus*, *Staphylococcus*, and *Streptococcus*. Isolates were speciated based on identification test strips and colony morphology. Forty-eight *E. coli* isolates were obtained from 12 rabbits. Of these, 83% were PCR-positive for *eae*, the gene encoding the virulence factor intimin in enteropathogenic *E. coli* (EPEC). This gene mitigates close association with intestinal microvilli, causing attaching and effacing lesions, inflammation, and severe diarrhea. All isolates were PCR-negative for the genes encoding Shiga-like toxins 1 and 2. Antibiotic susceptibility disc diffusion assays showed that no isolates were sensitive to cephalothin, 10% were sensitive to ampicillin, 79% to amoxicillin-clavulanic acid, 67% to gentamicin, and 71% to trimethoprim-sulfamethoxazole. All isolates were sensitive to enrofloxacin, thus validating the empirical treatment used. This study demonstrates the utility of a combined culture- and molecular-based approach in the diagnosis and treatment of colibacillosis and further supports the importance of rabbits as an EPEC reservoir.

P4 Epizootic of Shigellosis in Rhesus Macaques

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The following is a report of an epizootic of shigellosis that occurred in a free-ranging colony of rhesus macaque (*Macaca mulatta*). Several animals developed clinical signs of lethargy and recumbency with mucoid hematochezia followed by death. Necropsy of these animals revealed similar findings of hemorrhagic contents within the large intestine with a thickened mucosa. The mucosal surface of the cecum was ulcerated and diarrhea was evident throughout the small and large intestine. Histopathology results showed a severe, diffuse, and acute to subacute fibronecrotizing colitis). Initial bacterial culture for enteric pathogens was negative. Additional fecal samples of the affected animals were submitted to 2 different laboratories and results were positive for Shigella spp. In addition, several random fecal samples from the island also yielded positive results for *Shigella* spp. The *Shigella* isolates were susceptible to either triple sulfa or trimethoprim among other antibiotics. Affected animals were trapped and treated with trimethoprim-sulfamethoxazole via gavage. In addition intravenous or subcutaneous fluid therapy was administered. A total of 42 rhesus macaques were trapped and treated. Of these animals 38 were successfully treated and released back to the colony. Four animals had to be euthanized due to lack of response to treatment. The entire colony was treated with medicated feed containing trimethoprim-sulfamethoxazole. Clinical cases responded well to treatment and no complications were evident in the colony. Serial fecal cultures from the colony have yielded no positive results at the present time. This is the first report of an epizoonosis of *Shigella* treated with medicated food in a free-range colony of rhesus macaques. This report is of significance as the infection in rhesus macaques resembles the infection seen in humans. As a potential animal model, the rhesus provides a valuable opportunity to further study the pathogenesis, treatment, and potential vaccine application of shigellosis in humans.

P5 Strategy and Rationale for Anesthetic and Analgesic Drug Selection in Canine Surgery: Always Planning Ahead

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There is much discussion in the veterinary profession as to which anesthetics, analgesics and combinations of the 2 works most effectively in research surgery. We will discuss the best veterinary practices applied in the surgery program employed by our institution. We will describe the strategy and discuss the anesthetic and analgesic agents, and the techniques used to optimize the most comfort for canine and nonhuman primate surgery models created for our Safety Pharmacology and Pharmacodynamic and Metabolism partners. Multimodal analgesia and anesthesia dose, route, and administration techniques for these agents will be presented individually for distinct areas of cardiovascular telemetry, chronic cerebrospinal fluid collection, and chronic vascular access. In conjunction with anesthetic and analgesic agents, we have also evaluated the use of adjunctive nonpharmacologic and indirect techniques that do not interfere with study protocols and augment supportive care including the size and type of enclosures, ice/cold compressing the operative site, and the use of topical steroidal and antimicrobial agents in the management of wound healing and irritation. The methods to validate the use of these agents will be documented through pre- and postoperative assessment and observation: knowledge of animal behavior, individual animal personality, clinical vital signs (heart rate, respiratory rate, blood pressure, mucous membrane refill time and color, and others), response to palpation of affected and surrounding areas of the surgery, alteration in food intake, urine and fecal output; and any reduction or change in activity/ambulation. In conclusion, we believe our best practice standards are not only meticulous in surgical technique, anesthetic, and analgesic delivery, but it is of vital importance to have a robust plan for the patient in order to optimize animal care, welfare, and surgical success.

P6 Unilateral Testicular Teratoma in a Djungarian Hamster

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An 8-wk-old, male Djungarian or Russian Dwarf, hamster (*Phodopus campbelli*) within a conventional housing setting presented with gross abdominal distention. Physical examination revealed a firm, severely distended abdomen of unknown origin, approximately 3 to 4 times the normal size. The hamster was bright, alert, and in good body condition, with no other abnormalities noted. The animal was euthanized and a thorough gross necropsy performed, followed by histopathologic analysis of abnormal tissue. Completely contained within the vaginal tunic, the right testicle was noted to be grossly enlarged (approximately 3 cm × 5 cm × 1.5 cm), irregularly shaped, and expansile (causing a mass effect within the abdomen). The tissue was diffusely pale tan-white with multifocal to coalescing cystic cavities, the largest of which contained approximately 1.5 mLof dark red, serosanguinous fluid. All other organ systems were grossly within normal limits. Histopathologic analysis of the right testicle revealed a well demarcated neoplastic mass which completely effaced the normal testicular parenchyma. The mass was composed of a multitude of tissue types, including derivatives from all 3 germ layers. Neural, epidermal, and glandular were the most prevalent tissue types, but the mass also included skeletal muscle, cartilage, hair follicles, mammary tissue, respiratory epithelium, choroid plexus, and bone marrow. The gross and histologic findings, tissues from all 3 germinal layers and lack of malignant features, are consistent with a diagnosis of teratoma of the right testicle. To our knowledge, this is the first reported case of a testicular teratoma in a Djungarian hamster.

P7 Real Time and Long-Term Analysis of the Electrocardiogram with a Nonimplantable Telemetry System in Domestic Swine

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Telemetry is increasingly being used to monitor electrocardiographic (ECG) events in pharmacological and other cardiovascular studies due to its capacity to acquire continuous, long-term physiologic data in a minimally invasive manner. The purpose of the current study was to evaluate the feasibility of using a nonimplantable system to collect and analyze ECG data obtained from juvenile domestic swine during an approximately 30-d data acquisition period. A nonimplantable telemetry system was used to collect data from greater than 26 swine models of chronic myocardial infarction. The telemetry system allowed for real time ECG data collection and analysis. Most commonly, the system was used to retrospectively analyze ECG data after an acquisition period. The system allowed for long-term data collection with minimal disruption or lost data. A proprietary ECG interval and arrhythmia analysis software package was used for data analysis. Interval analysis was performed at selected time points and included heart rate, RR, QRS, QT, and QTc durations. Arrhythmia detection included identification of extra systolic and supraventricular beats, as

well as negative deflections of QRS complexes and T waves. In addition, arrhythmias were classified quantitatively for severity according to the absolute number of arrhythmic events per monitoring interval and whether the arrhythmias occurred as isolated events or as runs (>4 consecutive beats). Although continual data collection was made over an approximately 30-d period, selected time intervals were chosen for data analysis. Using the nonimplantable telemetry system, it was possible to acquire good quality ECG data and identify changes in cardiac electrical activity during real time acquisition or retrospective analysis. The telemetry software package was useful for performing both interval and arrhythmia analyses. This nonimplantable telemetry system provided an efficient, cost effective approach to monitor the effects of novel therapies for the treatment of cardiovascular disease in domestic swine.

P8 Enhancing Successful Outcomes in the Preparation of an Ovine Myocardial Infarction Model: A Case-Control Study

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Myocardial infarction (MI) remains one of the primary causes of death in humans; therefore, large animal MI models have been increasingly used for the development of new therapies in the preclinical research setting. There is an inherent risk for morbidity/mortality associated with MI model preparation. In the current study, historical approaches and outcomes were reviewed for the treatment of dysrhythmias associated with MI model preparation in sheep. The primary study objective was directed at whether a standardized treatment regimen would increase the success rate of MI model preparation. A case-control study was conducted to analyze morbidity/mortality in 2 equal groups (n = 23 per group) of sheep with and without standardized treatment of cardiac events. The approach to MI model preparation was the same for both groups, and involved an occlusion/reperfusion model technique. During model preparation, the electrocardiogram, blood pressure, and pulse oxymetry were continuously monitored for all study animals. All research subjects exhibited arrhythmias during MI preparation. The dysrhythmia treatment approach was not standardized for the historical group. However, a standardized antiarrhythmic therapeutic regimen was administered to all research subjects in the second group. Arrhythmias detected during occlusion/reperfusion were treated with β-blockers and antiarrhythmics. If ventricular fibrillation occurred, immediate CPR and electrical cardioversion were administered, while appropriate fluid and supportive therapy were provided. In the historical treatment group, 7 of 23 animals developed lethal refractory arrhythmias. In the second group, the number was 2 of 23 animals. Additionally, one animal in each group died suddenly several hours following full recovery from the procedure. There was an approximate 3-fold increase in successful CPR following implementation of a novel, standardized treatment algorithm. The procedural refinement resulted in increased success of model preparation and reduced the number of animals required for model preparation. The implementation of a standardized drug therapy protocol enhanced procedural efficiency and proved beneficial for veterinary technical staff training.

P9 Refining a CSF Collection Model in Cynomolgus Macaques (*Macaca fascicularis*) for Biomarker Studies

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Serial collection of cerebrospinal fluid (CSF) from the cynomolgus macaque for pharmacodynamic analysis presents challenges which lend themselves to opportunities for refinement to minimize pain and distress. Multiple percutaneous punctures can be performed at either the cisterna magna or lumbo-sacral junction; however, this method necessitates the use of sedation or anesthesia and requires a high level of proficiency to consistently collect CSF samples not contaminated by blood. Chronic access catheters attached to subcutaneous ports may be used with varying degrees of success due to variable patency rates and interactions when working with the monkeys. A pool of these surgically implanted monkeys was desired, however, by our Translational Medicine group in order to develop validated biomarker models inhouse. The successful development of this model within our institution for our biomarker program required close interaction between the Comparative Medicine group and the Translational Medicine team in order to yield data that was scientifically relevant. Many challenges recognized by both groups (both technical and organizational) required remediation in order to properly design and refine the final working model. Ultimately, the key to success lied with minimizing the pain and distress associated with all aspects of the conduct of the study and, when applicable, led to serial collection of both blood and CSF from outside the monkey's cage. We will describe the evolution of the final working model, technical aspects of the surgery and study conduct, on-going study plans, and future refinements that this functional partnership is helping to create.

P10 A Nonsteroidal Antiinflammatory Drug Improves Surgical Outcome in Hypophysectomized Animals

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The hypophysectomy procedure is performed on a modified stereotaxic apparatus using the transauricular approach. This approach is technically challenging, predisposed to postoperative mortality and clinical care challenges, and poses difficulty in evaluating postoperative animals for surgical success. In an effort to improve the postoperative care, we compared the impact of NSAID therapy on hypophysectomized animals. One hundred female Sprague–Dawley rats received pentobarbital anesthesia (30 to 50 mg/kg), buprenorphine analgesia (0.05 mg/kg), and fluid therapy (30 mL/kg); 50 of these animals received a supplemental dose of 5 mg/kg carprofen subcutaneously at the time of surgery. Rats that received the supplemental NSAID had higher rates of survival (94% with carprofen; 78% without carprofen) and lower incidence of morbidity postoperatively (bleeding: 29% with carprofen, 44% without carprofen; ataxia: 6% with carprofen, 16% without carprofen; weight loss: 2% with carprofen, 22% without carprofen) than rats that did not. Although the definitive mechanism by which the carprofen acts is not definitively known, it is hypothesized that contributions from the inhibited COX-pathways, particularly COX-2, may play a role in interrupting the onset and development of bleeding, ataxia, and ultimately weight loss. These 3 sequelae may then also contribute to and affect the mortality rate. In summary, this study offers a model for justifying additional or adjunctive analgesic therapy to refine analgesic protocols for rodent surgery, improve postoperative care and animal welfare, and reduce the number of animals used.

P11 A Practical Approach to Dystocia in Mice

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Dystocia is a common problem in rodent breeding colonies and is considered a medical emergency. Standard medical management of dystocia relies on the use of pharmacological agents such as calcium gluconate (100 mg/kg) and oxytocin (1 USP unit), subcutaneously. These treatments have not yielded positive outcomes at our institution with respect to dam survival and live births. Thus, we modified our strategy to focus on clinical signs and progression of the birthing process for establishing treatment paradigms. Dams found in dystocia are categorized based on body condition: good, compromised, and moribund. Females classified as "good" have a sleek coat with normal posture and activity level. Minimizing handling, these animals are visually checked for vaginal dilation, discharge, and pups retained in the birth canal. If pups are present in the birth canal, manual removal is attempted. These dams are also provided moist, palatable food on the cage floor and thermal support. The veterinary staff rechecks animals in this category every 4 h for a 24-h period. If pups are retained the next day, we recommend euthanasia. Most dams are found in the "compromised" category. These females present with hunched posture, lethargy, and vaginal bleeding or discharge. These dams are checked as above. The abdomen is palpated and any pups in the vaginal canal are removed by gentle massage. Warmed subcutaneous fluids (1 to 3 mL, 0.9% sterile saline), palatable soft food, and thermal support are also provided. Animals are rechecked every 1 to 2 h to determine if pups are being delivered and to reassess dam health. At any point during the day the dam may become moribund or hypothermic. If this occurs, the dam is euthanized. With these new treatment paradigms dam survival increased from 25% to 65%. This 'wait and see' management approach has allowed us to minimize animal handling, decrease potential stress for the dams, and improve overall animal welfare.

P12 Perioperative Care and Maintenance of Paraplegic Spinal Cord-Injured Cats

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There are approximately 259,000 individuals with spinal cord injuries (SCI) living in the United States with 12,000 new cases reported each year. These can be devastating, life-changing injuries requiring extensive rehabilitation at great financial cost. Investigators our institution used a feline model to evaluate how noradrenergic and serotoninergic agonists, drugs which are known to facilitate the recovery of locomotion following a complete SCI, influence neuronal circuits within the adult mammalian spinal cord. The cat was the animal model of choice for this study as they have the most well known neurophysiology of all mammalian species and are used extensively in research for locomotion and SCI studies. A low thoracic surgical spinal cord injury model was used by transecting the spinal cord at the 13th thoracic spinal cord segment in 8 cats (n = 8). The spinal lesion produced complete loss of voluntary hind limb motor function, loss of voluntary micturition and loss of sensation from the hind limbs and perineal region. Without proper and timely care, these impairments can lead to severe medical complications including bladder wall injury, pressure sores, and skin irritation secondary to perineal scald. This model, therefore, required intensive postsurgical convalescent care for the 1-mo study duration to keep the animals comfortable, clean, and to minimize postsurgical complications. With the animals' welfare in mind, the veterinary staff developed general and topical anesthetic and multimodal analgesic plans in addition to an extensive convalescent care plan that together provided for effective pain management and environmental comfort. Care included daily bladder expressions, manual stool expression, grooming, and providing well padded enclosures to avoid pressure sore formation. The combination of these approaches minimized potential postoperative complications. The care plan developed was guided by Russell and Burch's 3Rs philosophy, particularly as it relates to refinement. Working with a paraplegic model of SCI can be a daunting task. Through our planning, research, and experience with this model, we hope to share our knowledge and educate our colleagues who might be contemplating a SCI study in a relatively large mammalian species.

P13 Technique for Intracolonic Administration in Rats for Pharmacokinetic Studies

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When being involved in pharmacokinetic research studies, there are times when the dosing and collection technique requested by clients are not common procedure. Often it requires team effort to suggest and evaluate a method for successful dosing and sampling techniques while minimizing stress to the animals and maintaining valid data. The problem was to evaluate if it was possible in rats, to administer drugs of various consistencies in the distal colon via the rectum and ensure proper retention of the dose administered. Following dose administration, systemic and tissue distribution sampling needed to be developed to characterize the absorption of the test articles. A second problem was time management, since the study required a fairly large number of animals that needed to be dosed at specific times to meet the clinical observations and sample collection schedule. We approached this problem by evaluating a method that can be used to administer either liquid or gel-like compound (maximum volume 3.5 mL/kg of either liquid or gel-like substance) via the rectum without causing internal injury to the animal, loss of product post administration, and prevent the animal from ingesting the product once administered. This needed to be evaluated on awake and anesthetized animals to determine the best method and meet the clinical observation time points. A pilot study protocol was submitted. This procedure is more efficient when done on lightly anesthetized animals (using medical air/isoflurane at 2% to 4% delivery rate) rather than on awake animals. Minimal to no loss of the test compound was achieved depending on dose volume administered. The procedure was a success. It was possible to have a multistation anesthesia set up with animals positioned at several stations and dosing was done within seconds of each animal by the technical team. Clinical observation was clearly defined post administration as well as plasma and colonic tissue collection schedule. The raw data collected became valuable asset for this research model.

P14 Development and Utilization of an Ultrasound Imaging Service Core for Mouse Models

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Establishing a multidisciplinary service for high-resolution ultrasound of mouse models introduces technical and logistical challenges, but results in diverse offerings of improved minimally invasive research approaches. An ultrasound system was added to the central imaging core within a mouse barrier facility in the fall of 2009. It has since provided more than 300 h of direct technical assistance to research protocols visualizing anatomic structures, measuring tumors, and assessing hemodynamic functions. Lymph node metastatic breast cancer and orthotopic pancreatic cancer models were established using ultrasound-guided injection of cancer cells, avoiding the surgical intervention and postoperative care requirements of surgical orthotopic injection models. Fluorescence and bioluminescence of injected tumor cells and histopathologic confirmation postmortem supplemented these studies. Prostate cancer progression in the TRAMP mouse model was monitored earlier and longitudinally in individual mice long before palpable tumors were detected. Tumor volumetric measurements, tumor neo-vasculature development, and metastasis detection supplemented longitudinal cancer biology studies, including a hepatic tumor xenograft mouse model. Visualization in utero of early embryonic development allowed for the precise staging and collection of embryos used in various developmental assessments. Animal use and discomfort were reduced, potential clinical complications associated with more interventional methods were avoided, and the integrity and reproducibility of data were augmented by establishing a multidisciplinary ultrasound service for mouse models.

P15 Use of Histopathology to Solve Parasitology Mystery

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Rhesus macaques (*Macaca mulatta*) were housed individually, in pairs or groups depending on the research protocol and the characteristics and needs of each animal. These macaques underwent regular health checks and diagnostic tests every 6 mo, including TB testing and fecal bacteriology and parasitology. All received a subcutaneous injection of ivermectin (0.2 mg/kg) twice a year. On several occasions, animal attendants reported seeing worms in the cages of several animals. These findings were reported to animal health technicians and attending veterinarians. The white elongated structures were 1 to 2 mm in diameter, round to flattened and measured 4 to 8 cm in length. One end was usually tapered over a few centimeters. Feces were brought to the diagnostic laboratory on several occasions for endoparasite diagnostic investigation, and were processed both by flotation and by centrifugation in zinc sulfate. Following several negative results, the white elongated structures were fixed in 10% neutral buffered formalin and sent to the histology core for processing and H&E staining. Histopathologic analysis revealed a dense coagulate of protein with interspersed mature spermatozoa. It was concluded that these structures were in fact urethral sperm casts resulting from the coagulation of sperm in the penile urethra, and apparently expelled upon urination. In hindsight, the urethral sperm casts were mostly observed in singly housed, large unneutered male rhesus macaques that had visual contact with females. In this case, histopathology was useful in resolving a parasitology mystery.

P16 Comparison of Serum Cortisol Levels in Blood Collected via Central Ear Artery and Jugular Venipuncture in New Zealand White Rabbits

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Both the central ear artery and jugular vein are common venipuncture sites in rabbits. Blood samples were collected in conscious rabbits for comparison of cortisol levels in response to the blood collection technique used, with 5 rabbits being used for each collection technique. The goal of the study was to identify which method caused the least stress as measured by elevation in blood cortisol levels. Prior to collections, rabbits were acclimated to both restraint methods used

for the ear artery and jugular vein bleeding techniques. The rabbits were placed in a restrainer for ear artery blood collection and were restrained manually for jugular blood collection. For each technique, a total of 4 samples were collected from each rabbit. Baseline blood samples were collected, representing a background cortisol level for that rabbit. After 20 min elapsed, we collected a repeat sample using the same method. The assumption was that the cortisol level in the second sample would be reflective of the stress response from the procedures used to collect the first sample. In addition, samples were collected in this manner in the early morning and late afternoon, following a 1-wk washout period, to capture any cortisol fluctuation based on time of day. Paired *t* test was used for comparing the hormone fluctuations within the same rabbits, and unpaired *t* test was used for comparing the difference between the jugular sampling and ear sampling. There were no significant differences found between mean cortisol levels for the 2 collection techniques, although the ear sampling demonstrated a greater cortisol response range (jugular samples ranged from 0 to 0.44 µg/dL cortisol response, ear samples 0 to 1.11 µg/dL). There were no differences observed based on the time of collection ($P = 0.85$ for jugular assay; $P = 0.78$ for ear assay). There was, however, a statistically significant smaller variance observed in jugular sample data compared with ear artery data (*P* value of 0.006 in an unpaired *t* test), demonstrating that fewer animals were required to obtain stable cortisol data using the jugular collection method than the ear collection method.

P17 Collaborative Effort to Promote the Physical and Social Wellbeing of an Orphaned African Green Infant through Fostering

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At 72 d of age, an infant African green monkey (*Chlorocebus pygerythrus*) became orphaned due to its dam succumbing to complications from gastric dilation. This left the technical, husbandry, behavioral, and veterinary staff with the challenge of how to raise the infant. While orphaned nonhuman primates housed in conventional caging are typically managed by placing in a nursery setting, this can lead to later behavioral abnormalities. Therefore, we sought to identify a suitable surrogate female African green monkey so this infant could be pair-housed and raised in a social environment, while simultaneously providing clinical support to ensure its continued ability to thrive independently of its dam. A feeding plan, in conjunction with a clinical observation schedule, was established and implemented. The infant adapted to eating independently and continued to progress normally, which supported the option of placing the infant with a surrogate. We found that while data on both subspecies of African green monkeys, *Chlorocebus pygerythrus* and *Chlorocebus aethiops*, is limited, many species of nonhuman primates both in their natural habitats and in field station environments within a research setting live in established matrilineal societies in which the females share in the maternal responsibilities of the society's offspring and will readily accept a cross-fostered infant if presented the option during the first few days of the postpartum period. While the aforementioned scenarios appeared most conducive to a successful cross fostering of the infant; we did not have the option of placing this orphan with a postpartum female. Alternatively, an experienced mother who had recently aborted a pregnancy was chosen from the existing African green colony as an ideal candidate for the cross-fostering process and a pair-housing plan was created. The husbandry staff modified a cage that allowed the infant and surrogate to acclimate during the fostering process. We custom fabricated a cage to meet the specific needs of this case due to the fact there were no primate breeding or weaning cages commercially manufactured and available. The cage is unique in that the custom access tunnel design allowed for the infant's unrestricted access to the surrogate as well as an escape route to his own cage if necessary. However, the tunnel was rarely used for escape by the infant, as she proved to be receptive to him by allowing him to cling to her. In conclusion, the collaborative effort and innovative ideas of our team resulted in identification of a suitable foster mother and a successful socialization of the orphaned African green infant.

P18 Viral Bronchointerstitial Pneumonia in a Litter of New Zealand White Rabbit Kits (*Oryctolagus cuniculus*)

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A 1-mo-old intact female audiogenic New Zealand white rabbit kit presented acutely for bilateral mucopurulent nasal discharge and rapid and shallow breathing. Physical exam findings included loud crackles and wheezes from all lung fields and poor body condition. At necropsy there was cranioventral lung consolidation with discrete pinpoint foci of suppuration. Histology showed a suppurative bronchopneumonia as well as a severe necrotizing and proliferative bronchointerstitial pneumonia with multinucleate epithelial syncytial cells and interstitial fibrosis present. Brown and Hopps stain showed gram-negative plump rods consistent with Bordetella *bronchiseptica*, confirmed by aerobic culture. Feulgen stain showed occasional atypical epithelial nuclei suggestive of Cowdry type A inclusions. Based on culture results from the nasal cavity, treatment of the remaining kits in the litter with enrofloxacin was initiated. One kit died spontaneously 1 mo after completion of the treatment regimen with no apparent clinical signs; a second kit underwent an additional course of enrofloxacin due to clinical signs of respiratory illness but was euthanized due to declining health. Both kits had similar gross and histologic lesions compatible with bronchointerstitial pneumonia. Fresh and paraffin-embedded lung tissue from 2 of the rabbits was negative for Sendai virus by RT-PCR. Other differential etiologies include parainfluenza virus and an uncharacterized α-herpesvirus associated with epizootic systemic disease.

P19 Cage Washer Selection: Energy Efficiency and the Environmental Footprint

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Cage washing and autoclaving processes can be both energy and water intensive. Reducing the operating and utility costs and carbon dioxide emissions of these processes are, therefore, major operational objectives. We will identify metrics that focus on reducing energy and water consumption and greenhouse gas (GHG) emissions (carbon dioxide and methane) during routine cage wash operations and new equipment selection. The introduction of these metrics into a cage/rack wash equipment selection protocol produced an annual saving of 3500 m^3 of water, 33,500 m^3 of methane and avoided the production of 76,750 kg of CO^2. Equipment selection metrics also included efficient space utilization, maximum washing efficiency (assessed by cycle time and microbiologicly validated cycles), alternative use as a decontamination chamber, and reduced operating costs.

P20 Rare Cardiac Disease Cases in 4 Cynomolgus Monkeys (*Macaca fascicularis*)

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There is almost no information about congenital cardiac disease in nonhuman primates. We identified 2 types of congenital cardiac diseases in 4 cynomolgus monkeys in regular healthy inspection. Four cynomolgus monkeys that were born in a primate research center underwent radiographic and echocardiograph assessment and atrial (ANP) and brain (BNP) natriuretic peptide levels were measured. Two monkeys had a double chambered right ventricle (DCRV; cases A and B) and another 2 had ventricular septal defect (VSD; cases C and D). Movement limitation was initiated but animals did not show abnormal clinical signs. Medical therapy was provided such as furosemide after clinical signs had appeared. However, treatment was unsuccessful in 2 DCRV cases and one VSD. Echocardiography in DCRV cases A and B revealed high-speed turbulent flows to the pulmonary artery and remarkable expansions of the right atrium. Their ANP and BNP levels indicated high values. By pathologic findings, case A was diagnosed as congenital right ventricle stenosis by abnormally muscle bundle on RVOT. Case B was acquired stenosis by intramural thrombus on RVOT. VSD case C had turbulent inflow to the right ventricle from ventricle septum membrane foramen. VSD case D was revealed ventricular septum muscles foramen by echocardiography. We will present our diagnosis, treatment, and postmortem findings of rare

cardiac disease. Echocardiography and cardiac peptide hormone test were useful for nonhuman primate cardiac disease diagnosis. These noninvasive diagnostic methods may be especially useful in the case of cardiac morphologic abnormalities in nonhuman primates.

P21 Successful Surrogate Adoption of Twin *Macaca fascicularis* on a GLP Reproductive Toxicology Study

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The cynomolgus macaque (*Macaca fascicularis*) is a well established animal model for reproductive toxicology studies. Two fetuses were detected during a routine study ultrasound on gestational day (GD) 31. Naturally occurring twins are rare in macaques. A primary measurement in reproductive studies is serial assessment of embryo-fetal measurements via ultrasound. At our facility, review of ultrasound data shows that fetal losses before GD100 are common. Embryo-fetal measurements were assigned to the appropriate twin using landmarks as follows: fetus no. 1 was located closest to the cervix and fetus no. 2 was located farthest from the cervix. Ultrasounds were performed by the same technician each time for consistency. For the health of the animals, the decision was made to deliver the twins via cesarean section at term on GD162. Following cesarean section, the infants were hand-raised for a short period. Several attempts were made to introduce the twins to the natural dam over a 2-d period, but introduction was not successful. Introductions to 3 surrogate dams were then attempted with the third dam successfully accepting the twins. The successful surrogate had a term stillbirth on the same day as the introduction of the twins, and then raised the twins until weaning. The infants exhibited normal growth patterns both in utero and after birth. These values were comparable to infants born and raised singly by the natural dam. There has been no evidence of any behavioral issues. In conclusion, proper procedures and adequate animal care can enable surrogate raising of infants in cynomolgus monkeys.

P22 Minimizing Stress in Rabbits during Manipulation

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One of the most commonly used rabbits in research today is the New Zealand white (*Oryctolagus cuniculus*). Due to their delicate nature, manipulations may induce stress causing fear, excitement, and possible devastating injury to the animal. Commercial restraints often induce unnecessary stress during placement. The veterinary technicians decided to develop a soft restrainer that allows for manipulation of the ears, feet, lumbar, and caudal region of the rabbit while minimizing stress. The restrainer is composed of 100% polyester, a material that is durable, washable, and autoclavable. As an initial trial, 8 rabbits were individually placed into the restrainer, administered an intramuscular injection, and observed. The rabbits remained calm during placement into the restrainer, at the time of administration, and immediately following the injection. This simple restrainer minimized stress in the animal and made a routine technical procedure easier for the technician. The veterinary staff has since adopted this novel restraint for inhouse use during routine manipulation such as injections, nail trims, and blood draws and with new technician training protocols.

P23 Development of a Recordkeeping System for the Assessment of Nonhuman Primate Behavior Using Commercially Available Software

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The Animal Welfare Act regulations require institutions using nonhuman primates (NHP) for biomedical research to develop and document an environmental enhancement plan for NHP to promote their psychologic wellbeing. Evaluating the effectiveness of a plan should be based on outcomes, that is, the behavior of the NHP. At the authors' institution, the behavior of each NHP is assessed on a monthly basis, and a behavioral record is initiated for those exhibiting signs of psychologic distress, such as overgrooming or stereotypical behavior. At the time this program was initiated, all health records were paper-based. Because a paper-based behavioral recordkeeping system would be cumbersome and inefficient, we established an electronic system that was quick to develop, user-friendly, searchable, portable, and allowed documentation of hair loss patterns with real-time access to historical information. A note-taking and information-management program was easily configured into a format similar to our paper-based system. Using a tablet PC, cage-side documentation can be made, with areas of alopecia precisely illustrated on an anatomic diagram using the touchscreen and stylus. Navigation and searching is simple and the software is relatively easy to learn. A built-in tagging feature is a useful tool for flagging reminders and notes. The primary disadvantage of this software is the lack of document protection, so text boxes or images can be inadvertently altered by the user. Another disadvantage is the absence of user tracking. However, at the authors' institution, these disadvantages are considered minor inconveniences that have been addressed with training, internal policies, and frequent file maintenance with data backup. In summary, the use of this system has been well-accepted, and has substantially increased the efficiency of our NHP behavioral monitoring program.

P24 Use of Pole and Collar Restraint of Cynomolgus Macaque without the Collar

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Serial collection of cerebrospinal fluid (CSF) in unsedated cynomologus macaques has been an important aspect in studying selective biomarkers in developing novel therapeutics for neurodegenerative diseases at Abbott Laboratories. These monkeys are surgically implanted with chronic access catheters in the cisterna magna and femoral vein. The CSF catheter requires repeated flushing with phosphated buffer solution within the first 2 wk of surgery to maintain patency. Since our typical method for handling and restraint is using the pole and collar, retrieval from the cage for flushing becomes a challenge since the collar is removed during this recovery period to facilitate proper healing of the 4-in. dorsal cervical incision. Chemical restraint (ketamine and midazolam) was used each time for each flush which negatively affected their appetite and resulted in undesired weight loss. A solution was required that would eliminate the need for chemical restraint, but still allow the required flushing to occur with minimal stress to the animals. Several technicians collaborated with the vendor that supplies our jackets and designed a suitable jacket to allow safe retrieval and restraint of these monkeys without the use of a collar. After testing several design prototypes, a suitable jacket design was adopted and implemented into our procedures. The jacket was created with a low dorsal neck line, a wider padded back and sturdy rings strategically placed for pole attachment. The zippers were replaced with snaps to prevent additional trauma to the skin overlying the newly implanted port. Prior to surgery, all monkeys were acclimated to both the jacket and study procedures to reduce stress during surgery recovery. As a result of reducing the amount of chemical restraint in this vital time of recovery, the monkeys have maintained a more normal appetite and have minimized the weight loss that was experienced prior to using the jacket. A quick recovery is paramount for use in a CSF collection study since CSF catheter patency duration is not predictable.

P25 Adaptation of Submandibular Blood Collection in Rats

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The laboratory rat is a commonly used species in research. It has limited acceptable locations for blood collection without chemical restraint. Due to animal welfare concerns, bleeding from the orbital sinus is discouraged as a site for blood collection. The saphenous vein may be used, but requires a restraint device and excellent technique to obtain an optimum blood sample. This poster will describe how this submandibular blood collection has been adapted for successful blood collection in the rat. The technique is safe, quick, easy to learn, and requires only a short period of manual restraint. Blood collection using this technique provides a good quality sample, while at the same time causing minimal distress to the animal. The technique for restraining rats is scruffing tightly behind the ears using the whole hand and fingers. The scruffing technique will include as much loose skin as possible. White rat: located on the jaw line, directly below the lateral canthus of the right eye, a grey dot is seen. This is the

location for insertion of the 5.5-mm lancet. Colored rat: there is no dot; therefore, the 5.5-mm lancet will be inserted at a point from a line drawn straight down from the lateral canthus of the right eye to the jaw line. Following collection of the blood sample in the rat, the vein must be held off to stop bleeding. Pressure is applied to the area of the venipuncture with gauze until the bleeding stops. There are different sizes of animal lancets used for different strains of rats depending on their age and weight.

P26 A Different Approach to Hemostasis of Femoral Vessels in Swine

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Swine have been used extensively in procedures involving the use of cardiovascular catheterization laboratories, fluoroscopic imaging, and interventional radiology techniques. The vessels most commonly used for these procedures are the femoral artery and vein. Percutaneous access of these vessels provides minimal damage to the catheterized vessels, less trauma to the animal, shorter healing time, and possible reuse of the same vessels for future procedures. However, due to the location of the artery in the deep muscles of the leg, direct palpation of the artery is not possible necessitating utilization of anatomic landmarks. As soon as the procedure has concluded, removal of the catheter sheath is done once the activated clotting time (ACT) has returned to baseline. Because of the location of the vessels deep within the muscle, direct digital pressure applied to the vessels is difficult and can be insufficient. The overlying muscle layers assist with this process but it is critical to obtain hemostasis prior to recovering the animal. Without reestablishment of hemostasis, there is increased risk of hematoma development and possible death. In lieu of applying digital pressure, we have found a hands-free device used on humans that expedites bleeding cessation. It is an integrated manometer that allows pressure to be adjusted based on patient status. It has an inflatable transparent dome that offers controlled pressure and ensures puncture site visiblity for effective hemostasis. In our studies, it was found that the device worked well on all size pigs gradually decreasing the pressure over 30 min. However, on application with a sheath larger than a 12 Fr extra care must be taken with regard to placement and positioning over the puncture site. Compared with manual compression, this technique has been shown to help achieve hemostasis more quickly and more comfortably.

P27 Fecal Transplant for Treatment of Persistent, Intermittent Bloody Diarrhea in the Beagle Dog

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A study-naïve, 1-y-old telemetry-implanted, 9.6-kg male beagle presented with bloody diarrhea. Physical exam revealed no discomfort or pain upon abdominal palpation (although soft and gaseous) and normal stools mixed with pink/red stains upon rectal exam. Further diagnostics consisted of CBC/chemistry panel, fecal floatation, and Giardia ELISA. Neutrophil bands were slightly elevated and ELISA was Giardia positive. Metronidazole treatment was initiated at 250 mg twice daily for 10 d, retested for Giardia (negative results) at 7 d, followed by 5 d of sulfadimethoxine treatment. The occurrence of bloody diarrhea persisted over the next 3 mo upon which numerous therapies (metronidazole, fenbendazole, sulfadimethoxine, sulfasalazine, and a nutritional supplement) were attempted, as well as switching the diet to an allergen-free diet and removing all other treats. Additional diagnostics included repeat CBC/chemistry panels, survey and barium series radiographs, and endoscopy, but none revealed any significant findings. The consistency of the stool ranged from normal to liquid with intermittent frank blood. An alternative therapy presently performed in humans known as "stool transplant" was suggested. Thirty grams of feces from enteric parasite-free tested dogs was blended with approximately 70 mL saline and then filtered through gauze to obtain approximately 30 mL of the suspension. The suspension was administered to the recipient dog through an orogastric tube directly into the stomach. Daily nutritional supplement administration was reinstituted after 3 wk of no improvement. A second fecal transplant was performed 4 wk from the initial, which produced an immediate improvement with normal stool the following morning. After approximately 2 wk after transplant, normal chow was gradually reintroduced over 1 wk with the continuation of the nutrional supplement. The supplement was eventually discontinued and the dog has produced normal stools to date (approximately 1 y). Based on these findings, fecal transplant may prove to be a rational alternative to consider as a part of the treatment plan in persistent bloody diarrhea.

P28 Effectiveness of Medicated Feed in Eradicating of *Helicobacter* spp. Infections in Genetically Modified Immunocompetent Mice

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Twenty-two lines of genetically modified mice submitted by investigators for *Helicobacter* spp. eradication were treated using medicated diet. Thirteen genotypes were weanlings (4 to 6 wk), 8 genotypes were adults (≥10 wk) and one genotype ($IL10^{-/-}$) was represented in both groups. Mice were treated for 6 wk and tested by PCR of fecal specimens at 0, 6, 9, 12, and 18 wk or later. All mice were free of a wide range of murine pathogens and were *Helicobacter*-positive at the start. Twelve of 14 weanling genotypes and 6 of 9 adult genotypes tested negative at 6 wk and remained negative throughout the follow-up period. Lack of obvious immunodeficiency did not guarantee success: weanling and adult $IL10^{-/-}$ mice failed to achieve *Helicobacter* spp. eradication although weanlings did test negative at 6 and 9 wk. The remaining 3 unresponsive genotypes had no known immune deficiencies yet remained positive on all tests. *Helicobacter* species did not predict success either: both successfully treated mice and mice that remained positive were initially infected with combinations of *H. hepaticus*, *H. typhlonius*, *H. ganmani*, and *H. bilis*. When $IL10^{-/-}$ results were excluded, tests at the immediate conclusion of treatment correctly predicted eventual treatment success. However, results in $IL10^{-/-}$ mice indicated that further testing at 6 wk after treatment was necessary to detect suppression of shedding without eradication. The 82% successful eradication rate in this study suggests that although medicated feed is not universally successful, 6 wk of treatment is a viable option for eradication of *Helicobacter* spp. in genetically modified mice.

P29 A Case of Spontaneous Sperm Cystolithiasis in a SIV-Infected Rhesus Macaque (*Macaca mulatta*)

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A 6-y-old, 14.4-kg, SIV infected male rhesus macaque (*Macaca mulatta*) presented with a history with acute hematuria. Differential diagnoses included cystitis, prostatitis, urolithiasis, urethral pathology, and neoplasia. Initial physical exam revealed an enlarged, firm urinary bladder. Complete blood count and chemistry revealed a mild thrombocytopenia. Initial urinalysis revealed hematuria, proteinuria, and pyuria. Enrofloxacin and buprenorphine therapy were initiated for suspected urinary tract infection. Abdominal survey radiographs were unremarkable. Subsequent urinalysis revealed numerous small fragments of pale colored debris throughout the urine sample. Histology of these fragments demonstrated a mixture of spermatozoa and rare inflammatory cells. Double contrast cystogram and ultrasound examination revealed numerous irregularly shaped radiolucent filling defects or masses within the lumen of the urinary bladder. After 5 d, the animal was euthanized for experimental purposes. At necropsy, gross findings included a bladder lumen containing multiple angular amorphous masses approximately 2 to 5 cm in diameter that corresponded to the masses observed on diagnostics. Similar calculi were also present near the ejaculatory duct opening of the proximal urethra and pinpoint ulcers were present on the trigone mucosal surface of the urinary bladder. Histopathology revealed that these masses were composed of semen matrix. These findings taken together support a diagnosis of retrograde ejaculation into the urinary bladder causing semen coagulation and formation of calculi. Retrograde ejaculation is a published side effect of routine electroejaculation methods used to collect spermatozoa for scientific investigations in nonhuman primates. Current medical literature suggests this side effect is due to asynchronous stimulation of nerve tracts. It has been documented in rats that sperm urolithiasis can be induced by inseminating sperm into the neck of the urinary bladder. The effect of SIV infection in nonhuman primates on semen characteristics and sperm coagulation is currently unknown. To the authors' knowledge, this is the first report of a spontaneous case, in the absence of electroejaculation, of sperm cystolithiasis in a rhesus macaque.

P30 Intramedullary Pressure Transients during Initial "Flush" of Intraosseous Cannulas

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Intraosseous (IO) cannulation provides rapid vascular access for the emergent delivery of drugs, fluids, and blood products. IO cannulation has long been used in veterinary practice as a means to achieve vascular access in neonates or in species in which venous access is difficult. The advantage of intraosseous access is that bone marrow provides a rich network of noncollapsible venous sinusoids. However, bone marrow is confined by a rigid compartment and offers greater resistance to fluid infusions than intravenous sites. It is common practice to flush the IO cannula prior to establishing infusions by gravity feed or a pressurized bag. Little is known about medullary events associated with IO flush. The objective of this study was to assess intraosseous pressure changes during IO flush procedures. Twenty-one veterinary support or research staff within an animal research facility were asked to flush an IO cannula (15 gauge) with 10 cc of saline (0.9% NaCl). The cannula was placed in the distal femur of an isolated cadaveric preparation obtained from a 42 kg swine (*Sus scrofa*). A second cannula was placed in the proximal femur and bone marrow pressure recorded. Peak IO flush pressures varied over a wide range (57 to 1100 mm Hg) with mean ± SD = 587 ± 304 mm Hg. The median peak flush pressure was 615 mm Hg. The maximum rate of increase and decrease in intramedullary pressures ranged from 105 to 2300 mm Hg/s and −65 to −6200 mm Hg/s, respectively. Prior investigators have demonstrated bone marrow fat embolism with IO infusions and others have identified the role of high medullary pressures for embolism in orthopedic procedures. The present study raises concern that high pressure transients during flush may be a contributing factor for fat embolism with IO infusions.

P31 Go for the Jugular! Blood Draw Refinement in Rats

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During routine retroorbital blood draws from rats, the technicians noticed that there was occasional trauma to the eye after the procedure, so other means of obtaining blood from the rats were explored. The ideal blood draw technique would provide the quantity of blood needed with the least amount of stress to the animal while not incurring high costs or requiring extensive time. Mandibular and tail-stick bleeds did not provide enough blood required for the studies being performed. Tail-nick bleeds did not heal very well, and retroorbital bleeds required anesthesia and could occasionally cause trauma to the eye. The best solution was the jugular bleed. The procedure was simple, quick, and best of all, caused little to no stress to the rat. The task could be performed by a single individual, each technician restraining and bleeding their own animal. No anesthesia, heat lamps, or restraint devices were needed and it was easy to obtain the amount of blood needed. The rat was restrained in one hand with the thumb and last 3 fingers restraining the forelimbs behind the back while the index finger drew back the head. The thorax was shaved to access the jugular vein and swabbed with an alcohol pad. The needle was inserted above the nipple at a 20° angle with a minute vacuum, until blood was drawn. Avoiding continuous pressure on the syringe plunger prevented the collapse of the vein. After blood collection was complete, gauze was held in place on the site of the blood draw until hemostatis was obtained. Once the technicians mastered the manual restraint of the rat, the blood draws from the jugular vein were exactly what they had been seeking. A standard operating procedure was put in place to inform the other technicians and teach incoming staff the refined procedure.

P32 Obtaining Blood from the Superficial Temporal Vein on Mice with Different Morphologies

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Obtaining blood from the superficial temporal vein of mice is becoming a more popular method of blood collection. Also referred to as submandibular bleed or cheek bleed, it can often be difficult to visualize the venipuncture site to ensure successful blood collection. From mice with significant anatomic differences, our standard venipuncture technique is less than 100% effective. To help develop a consistent technique among mice with different morphologic features we have necropsied the following mouse strains: C57BL/6, A/J, TALLYHO, SEA/Gn, P/J, and SWR. These commonly used strains were chosen based on morphologic variation of the skull. Based on necropsy findings, our standard method of identifying the sebaceous gland as a reference point to base the proper venipuncture location worked for the following strains: C57BL/6, A/J, and SWR. For the strains with atypical morphology, it was observed that the method used for identifying the blood collection site was not effective. For example, necropsy results of the TALLYHO strain indicated that the sebaceous gland location was too ventral for our landmark points of reference. The SEA/Gn results showed that the sebaceous gland starting point was too ventral and too rostral, and the P/J too rostral. Based on our findings, anatomic variation does occur between strains and must be considered when obtaining blood from the superficial temporal vein of specific mouse models.

P33 Multiple Masses on the Femur: A Case of Osteochondromatosis in a Rhesus Macaque (*Macaca mulatta*)

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A 5-y-old, male, rhesus macaque (*Macaca mulatta*) presented with a prominent mass slightly anterior to the right knee. On exam, under ketamine anesthesia, multiple radiopaque masses were identified protruding from the distal femur. Lateral and AP radiographs were taken of the right stifle region revealing multiple exophytic masses arising from the distal femur with mild bony reaction of the proximal tibia. Samples were taken for CBC, serum chemistry panel, blood culture, urinalysis, and urine culture. All tests were performed inhouse and all results were within normal limits. A bone biopsy was performed on one of the masses. Histologic examination of tissues stained with hematoxylin and eosin revealed woven and lamellar bone with granulation tissue and skeletal muscle. The presence of woven bone and granulation tissue suggested an old fracture site; however, neoplastic disease could not be completely ruled out. Since the animal was exhibiting no lameness or signs of pain associated with these lesions the decision was made to monitor the progression, if any, of these masses. Minimal change was noted in the masses during the time leading to study termination at 6.5 y of age. On gross necropsy examination, the bony masses were cartilage capped lesions arising near the growth plate of the distal femur and midshaft of the femur and tibia. Histologic examination revealed cartilage capped spongy chondro-osseous exophytic growths that blended imperceptibly with the cortex and spongiosa of the femur. The clinical, gross, and microscopic findings support a final diagnosis of multiple osteochondromas and, according to a literature review, is the first reported case in a rhesus macaque.

P34 Malignant Medulloblastoma with Secondary Hydrocephalus in an Infant Pig-Tailed Macaque (*Macaca nemestrina*)

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A 4.5-mo-old intact male pig-tailed macaque (*Macaca nemestrina*) that was part of a specific pathogen-free breeding colony presented with an enlarged cranium and ataxia. On physical examination the animal was in good body condition, well hydrated, and had a grossly enlarged cranium, estimated as 50% to 75% larger than average circumference. Open cranial sutures and nystagmus were also observed. Cranial radiographs were unremarkable. Hydrocephalus was the working diagnosis. The animal had progressive difficulty supporting the cranium and nursing from the dam, and despite supportive care, the macaque was found dead 4 d after presentation. On gross necropsy, cranial sutures were approximately 2 cm wide, the lateral ventricles were moderately distended, and the third and fourth ventricles were severely distended. About 150 mL of normal appearing cerebrospinal fluid drained from the enlarged ventricles. An approximately 2 × 2 × 2 cm, round, red mass was present between the caudal cerebellum and brainstem. Histologically the mass consisted of nests, cords, and sheets of small- to medium-sized, crowded, and piled

round cells in fine fibrovascular stroma, and with irregular, round to oval nuclei, and scant eosinophilic cytoplasm. There were areas with high mitotic index, multifocal necrosis, and regions of infiltration into adjacent parenchyma. Brain ventricles were uniformly moderately or more enlarged. Histology was consistent with a malignant medulloblastoma and secondary hydrocephalus. Medulloblastomas are the second most frequently diagnosed malignant brain neoplasms of children. In animals, this neoplasm is seen most frequently in young cattle and dogs, and has also been reported in pigs, cats, rats, and a baboon. To the authors' knowledge, this is the first reported case of a medulloblastoma in a macaque.

P35 Hypermature Cataract in a 1-Y-Old Sprague–Dawley Rat

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A 1-y-old, intact female, experimentally naïve Sprague–Dawley rat (NTac:SD), used as a soiled bedding sentinel, was noted to have buphthalmia of the right eye. There were multiple foci of white tissue within the anterior chamber in addition to a Y-shaped band of tissue (2 mm in diameter) present within the posterior chamber. The vasculature surrounding the pupil was prominent and the anterior chamber was cloudy. Apart from the ocular lesion, the animal appeared to be in good health. The rat was monitored and the lesion showed no signs of progression. The animal was later euthanized for routine sentinel testing and was found to be free from internal and external parasites and tested negative on serology for a panel of 12 pathogens. Histopathologic evaluation of the right eye revealed a misshapen lens with a wrinkled capsule. Multifocal extensive areas of loss of subcapsular lenticular fibres were evident and replacement by mineralized debris noted. Hyperplasia of the lenticular epithelium was apparent with migration of the epithelium to the posterior region of the lens. Several lenticular epithelial cells showed evidence of fibrous metaplasia. Peripheral posterior synechiae were visible and extensive fibrovascular membranes were adhered to the ciliary body, posterior lens capsule and choroid. The retina was markedly atrophied. Edema, fibroplasia and an inflammatory infiltrate resulted in thickening of the choroid. The infiltrate consisted of numerous lymphocytes, moderate numbers of plasma cells, eosinophils, neutrophils, and hemosiderin-laden macrophages. Vascular congestion of the iris was present, and the anterior chamber was filled with eosinophilic proteinaceous fluid consistent with plasmoid aqueous. The other eye was histologically normal. The microscopic changes in the eye are consistent with a chronic, subcapsular, hypermature cataract. Other changes within the eye suggest phacolysis leading to a secondary panuveitis. The Y-shaped appearance of the cataract resembles that of Y-suture congenital cataracts in humans and may also be observed as a late change in advanced cataracts of other etiologies. Cataracts are commonly associated with aging in various rat stocks and strains including the Sprague–Dawley rat.

P36 Eye Problems: Why Euthanize When It Can Be Treated?

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The health and welfare of research is of paramount importance. With the increased complexity of genetically engineered mice, individual subjects can be extremely valuable. Avoiding euthanasia for conditions that can be treated allows principal investigators to keep precious breeders or obtain important data from animals already on study. Common clinical conditions affecting the eye can easily be diagnosed and treated thus improving the welfare of laboratory mice while saving their experimental value. Rigorous staff training as well as an efficient veterinary care program are key elements to ensure that these conditions are adequately managed. Clinical manifestations such as conjunctivitis, corneal ulceration, orbital masses, and exophthalmia are common in mice. They can be related to a strain phenotype, a bacterial infection, or environmental factors. The medical and surgical strategies to detect, report, treat, or control these conditions will be presented. When the first clinical signs such as ocular discharge, swollen eyelids, or redness are observed in a mouse, a topical treatment is applied for a period of 7 d. After this period, if there is no evident improvement, the medication can be changed for a different antibiotic for another 7 to 14 d. When the animal is still not responding to either treatments, oral medication can be given in drinking water. The material used in regular husbandry can also be changed to help the animal recover. If the eye status reaches an endpoint such as ulceration, enucleation can then be performed. By finding adequate treatments to common ocular problems, the number of animals used can be reduced and their welfare can be improved, hence addressing 2 of the 3Rs.

P37 Reduced Rat Usage through the Use of an Automated Blood Sampling System

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The use of bile duct cannulated (BDC) rats is standard procedure for absorption, distribution, metabolism, and excretion (ADME) studies. Historically, BDC rats have been maintained in metabolism cages to allow collection of fractionated urine, feces, and bile in an effort to calculate mass balance of the test article, as well as evaluate parent compound and metabolites in the excreted matrices. Additionally, when these excretion matrices are analyzed in comparison to the animals' plasma samples, pharmacokinetic parameters such as biliary excretion and area under the curve (AUC) can be calculated. One limitation of traditional rat metabolism caging has been the ability to continuously collect urine, feces, and bile while serially collecting multiple time-point blood samples from the same tethered animal. Because of this limitation, a satellite group of intact terminal rats have been historically used to obtain blood samples at terminal time points. In an effort to adopt animal welfare principles of reduction, refinement, and replacement (3Rs), an alternative method using automated blood sampling technology was evaluated for ADME studies. Use of an automated blood sampling system was evaluated for collection of fractionated urine, feces, and bile while simultaneous serially sampling blood from the same animal. In regard to the 3Rs, both a reduction and refinement was accomplished. Following implementation of an automated blood sampling system, a 30% reduction in the overall number of animals used for an ADME study was achieved. This resulted from the elimination of the rats used in satellite groups to obtain timed terminal blood samples. Additionally, a noticeable refinement to the procedure was implemented through the use of the automated blood sampling system, which eliminated manual manipulation of the animals during blood sampling, thus reducing the stress experienced by the animal.

P38 Preparation and Monitoring of Swine during Computer-Assisted Robotic Surgical Procedures

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Computer-assisted robotic surgical platforms perform complex surgical procedures using miniaturized wristed instruments that translate the surgeon's hand movements at a remote console into precise micromovements within the patient while being visualized via a 3D high-definition camera. Like traditional endoscopic approaches, it provides a minimally invasive approach that accelerates postoperative recovery. Unlike endoscopic surgical methods, this minimally invasive approach has a much broader range of complex surgical applications, including cardiothoracic, colorectal, gynecologic, urologic, and general surgical procedures. Robotic arms introduce miniaturized instruments and a camera, and require that specific modifications be made to patient support and monitoring equipment and techniques. Prior to training protocols using robotic surgical platforms, general surgical preparation of anesthetized model swine includes as a minimum endotracheal intubation, placement of a Foley catheter, and establishing intravenous access. Extensions must be added to SpO_2, CO_2, respiration, and temperature monitoring lines, as well as to intravenous tubing. Longer parallel Wye pieces in anesthetic rebreathing circuits are necessary to ensure that an effective surgical plane of anesthesia is maintained without hampering the necessary movements of the robotic arms. Extensions can occasionally pose problems with ineffective electrical contact, intertwining with each other or the robotic device. Anesthetic partial pressures must be carefully monitored as the insufflated abdomen causes an inclination toward positive-pressure ventilation during long surgical procedures. With careful planning and preparation the unique challenges of

minimally invasive surgical training using computer-assisted robotic surgical platforms can be successfully addressed.

P39 Development of a Nonhuman Primate Special Care Nursery

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In the United States, 1 in 8 babies (12.8% of live births) are born too early. The most prevalent underlying cause of preterm labor is intraamniotic infection caused by *Ureaplasma* species. Previous studies at our institution have focused on early detection and interventional therapy for the prevention of fetal organ injury and preterm labor. In order to develop our studies into a neonatal model it has been necessary to expand the scope of newborn infant care at the primate center by establishing a Special Care Nursery which is analogous to a human neonatal intensive care unit. Our team of investigators includes neonatologists, perinatologists, neonate registered nurses, clinical/surgical veterinarians, and primate neurobehavioral specialists. We have successfully survived the first ever prematurely born nonhuman primate neonates exposed to *Ureaplasma* species and antenatal antibiotic therapy. Careful consideration was taken to integrate both human neonatal and veterinary based equipment and care guidelines to ensure low morbidity and mortality. This unique special care nursery can now accommodate prematurely born infants that require 24-h intensive care, respiratory support (that is, oxygen supplementation, mechanical ventilation and/or continuous positive airway pressure), continuous intravenous fluids, and drug administration specific to the needs of the neonate.

P40 Use of a Custom Syringe Set for Collection of Whole Blood from Laboratory Cats

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Feline whole blood or blood components are often required for in vitro laboratory studies. Often the volume required exceeds the conventional blood collection volume of 5 to 10 cc via a small syringe or vacutainer system. Though easily adaptable for dogs, commercial blood collection sets for human use are not an option when collecting blood from cats. To provide a closed blood collection system for collecting large volumes (45 to 50 mL) of blood from cats we designed a specialized blood collection system. The set consists of a 60 cc syringe with a 3-way stopcock; integrally attached to the 2 ports of the 3-way stopcock are a 19-gauge butterfly needle and a multipurpose sterile blood collection bag. The system is also designed to accommodate an optional integrally attached transfer bag for removal of plasma from the sterile blood collection bag. With this system the user can aseptically collect up to 50 mL of whole blood from the cat and either store the blood in the primary storage bag or transfer blood components such as plasma to the satellite bag. This system represents a refinement of the traditional syringe only method of blood collection from laboratory cats and allows for storage of the blood in the original collection bag maximizing sterility and viability of the blood product.

P41 Development of a Minimally Invasive Orthotopic Pancreatic Cancer Mouse Model Using Ultrasound-Guided Injection

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Orthotopic mouse models more closely resemble human cancers than do subcutaneous cancer xenograft models. However, they require a major surgical procedure and postoperative care established by surgical orthotopic injection during direct visualization of the organ of interest. In order to establish an orthotopic pancreatic cancer mouse model without surgical intervention, without exteriorizing and injecting directly into the pancreas, we compared the efficacy of direct surgical orthotopic injection of cancer cells into the exteriorized pancreas to ultrasound-guided injection of cancer cells into the pancreas. HCT116 cancer cells engineered for fluorescence imaging were injected into the pancreas either intraoperatively by surgical orthotopic injection while directly visualizing the pancreas or by ultrasound-guided injection without surgical intervention. Mice were imaged weekly by both ultrasound and by fluorescence imaging. Tumor growth was monitored for up to 4 wk, then mice were euthanized and the pancreas and other tissues evaluated postmortem by both ex vivo fluorescence and histopathology. Cancer in the pancreas with comparable tumor volumes was confirmed by both ultrasound and fluorescence imaging within 2 wk post injection using either the surgical orthotopic injection or ultrasound guided injection method. Cancer in the pancreas and only in the pancreas without leakage of cancer cells into other abdominal tissues was confirmed by histopathology in all HCT116-xenografted mice using either method. The ultrasound guided injections orthotopic pancreatic cancer model is more clinically relevant than the subcutaneous model, is comparable to the direct visualization surgical orthotopic injection method, and avoids a major surgical procedure and postoperative monitoring and care to create.

P42 Septicemia Associated with the Opportunistic Bacteria *Burkholderia cepacia* in Cybb Mice

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Several Cybb (B6.129S6-Cybbtm1Din/J) mice in a small inhouse breeding colony were found hunched, lethargic, moribund, or dead. At necropsy, mice with clinical signs had pale livers with diffuse petechial hemorrhage. Liver, kidney, spleen, blood, feces, and oropharyngeal swabs were submitted for microbiologic culture. Tissues were submitted for histopathologic evaluation. CBC were performed on mice that appeared clinically ill as well as healthy mice from the same colony. Mice were housed in microisolation cages with autoclaved hardwood bedding, autoclaved diet, and acidified reverse osmosis, deionized (RO-DI) drinking water. Husbandry was performed in a microisolation workbench after spraying with chlorine dioxide. Biochemical assays identified the cultured organism as *Burkholderia cepacia*. Bacterial isolates were submitted for16SrRNA PCR and sequencing of which 1136 nt were 99.3% identical to *B. cepacia* (GB accession FJ870551). Histologically, the liver and spleen demonstrated lesions consistent with generalized septicemia. The degree of necrosis and vascular thrombosis in the liver and spleen was suggestive of disseminated intravascular coagulation. Neutrophilia and lymphocytosis were found in clinically ill mice. Neutrophils were heavily vacuolated and contained gram-negative rods consistent with culture results. Bacterial infections are a serious complication in immunocompromised patients. *Burkholderia cepacia* is a gram-negative opportunistic pathogen associated with infections in patients with chronic granulomatous disease (CGD) and cystic fibrosis. Cybb mice are an animal model for CGD and require special housing and handling to limit exposure to opportunistic pathogenic bacteria. The disease outbreak reported in this abstract was a direct result of a break in specialized husbandry procedures. The source of *B. cepacia* was not determined, but it is typically found in soil, plants, and water in the environment. The colony was depopulated, operating procedures reviewed, and a new colony established from vendor source animals.

P43 ICLAS Performance Evaluation Program for Diagnostic Laboratories: A Tool for Monitoring Diagnostic Performance

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The Performance Evaluation Program for Diagnostic Laboratories (PEP) was set up in 2007 to promote and maintain the use of high quality animal models in research by providing a tool for diagnostic laboratories worldwide to monitor the sensitivity and specificity of their health monitoring assays. Serum and microbiologic specimens are prepared and confirmed by internationally recognized laboratories and sent to subscribing participating laboratories around the world for analysis. All samples are generated under stringent conditions and extensively characterized. Agents are obtained from known sources and identified phenotypically and genotypically to confirm identity. Then the masked specimens are sent to participating laboratories as unknown samples. The current PEP specimen library includes all common agents that can infect rats and mice. Batches of 10 serum and/or microbiologic specimens are sent annually to subscribing

participating laboratories around the world for analysis. Participating laboratories then request expected results and a comparison of results enables them to self-asses their diagnostic performance. There are currently 14 participating laboratories from: Asia (4), Australia (2), Europe (5), and North America (4). For participating laboratories the main benefits of participating in PEP are: 1) use of a scientifically robust program to monitor diagnostic performance, 2) access to expert help and advice from internationally recognized diagnostic laboratories, 3) participation in PEP enables the labs to implement a quality assurance program, and 4) the labs contribute to improved animal quality in research institutions. Details of PEP can be found at http://www.iclas.org/NetworkPEP.htm.

P44 Effects of Developing *Haemonchus* Resistance in Antibody-Producing Sheep and Goats

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Haemonchus is an internal parasite that in any sheep and goat herd can have a significant negative health impact. One example of the negative effect is development of multiple anthelmintic resistance. Several factors led to the discovery of anthelmintic resistant *Haemonchus* in our group of antibody-producing sheep and goats. Among these factors were seasonally declining packed cell volume results and persistent findings of stongyle ova in fecal examinations. A factor that likely contributed to the resistance development was the implementation of a large animal enrichment pastures. The intense stocking density of the 1.1-acre area set the stage for the anthelmintic drug resistance that we encountered. The following factors and actions were used to monitor the health of the small ruminants: daily technician observations, weekly veterinary inspections, observation of packed cell volumes in production bled animals, quarterly weights, body condition scores, animal ages, routine composite fecal examinations, and treatment for the specific internal parasites encountered. The Wisconsin fecal flotation method along with typical small ruminant dewormers were used from 2004 to 2009. Beginning in 2010, currently available anthelmintics such as benzimidazole and ivermectin were used in combination with fasting, complete oral dosing, and follow-up fecal analysis, which resulted in decreased fecal egg counts and normal packed cell volumes. Available resistance screening assays combined with our continued nutritional support for the age and condition of the animals pinpointed the specific animals that were parasite reservoirs in the herd. The results suggest parasite management, at the individual pen/group level, can be employed to control the onset of anthelmintic resistance in small ruminants in a research environment. Educated staff along with investigational screening methods combines to create an effective and efficient parasite control strategy.

P45 Novel Method of Oral Gavage in Neonatal Mice

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Oral gavage is a commonly used method of pharmaceutical compound delivery in adult rodents. Unfortunately, there is a paucity of delivery methods that can accurately and reliably deliver drugs into the gastrointestinal tract of neonatal mice. Gavaging neonatal mice with commercially available gavage needles proves difficult at best. Here we describe a novel method using a self-made gavage needle using polyethylene tubing and tuberculin syringe with needle, to orally administer compounds in neonatal mice. This rapid, relatively safe, and easily learned procedure was performed in neonatal mice aged postnatal day 4 through postnatal day 5. Oral doxycycline was administered up to a maximum volume of 10 mL/kg per neonatal mouse. In conclusion, we were able to reliably and safely orally administer drug compounds to neonatal mice. This technique offers a simple, rapid, practical, and relatively inexpensive alternative method to administer compounds to neonatal mice.

P46 Using Chlorhexidine to Treat Dermatitis in Mice

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Dermatitis is a common problem in laboratory mice for which treatment can be challenging. Systemic and topical antibiotics are frequently used to treat dermatitis, but can lead to the development of antibiotic resistant strains of bacteria, and may interfere with some types of research. In mice, dermatitis is commonly seen on or around the ears, neck, shoulders, and thorax region. Our veterinary staff needed a cost-effective, readily available, and successful treatment for mouse dermatitis. Chlorhexidine has many uses, one of them being a topical antiseptic effective against both gram-negative and gram-positive bacteria. Chlorhexidine was diluted to 1 oz/gal of distilled water. Distilled water dilutions provide up to a 6-wk shelf life compared with a 7-d shelf life with tap water. The solution may be used alone to clean and protect the irritated skin or used to remove dead skin cells and debris before applying topical medications. At our institution, we purchased 0.5-oz. plastic boston round bottles to hold the chlorhexidine solution. The solution was squeezed onto a cotton tip applicator or gauze pad and applied to the affected areas. In addition, trimming of the hind toenails was performed in all pruritic cases to prevent further tissue damage by scratching. The type of skin lesion determined the frequency of cleaning. Mild redness and irritation was reduced or resolved with cleansing 3 times a week for approximately 1 to 2 wk. More severe ulcerative lesions were treated with a combination of cleansing with chlorhexidine and triple antibiotic ointment 3 times weekly, and resolution of skin lesions was also observed within 1 to 2 wk. The use of chlorhexidine as a dermatitis treatment at our institution has greatly improved murine quality of health, convenience for research staff and offered significant cost savings for the Division of Animal Care.

P47 Personalized Recording Chambers for Nonhuman Primate Cortical Recordings

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While neural recording chambers for nonhuman primates can be purchased commercially, these generic chambers do not contour to the animal's skull. In order to seal gaps, a cap of dental acrylic (methyl methacrylate) is often applied around the chamber. This method is not ideal for several reasons. Applying acrylic delays and further complicates surgical procedure, and overheating during the curing process can cause damage to the bone. Postsurgery, acrylic margins can give rise to bacterial growth and infection. This study presents a method to develop custom implants that conform to the individual's skull by combining CT and MRI data, thereby eliminating the need for acrylic. The chamber is cylindrical in design and made of polyether ether ketone, and is mounted to the skull using four self-tapping bone screws. In the 4 mo since implantation on one rhesus macaque, scabs have not formed around the perimeter of the implant and infection has yet to be seen. In summary, this method shortens surgery time and significantly improves the hygiene of chamber margins.

P48 Clinical Symptoms and Supportive Care Provided to Rhesus Monkeys (*Macaca mulatta*) Infected with a Novel Simian-Human Immunodeficiency Virus

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Five rhesus macaques (*Macaca mulatta*) were infected with a novel simian-human immunodeficiency virus (SHIV) by intravenous route. Our purpose is to show clinical symptoms observed, describe hematological findings and discuss the supportive care provided. SIV is genetically similar to HIV2. It was discovered that SIV, which is endemic in African old world primates, can be pathogenic in Asian macaques. The Asian macaques developed CD4 T cell depletion and clinical symptoms very similar to AIDS patients. SIV induces an immunodeficiency syndrome in infected macaques similar to human AIDS. SIV-infected macaques develop AIDS after chronic phase (1 to 2 y), although several symptoms are observed, including diarrhea, rash, and lymphadenopathy, within 14 d after infection. These acute symptoms are generally not severe. However, the newly generated chimeric SHIV, SHIVAD8, causes severe diarrhea and dehydration in inoculated rhesus macaques within 5 to 14 d after infection. Similar early symptoms are previously reported in pig-tailed macaques infected with one SIV strain, SIVPBj, sharing the same amino acid changes in viral NEF protein. Without appropriate treatments, diarrhea and dehydration caused by these viruses would be lethal. Once the animals recover from the acute symptoms, they usually survive and develop AIDS after 1 to 2 y of asymptomatic

phase. Diarrhea and weight loss can be used as indicators of viral pathogenicity. Those who received immediate supportive care at the onset of diarrhea had survived longer than those with delayed treatment. Early identification and intervention during this acute stage helped to slow the animal's deterioration. The present study demonstrated that intervention as early as the first day after the onset of symptoms; prevented the deterioration of physical condition.

P49 Perianal Swelling in a Crossbreed Pig

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Here we report an emergency clinical case of perianal swelling in a 4-mo-old, castrated male, domestic Yorkshire/Hampshire cross pig housed in a biomedical research facility. Treatment involved topical hypertonic sugar solution, surgical resection of the affected portion of the rectum, providing analgesia with supportive care and administration of soft feed. Postoperatively the pig recovered normally and was successfully able to resume its experimental purpose. At euthanasia 4 mo later, gross necropsy and histopathology of the gastrointestinal tract were performed. Histologic sections from the surgical site revealed a mature postsurgical scar, but no areas of devitalized rectal tissue or other significant abnormalities. Rectal prolapse is one of the most common gastrointestinal problems in swine. Though there are multiple possible etiologies, the underlying pathomechanism is thought to be from the weakening effects of increased abdominal pressure on the pelvic musculoskeletal system supporting the rectum. Rectal prolapse can cause health problems and result in early removal of swine from research studies.

P50 Needle-Free Injections via the Subcutaneous Route in Rats: An Alternative to Dosing Preclinical Species

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The industry standard for subcutaneous and other parenteral routes of drug administration has historically involved the use of a needle and syringe system. However, there are many drawbacks to needle usage in preclinical and clinical settings such as: passage of infectious diseases, potential fear of needles, and accidental needle sticks. Alternatively, needle-free injection systems (NFIS) provide an empowering technology that work by forcing liquid medications at high speed through a tiny orifice held against the skin. This creates a fine stream of high-pressure fluid penetrating the skin and depositing medication in the tissue beneath in a fraction of a second. The novel technology of NFIS has been used recently in preclinical and clinical research but has not been previously used in rodents via all routes of injection: intradermal, intramuscular, and subcutaneous. In a recent 2-phase study conducted to evaluate and characterize the acute toxicity and estimate the maximum tolerated dose following a single subcutaneous dose, and evaluate the toxicity and toxicokinetics of the test article following 7 d of repeat subcutaneous dosing in CD [Crl:CD(SD)] rats, NFIS was used as an alternative to the traditional needle and syringe system. The NFIS device had not been previously used for subcutaneous injection in rats and we will describe the different trials used to determine the best technique for administration. Several techniques were employed to determine the ideal method to deliver the test article formulation in the subcutaneous space consistently and accurately that would result in predictable and repeatable result. The technique used on study involved "tenting" the skin with the injection taking place perpendicular to the animal.

P51 Development of a Model of Heart Failure in the New Zealand White Rabbit: Echocardiographic Data

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The New Zealand rabbit (NZR) surgical model of left ventricular failure (LVF) is known. In our lab, precision and reproducibility of this substrate is validated by the use of transthoracic echocardiography (TTE) during the surgical procedure and at regular intervals throughout the development of LVF. NZR underwent the 2 stage procedure to develop LVF. In stage 1 (volume loading), aortic regurgitation (AR) was created by catheter perforation of the aortic valve. In stage 2 (pressure loading), increased afterload was induced by banding the abdominal aorta. Baseline and serial TTE was performed with emphasis on left ventricular (LV) dimensions: end systolic (LVIDs) and diastolic (LVIDd) diameter, posterior systolic (LVPWs) and diastolic (LVPWd) wall thickness and interventricular septum systolic (IVSs) and diastolic (IVSd) thickness. Fourteen rabbits underwent this procedure. In addition to perioperative TTE, each rabbit was followed every 2 to 3 wk with a TTE evaluation. Eight rabbits (57%) survived both surgical stages and successfully developed LVF, having reached the target LVIDd (50% increase). TTE was able to predict mortality in 83% of nonsurvived rabbits. The TTE data was analyzed for the change in LV dimensions and degree of AR to determine that LVF was established. To our knowledge, this is the first study to use TTE as guidance to stage 1 (AR creation). TTE can reliably diagnose and guide management of developing LVF in NZR. Quantitative echocardiographic assessment of LV dimensions are correlated to critical markers of cellular myocardial failure and help to determine optimal timing for harvesting of the LVF heart for cellular evaluation of failing myocardial tissue.

P52 Eradication of *Helicobacter* spp. in an Inbred Rat Colony Using a Medicated Diet

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There has been increasing awareness and concern among investigators worldwide on the effect of *Helicobacter* spp. infections in research animals, and data integrity that may be affected due to infection, specifically in research areas involving autoimmunity, immunology, gastrointestinal disease, and other related fields. Therefore, the presence of *Helicobacter* spp. within research animal colonies has been deemed pathogenic and undesirable. Although many methods of eradicating *Helicobacter* spp. have been previously reported, use of a diet containing a formulation of antibiotics is the most humane. The study described herein evaluated the effectiveness of a commercially available medicated diet, the *Helicobacter* diet, at eradicating *Helicobacter* spp. in an infected inbred rat breeding colony. The breeding colony where the study animals were selected was positive for *Helicobacter* spp., β-*Streptococcus* spp. group B (GBS), and *Staphylococcus aureus*. Weanling animals were selected as future breeders/founders and continuously treated with the medicated diet while under stringent husbandry practices for 9 wk. Fecal PCR testing as well as live animal full-panel health screenings were conducted by an independent laboratory at 5 and 7 to 8 wk during treatment, as well as 1, 4, 5, and 7 mo after treatment. All results during and after treatment were negative for *Helicobacter* spp. The live animal health reports had also revealed negative test results for both GBS and *S. aureus* pathogens during treatment and were negative for GBS after treatment in immunocompetent strains. The medicated diet was not able to eliminate GBS from the immunocompromised rat strain. Additionally, *S. aureus* reemerged once the treatment was halted. Animals from this study and their offspring have remained *Helicobacter*-free for over 9 mo. These data suggest that the medicated diet was effective at eliminating the *Helicobacter* spp. in both immunocompetent and immunocompromised rats, suppressed *S. aureus* infection during treatment, and eliminated GBS in immunocompetent animals.

P53 Screening for Rodent Pathogens in the Wild Mouse (*Peromyscus leucopus*) Population in Southeastern Connecticut

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Laboratory mice are widely used in biomedical research. A rigorous surveillance and biosecurity program is crucial to any laboratory animal program; however, maintaining a colony of pathogen-free mice can be challenging. The most commonly seen pathogen in our vivaria is mouse parvovirus (MPV) with approximately 2 to 5 outbreaks per year. There are many opportunities for the introduction of rodent pathogens. Although no wild rodents have ever been captured in our vivarium, there is the possibility that contamination from the wild

rodent population is a cause of these outbreaks. This study endeavored to elucidate what pathogens, if any, could be found in the population of wild mice surrounding the vivaria in Groton, CT. Specifically of interest was the prevalence of MPV. This information might allow the veterinary staff to determine if the wild population poses a potential threat to the research colony, which in turn might also allow for a review of the current garb policy at the site. Mice were humanely trapped, euthanized, and sampled for viruses and specific bacteria. Fecal pellets, mesenteric lymph nodes, and sera were collected from each mouse. The mice were visually inspected for ectoparasites. A cohort of the trapped mice was also screened for pinworms. The wild mouse population had a 65% prevalence of mouse adenovirus 2 (MAV 2) and a 10% prevalence of theilovirus (GDVII), epizootic diarrhea of infant mice (EDIM), and mouse thymic virus (MTLV). *Helicobacter* species was identified by PCR in 95% of the fecal pellets submitted. Eleven mice were screened for pinworms and were negative. None of the wild mice trapped were positive for MPV. The results of this study indicate that the population of wild mice in Southeastern Connecticut is not the source of the MPV outbreaks in the vivaria. However, the results indicate that the wild population does carry rodent pathogens that could potentially threaten the research colony.

P54 Vaginal Septum Is a Possible Cause of Reduced Reproductive Performance in BALB/cByJNarl Mice

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Vaginal septum was noted in our specific pathogen-free BALB/cByJNarl mice breeding colony with a prevalence rate of 14.4% (216 of 1505). To verify whether this defect would affect the reproductive performance, we compared the fertility of normal and affected animals on a prospective study with BALB/cByJNarl breeders at 9 to 12 wk of age in a monogamy pair. The area of the vaginal orifice of mice with vaginal septum was significantly smaller than those without septum (1.46 ± 0.11 mm^2 compared with 2.77 ± 0.33 mm^2, unpaired *t* test, $P < 0.005$). The rate of copulatory plugs in normal female mice (72%, 49 of 68) was significantly higher than in mice with vaginal septum (51%, 23 of 45) (χ^2, $P < 0.05$). The numbers of spermatozoa in the vagina of normal mice at 3 h after mating ($1.45 \pm 0.08 \times 10^8$/mL) were significantly higher than those of mice with vaginal septum ($1.84 \pm 0.97 \times 10^7$/mL) (unpaired *t* test, $P < 0.001$). The pregnancy rate was also significantly higher in the normal mice (54%, 37 of 68) than the affected counterpart (27%, 12 of 45) (χ^2, $P < 0.01$). Among all normal females having copulatory plugs, 75.5% (37 of 49) had successful pregnancies while only 52.2% (12 of 23) of the affected mice did (χ^2, $P < 0.05$). No dystocias was noted in the normal mice group, while 3 cases were confirmed in the affected animals (Fisher exact test, $P < 0.05$). Only 6.1% (8 of 131) of the offspring from normal female inherited the vaginal septum, while 15.5% (24 of 155) of the offspring from the affected mice carried the trait (χ^2, $P < 0.05$). Together, our observations suggest that vaginal septum should be considered as a criterion for the selection of future breeders in a mouse colony with high prevalence of vaginal septum.

P55 Reduced Rat Usage through a Comparison of Catheter Placement for Serial Blood Sampling in Rats

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We use male Sprague–Dawley rats for absorption, distribution, metabolism, and excretion (ADME) studies. The dosing procedure used for these studies involves injecting a radiolabelled compound intravenously. Following dosing, collection of fractionated urine, feces, bile, and plasma is performed in an effort to calculate mass balance of the test article, as well as evaluate parent compound and metabolites in the excreted matrices. An automated blood sampling unit was recently introduced to obtain recovery and serial blood samples from the same rats, and femoral vein catheterized rats were initially used. The rats were purchased from a vendor bile duct cannulated and femoral artery catheterized. This process included recovery time at the vendor and the acclimation period once the rats were received inhouse. Due to shipping time and acclimation period, patency issues developed with the catheters. Due to the decrease in the number of rats that had patent catheters, a higher failure and missed sample rate was experienced. The number of animals ordered per study was increased by 30% to ensure a complete and successful study. In an effort to refine the procedure, a search was performed for an alternative method that would prove to be more successful. A comparison was performed with femoral vein and femoral artery catheters in rats to evaluate catheter placement compared with patency. Through evaluation, the femoral vein catheterized rats exhibited a 60% success rate in comparison to femoral artery catheterized rats which exhibited a 90% success rate. Following implementation of femoral artery catheterization as the primary method to catheterize serial sampled rats, a 30% reduction in the number of rats used on study was achieved. Additionally, due to the placement of catheters, this increased patency and the number of missed samples at time points was drastically reduced.

P56 Animal Care Staff Exposure to Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Commercial Rodent Populations

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Staphylococcus aureus is present in 25% to 30% of the general population. Methicillin-resistant *Staphylococcus aureus* (MRSA), is present in 2% to 5% of the general population, and is a major cause of nosocomial, life threatening infections in hospital settings such as intensive care units. Recently, MRSA has been reported in bedbugs. MRSA has been associated with an asymptomatic carrier state in companion animals and livestock and transmission between animals and humans has been documented. The incidence of *S. aureus* in commercial rodent vendor rooms ranges from less than 5% to 90%. Our Occupational Health and Safety Program staff questioned whether our rodent population could be a potential source of human-acquired MRSA. Over 100 *S. aureus* isolates from clinically ill, resident or incoming animals were evaluated to determine methicillin resistance. Isolates were cultured on commercially available trypticase soy agar with 5% sheep blood, *S. aureus* chromogenic agar, oxacillin screen agar, 2 types of chromogenic MRSA agar plates and susceptibility tests were performed via the Kirby–Bauer disk diffusion method using cefoxitin (30 µg) and oxacillin (1 µg) discs in order to determine methicillin resistance. Positive control MRSA isolates were used for comparison. The absence of growth on the 2 types of chromogenic MRSA agar plates or on the oxacillin screen agar indicated that all *S. aureus* isolates evaluated were not MRSA. Also, all of the isolates were sensitive to cefoxitin (average zone = 26 mm) and oxacillin (average zone = 18 mm) confirming that *S. aureus* isolates were not methicillin resistant. Our results agree with other reports that cefoxitin discs are a better indicator of methicillin resistance than oxacillin discs due to clearer zones and easier readability. It was concluded from the isolates we evaluated, that our rodent population does not pose an occupational risk of MRSA to our animal care and scientific staff.

P57 The Use of Plastinated Models as a Teaching Method for Blood Sampling in Rats

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Blood collection from rodents is necessary for many experimental or diagnostic procedures. Retroorbital or facial vein blood collection in rodents can provide moderate to large amounts of blood; however, severe injuries may occur to animals if this procedure is not done properly. Tail bleeding is another commonly used method. These collection techniques require training, and are often accomplished in anesthetized animals. Plastination is a useful technique for preservation of biologic specimens. Plastination methodology consists of slowly replacing tissue fluids and a portion of the tissue lipids with a polymer, under vacuum. The results are clean, dry, odorless, and durable, real biologic specimens. The purpose of this project is to use the skull and plastinated heads and tails for training of multiple students, without sacrificing additional animals. Adult rats no longer needed in other projects, were euthanized by CO_2, and the heads and tails were obtained. Skulls were prepared first by freezing, then by thermal maceration, and last, mechanical removal of soft tissues. Each skull was then bleached with 30% hydrogen peroxide. Using other heads, some were left with the skin intact, and in others the skin was removed. They were fixed in 10% buffered formalin. Acetone was then used for dehydration, and silicone infiltration was performed. Tails

were prepared in a similar manner. Rat heads, and tails were obtained with and without soft tissues to provide models to demonstrate anatomy, and to provide training models for the collection of blood. Advantages of this model include reduction of the use of live animals. Initial use of anatomically real models may reduce student anxiety, as well as trauma induced to animals during subsequent learning attempts. Rat heads and tails are an excellent model for education, skill development and refinement of bleeding technique. This kind of teaching material can improve the teaching/learning process.

P58 5/6 Nephrectomy Rat Model of Compromised Renal Function: Comparison of Operated Rats from Different Vendors

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A rodent model of compromised renal function was needed to determine if renal function impacted pharmacokinetics of test article. The 5/6 nephrectomy is a 2-stage surgical model of compromised renal function. Two of our approved rodent vendors offer this model in rats. Studies to assess surgical quality and track selected clinical chemistry and hematological tests over time were conducted. Each vendor uses a slightly different surgical procedure. Both vendors start the first surgery with a ventral midline abdominal incision and exposure of a kidney. Vendor A places a ligature around each pole of exposed kidney at the 1/3 position. Ligatures are tightened. Renal tissue distal to each ligature is excised. Vendor B isolates renal artery and vein of exposed kidney. Upper and lower branches of the renal artery are ligated, causing avascular necrosis of renal poles. Middle branch maintains to supply blood to kidney. Incisions are closed, and rats recover for 1 wk. Then, both vendors perform the second surgery, excision of opposite kidney, through a dorsolateral lumbar incision. Five 5/6 nephrectomized male Sprague–Dawley rats from each vendor were euthanized at various times after arrival and necropsied. Surgical quality of both vendors was acceptable although small adhesions of partial kidney to other organs were seen in all rats. Five more 5/6 nephrectomized male rats were obtained from each vendor and followed for 8 wk. Rats were weighed on arrival and at least twice weekly. Blood was collected for selected clinical chemistry and hematological tests weekly starting 1 wk after second surgery. Rats from both vendors steadily gained weight although weights were at low end of normal for age. Range of blood urea nitrogen was 46 to 61 mg/dL at week 1 and 43 to 68 mg/dL at week 8 in Vendor A's rats and 30 to 39 mg/dL at week 1 and 29 to 108 mg/dL at week 8 in vendor B's rats. Range of creatinine was 0.6 to 0.9 mg/dL at week 1, and 0.7 to 1 mg/dL at week 8 in vendor A's rats, and 0.6 to 0.7 mg/dL at week 1 and 0.8 to 2.2 mg/dL at week 8 in vendor B's rats. By week 4, Vendor B's rats had a consistent wider range in both measures compared with Vendor A's rats. Since a uniform level of compromised renal function was desirable for our studies, vendor A was selected.

P59 Omphalitis in Immunomodulated Weanling Mice

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Four 21-d-old female littermate mice, transgenic for a modified T cell receptor, presented with 0.2- to 0.7-cm diameter, firm cutaneous masses in their umbilical areas. With the exception of this finding, the mice were bright and alert and in good body condition with adequate hydration. Physical examination of the sire, dam, and unaffected littermates was unremarkable. Cytology of the masses revealed mixed viable and degenerate neutrophils with fibrin and debris, suggesting bacterial infection. The lab opted to euthanize the affected mice, and 7 d after initial presentation, the mice were submitted for necropsy. Blood collected by cardiocentesis at the time of death revealed mild to moderate neutrophilia characterized by a left shift and toxic change. Fecal flotation was negative for ova or oocysts, and *Pasteurella pneumotropica* was detected by fecal PCR. Aerobic culture of the lesions yielded growth of *Enterococcus* spp. and *E. coli*, both commensal bacteria of the mouse gastrointestinal tract. At necropsy, each mass was found located at the cranioventral end of the median umbilical ligament, indicating umbilical origin. In addition to the cutaneous masses, 2 of the 4 mice had a 0.4- to 0.6-cm diameter dorsal extension of the lesion into the abdominal cavity. Histopathology revealed that the masses were abscesses. Omphalitis, or infection of the umbilical cord stump, is a generally localized neonatal condition that has been reported in several animal species and remains an important cause of human neonatal disease in developing countries. This is the first documented case of omphalitis in mice and, because in animals the condition is almost exclusively seen in immunocompromised individuals, suggests that the disease manifestation may have been influenced by the immunomodulating genetic manipulation of these mice. This case also illuminates the increasingly important role opportunists play in disease development in immunomodulated mice.

P60 Cost Analysis of Implementing Brown Paper Shred Enrichment Program

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Our institution wanted to implement the use of brown paper shred enrichment because it allows laboratory mice to mimic the natural nest-building behavior of mice in the wild and mice that have the opportunity to build a natural nest show less stress related stereotypical behaviors than animals kept in barren cages. However, one problem encountered with this change was that the soiled nesting material caused damage and back up of the wet bedding disposal system, resulting in costly repairs and downtime. Our facility performed an analysis of the costs to implement adding the brown paper shred enrichment to the cages in a manner that would not damage the wet bedding disposal system and compared it to the current labor model, as well as cost of replacement of the unit. The cost analysis included the cost of supplies, labor to build the caging with the additional enrichment, labor to remove the nesting material prior to reaching the Garbel, and repairs to the disposal system when nests create a backup. The results showed that there was an increase in labor cost to add the enrichment; however, there was no additional labor cost for the animal care technicians to remove the extra enrichment and dispose of it during cage changing. By removing the nests prior to cage processing, the incidence of Garbel breakdown due to nest disposal was minimized. Therefore, we determined brown paper shred enrichment can be implemented in a facility-wide program due to similarities to natural nests, benefits of decreased stereotypical behaviors, probable increased breeding success, and negligible time difference to discard the used nests. We also determined that it was not cost effective to perform a total replacement of the wet disposal system to a vacuum system at this time and to just manage the supplies manually.

P61 Prevalence of Abnormalities and Spontaneous Mortality Indentified by Husbandry Staff at a Major Biomedical Institution

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Identifying, monitoring, and responding to abnormalities and spontaneous mortality are a major undertaking at most institutions. However, there is no published data readily available on the prevalence of health concerns or mortality at biomedical research institutions. Unfortunately this means that there is no baseline reference for comparison to indicate whether these numbers are unusually high for institutions of similar size. Without that information it is difficult to imagine how issues can be addressed beyond the individual animal. Using an electronic tracking system at our institution, the prevalence of specific issues can be classified and characterized. At our institution, approximately 100,000 animals are maintained in the average daily census. Presented here are the numbers we have collected. Over a typical year time period there are approximately 6000 cases entered into our electronic database. Of those, 1692 (26%) were mortality reports, which leave 4270 clinical cases in the year, averaging 356 cases a month. Briefly the major types of reports include, barbering (719 cases, 17%), other (532 cases, 12%), tumor (470 cases, 11%), fighting (424 cases, 10%), no observation (335 cases, 8%), ulcerative dermatitis (317 cases, 7%), inflammation (133 cases, 3%), hunched posture (114 cases, 3%), rectal prolapse (77 cases, 2%), and dermatitis (68 cases, 2%). Of the clinical cases approximately 90% are mice, 6% nonhuman primates, 2% rats, 2% dogs, and <1% other species. Similarly for the mortality data approximately 95% are mice, 4% rats, and <1% other species. We hope this information can be used by other institutions for comparison with data they collect. Also we hope that it will open the door for sharing this type of information between institutions, so that benchmarks might be developed, enabling the possibility of addressing issues on a larger scale.

P62 Affordable Laboratory Animal Clinical Pathology for Emerging Economies

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Many developing nations are establishing or expanding biomedical research in their countries to develop drugs and vaccines for endemic diseases. In response to this trend and to serve the needs of colleagues in neighboring countries, we launched, in collaboration with ICLAS and funded in part with an AAALAC special projects award, a Latin America training fellowship in laboratory animal science. Fellows spend 3 mo in our department to learn current and innovative approaches to laboratory animal care. Veterinary clinical pathology is an important component of laboratory animal care at many institutions in developed countries, with specialized personnel and expensive, automated instruments. In emerging economies, clinical pathology services for laboratory animals are often absent or lack such instrumentation and staffing. Yet, without an effective health monitoring program, animal welfare and research quality may be compromised. An affordable clinical pathology approach was designed for research vivaria in underdeveloped nations, modeled for one fellow's institution in Uruguay. Practical testing needs for monitoring animal health were identified. A cost analysis was performed from which an appropriate panel of tests was selected. Using skills and understanding in clinical pathology, technical training and protocols were provided for methods to monitor animals for anemia, infection, and general health, including manual CBC and blood film reviews, urinalyses, fecal analyses, and cytology of blood and body fluids. Start up cost for this added laboratory animal service was estimated to be less than US$2000.00, with an expected average cost per test of less than US$1.00. With trained personnel, this panel of tests can be implemented for laboratory animal care programs in developing countries, thereby providing an affordable means to better monitor individual and colony animal health and promote quality research.

P63 Development of Strategy for the Attainment of Humanized Mouse Model for Studies of Sickle Cell Disease

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The mouse Hbatm1Paz Hbbtm1Tow Tg (HBA-HBBs) 41Paz/J corresponds to a humanized genetically modified model for studying sickle cell disease. It is a double knockout for mouse α and β globins and is transgenic for human α and β globins. Some hematological disturbances in the red cells and in the physiology of this animal model are parallel to the patient with sickle cell disease. However, these animals have reproductive difficulties. We breed sickle cell homozygous double knockout males with heterozygous females which have the mouse β chain and human α chain because the homozygous female is not able to produce live pups efficiently; although it induces anemia, it is not a severe profile anemia. However, this breeding combination produces very low rates of sickle cell pups. Our goal was to develop a breeding strategy for the production of more resistant homozygous knockout sickle cell pups generated by stronger, not anemic females, which might produce fetal hemoglobin that persists through adulthood. Therefore, we developed a strategy for programmed breeding of this sickle cell model with transgenic animals with the persistent fetal hemoglobin allele (g) from a Brazilian patient's mutation and the normal human β globin chain allele. The possible genetic combinations and their expected hemoglobin profiles were analyzed. The strategies included checking the genetic alterations of the offspring, which were products of backcross, by PCR for mouse α and β globins, as well as their respective knockout in order to get a completely knockedout globins animal. Real-time PCR testing was done using primers and taqman probes for the presence of the human β or βs globins differentiation within the transgenic alleles found in both transgenic animals. With these strategies we were able to successfully obtain a humanized animal that produces only human globins (α, β, and γpersistent) and does not depend on chimerical globins for its reproduction. This is an improvement on the process to achieve the sickle cell phenotype carrier animals when compared with the strategies used thus far.

P64 Evaluation of Biochemical and Hematological Parameters in Wistar Rats Exposed to Elevated Atmospheric Concentrations of Ammonia

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Although many effects of ammonia in rodents are known, there are divergences in literature regarding the concentration and duration of exposure which can cause morphologic and physiologic alterations in animals. This study was conducted to evaluate the hematological and biochemical profile of Wistar rats housed under adverse environmental conditions (ammonia build-up) during 5 (group 1, $n = 20$), 10 (group 2, $n = 20$) or 15 d (group 3, $n = 20$). For each experimental group, a control group was kept in another room in the Animal Facility (25 ± 5 ppm of ammonia), in order to serve as reference value for comparisons among the experimental groups. Increased levels of pollutants in the room was obtained by placing bedding (wood shavings) containing urine and feces and by reducing the ventilation rate in the animal room in order to achieve a concentration average of 90 ± 10 ppm within cages of all groups. The hematological and biochemical parameters were analyzed by the ANOVA test, followed by the Tukey test, for variables with normal distribution, or by the Kruskal–Wallis test for variables with asymmetric distribution. The hematological analysis revealed that the animals in group 1 had significantly lower hemoglobin (Hgb) and hematocrit (Hct) values than the other groups. In relation to biochemical parameters, again we see that group 1 statistically differs from groups control, 2, and 3 for aspartate aminotransferase (AST), amylase (AML) and gamma glutamyl transferase (GGT). The other measured parameters showed variable results among the groups and were apparently inconclusive. Finally, the data indicate that from the early exposure to ammonia until the fifth day there are alterations in the biochemical and hematological profile of the animals, which subsequently return to normal due to a possible adaptive response to adverse housing conditions.

P65 Evaluation of Intensity and Frequency of Noises Produced by Human Activity in Animal Facilities with Open-Cage System

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Laboratory animals are subject to a variety of noises caused by the routine care in the animal facility, which is something that may affect their welfare. Studies show that high frequency noises are capable of causing physiologic and behavioral changes in animals. Despite concerns about high-frequency sounds, low-frequency noises are dominant and originate from activities within the animal rooms. This study aims at assessing the intensity of low-frequency noises generated by human activity in animal rooms in an animal facility equipped with open-cage system. The noises were chosen by observing the work of technicians during daily routine at the central animal facility of UFRGS. The sounds were recorded using a class-2 decibel meter, calibrated in 20 hz to 20 khz frequency. The noises were selected and recorded on a laptop for later playback in 2 sessions of 15 min, simulating human work in the room. The selected noises were: 1) removing from and placing on the shelf a cage with rats, placement of feed and water dispenser in the cage, moving stacks of cages, placing the cage and the lid cage on the table; 2) cleaning of the room floor; 3) moving of room furniture; 4) opening and closing of a door; and 5) opening a faucet and letting the water run. Analyses were carried out with a digital audio editor. After the analyses, the following results were obtained for the each of activities: 1) 84.3 to 96.1 dB; 2) 80.4dB; 3) 84.6dB; 4) 110.1 dB; and 5) 77.7dB. We conclude that noises originating from work in animal rooms with open-cage system can reach levels exceeding those recommended and may compromise the welfare of animals.

P66 Why Are Indwelling Tail Vein Catheters Critical to MRI Imaging and How Do I Place One?

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Imaging modalities such as MRI and positron emission tomography (PET) have become prevalent within the research community. In vivo experiments, particularly in mice, have become an essential translatable model for human disease. The MRI team within the BioImaging COE (Center of Emphasis) at our institution has an increased demand for models studying CNS and oncology-related targets by way of contrast enhanced infusions. The Worldwide Comparative Medicine, Global Science and Technology group was challenged with the development of a system for continuous venous access while allowing for longitudinal imaging study conduct. With limited ability for long-term venous access in certain genetic strains and immune-suppressed mice, along with the difficultly of single injection access during scanning, we discovered that removable intravenous catheters were required for optimal success. These determinations led to the development of our technique for successful and sustainable tail vein catheterization and infusion. For this procedure animals were anesthetized under isoflurane while warm water, maintained between 39 to 40 °C, was used for vasodilatation of the tail vein. Materials used for this technique includes the use of a 26-gauge, 0.75-in. catheter, polyethylene-50 tubing, tape, and tissue adhesive. During development, we found many areas for refinement and adjustment to tail manipulations, equipment and material modifications, and addressed extension line dead space concerns for contrast delivery. While tail vein catheterization in the mouse can often be frustrating and time consuming for technicians, our refined technique led us to a high success rate of 99%. The challenge of repeated robust placement of tail vein catheters and contrast infusion enabled our research partners in the BioImaging COE to obtain high quality MRI data in support of our institution's portfolio.

P67 Challenges of Amphibian and Reptile Care and Use in ABSL3 Containment

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Research protocols involving infectious agents conducted at animal biosafety level 3 (ABSL3) containment introduce unique challenges requiring adaptations to standard practices of animal husbandry, care, and use already in accordance with the *Guide for the Care and Use of Laboratory Animals* and Animal Welfare Regulations since such practices must also comply with guidance provided in *Biosafety in Microbiologic and Biomedical Laboratories*. When the species involved in such protocols are not rodents, especially careful planning of logistics and practices is required to identify potential hazards and limit risks of occupational exposure. Studies involving the transmission and maintenance of eastern equine encephalitis (EEE) virus in American bull frogs, green anoles, and eastern garter snakes were conducted. Appropriate primary enclosures, living and nesting substrates, husbandry practices, and methods of animal handling were developed that limited splash and aerosol potentials and other risks of occupational exposure to the infectious agent. All procedures were conducted in a biosafety cabinet in a restricted ABSL3 facility. Individually ventilated microisolation caging (IVC) were used in static mode as primary enclosures with dry sphagnum moss and a perch provided for the anoles, dry sphagnum moss as an absorbent substrate for the snakes, and moist sphagnum moss as a living substrate for the frogs. Ceramic ramekins were used to provide a source of water. To aid in the handling of the animals, prior to opening primary enclosures of anoles or snakes, general inhalational isoflurane anesthesia was induced via the air supply valve on the IVC. Following frog anesthesia, waste MS222 in water was contained in a leak proof carboy. All waste was decontaminated by autoclaving out of the restricted facility. Animal care and use, ABSL3 containment, and research aims can be successfully achieved through creative cooperation between animal care, research, and biosafety staff.

P68 Withdrawn

P69 Development and Implementation of an Approved Enrichment Foods Directory for Cynomolgus Macaques

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In the research environment, animals do not have the opportunity to exercise as they would in a natural environment and overconditioning can be a problem. There may also be a tendency to overfeed animals with food rewards to the detriment of eating a nutritionally balanced ration. Overfeeding of enrichment foods and rewards can have an adverse impact on studies that require recording of food consumption. Furthermore, certain foods may not be compatible with study design because of adverse impact on study objectives. Finally, confusion may exist among staff as to what is or is not an acceptable food to be offered, the portion to offer, and for what purpose it is being offered. A search of nutritional reference resources was conducted to benchmark nutritional and caloric requirements of macaque species. Once created, the Directory was posted in the food preparation room in glossy poster format and bound in notebook form for training and reference. Criteria outlined included: supplier of product, approved product name, serving size, use categorization, Kcal portion offered, contaminant certification, and nutritional value. The Directory is easily replicated for the foods and portions offered and, when used by multiple personnel, provides consistency in the provision of nutritional enrichment foods. The establishment of an expansive directory from which to select food items has afforded a means for staff to experiment with many food choices, thereby providing variety for the animals and discovery of individual food preferences. The program promotes physical health and psychologic wellbeing of the animals while supporting research study design. Foods are used as an element of the environmental enrichment program for nonhuman primates, providing variety in the diet and foraging opportunities, as well as serving as rewards for desired behavior and medical treatment. The Approved Enrichment Foods Directory has provided a broad, clearly defined, quantified, and acceptable selection of foods with a well-defined diet implementation system.

P70 Rabbit Enrichment on a Budget

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While good husbandry is the cornerstone of a successful animal care program, a broad enrichment program is the key to ensuring laboratory animals' physiologic wellbeing. Our program houses a rabbit colony that, for study-related reasons, must be singly housed. Because of this lack of social interaction with their own species, we have endeavored to create a variety of novel environments that can stimulate our rabbits' physiologic and behavioral needs. An addition to our enrichment project is a rabbit condo. We created this condo with supplies we had around our facility, such as a Rubbermaid container, recycled trash cans, and reclaimed PVC piping. Not only does this enrichment environment satisfy behavioral needs, such as exploration, foraging, grooming, and digging, it is extremely cost effective. We allow our rabbits to get 30 min to 1 h twice per week of supervised exploratory time. We have created a roaming area, which can permit 2 rabbits at a time to interact. This roaming area is located within the existing rabbit room; it also has several toys for the rabbits to explore. This poster details these habitat ideas made with budget-friendly materials found in nearly all animal vivariums. These enrichment devices have been used successfully in our facility and have proven to reduce stress in our rabbits and encourage natural, inquisitive rabbit behavior. While no biologic experimental data was collected on stress reduction, behavioral indicators show that repetitive actions such as spinning, chewing on bars, and aggression have been drastically reduced within our rabbit population.

P71 Cost Saving Ideas for Animal Care Programs: Rodent Sentinel Testing

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In our current economy most state and federal budgets are facing cuts in spending, which in turn may impact the funding received by universities and research programs. In our department adjustments to our rodent sentinel program were explored as a way to trim our yearly budget without compromising quality care. A full overview of our sentinel program was undertaken to determine at which locations allocated funds were "appropriate" and where funds were being "wasted." A 2-tier testing paradigm was developed in which our specific pathogen-free facilities would receive the most testing whereas endemic disease facilities would receive less. The cost-saving strategies implemented for endemic disease facilities included: 1) creating a less expensive custom serology panel which eliminated testing for endemic pathogens, 2) reducing testing frequency from

quarterly to biannually, and 3) developing a targeted testing strategy to accommodate health requirements for inter- and intrainstitutional rodent shipping. For specific pathogen-free facilities changes include: 1) pooling of fecal samples per room for *Helicobacter* PCR and 2) standardization of testing in clean facilities. By implementing these strategies the cost reductions to our program were over 40%, even taking into account the cost of targeted testing when needed for the export of rodents. These savings were realized through decreases in serologic testing, sentinel animal purchasing, sample shipping, and per diem costs. In addition, our sentinel collection personnel saved over 160 h/y in our endemic disease facilities due to the reduction in testing frequency and subsequent sample processing. Rodent sentinel testing can prove an expensive endeavor and in cases of facilities with endemic disease frequent testing it is often redundant; however, significant cost savings to a program can be achieved by reevaluating the goals and strategies of a sentinel program.

P72 Refinement of Blood Collection Techniques to Permit Automated and Low-Stress Sampling from Canines

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Repeated blood sampling, such as that done for PK studies, is often performed using manual sampling techniques. While trained technicians act to minimize pain and distress to animals, manual blood sampling often includes repeated needles sticks, restraint, sensory stimulation, additional blood loss and danger of technician injury. Automated blood sampling (ABS) facilitates safe, efficient collection of whole blood from freely moving laboratory animals while maintaining animal health and comfort. ABS has many advantages over manual sampling: accurate sample volume, sample timing, and sample reliability. Due to the many benefits of ABS, we hoped to incorporate this method into our lab routine. During implementation, we encountered several small hurdles as we developed a working technique. Observed problems generally fit into one of 3 categories: 1) function of peripheral equipment (tubing, VAP, Huber needle), 2) operator error, and 3) surgery related issues. Each of these issues was addressed and rectified. After the initial trial and error period, we were able to perform a successful series of blood collection studies. A total of 6 dogs were used over 4 separate dosing/sample collection periods. Beagle dogs were implanted with a subcutaneous vascular access port (VAP) over the rib cage with the catheter inserted into a femoral vein. During dosing/collection periods, ranging from 6 to 48 h, dogs were housed in stainless steel cages. For all collection periods, either a Huber needle or cath-in-cath were used to access the VAP. The VAP was protected by a jacket and connected by a stainless steel tether to a single channel swivel connector on the cage ceiling. The swivel connector was connected by a second length of tubing to our ABS system. Catheter patency was good throughout all collection periods except for one instance of positional obstruction. Of the total of 156 samples programmed over the 4 sample collection periods, one sample was missed due to positional catheter occlusion unrelated to the ABS system.

P73 Development of a Technical Services Team for Husbandry Staff in a Large University Setting

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Animal resource programs provide a variety of services to investigators conducting animal research, including routine husbandry, animal procurement, veterinary care, compliance oversight, personnel training, specialized research support cores, and technical services. In 2006, our staff began to offer technical services to investigators. A group of animal technicians were trained to provide basic services, such as blood collection, ear tagging, tumor monitoring, and tail biopsies. The first year only 25 h of services were delivered but by 2008 nearly 400 h were provided. The dramatic growth in services created many challenges such as scheduling conflicts for technicians, inconsistent management of the program, lack of veterinary oversight, and instances of regulatory noncompliance. Over time, the compounding problems led to a decline in the number of services provided. To address these issues the program was redesigned and new organizational structure was created. The structure included a dedicated manager, a primary technician team and a secondary technician team. Technical services became the principal duties of the primary team technicians. An expanded training program for team members was created, a streamlined request system was initiated, marketing materials were generated and distributed throughout campus, and a process was developed to track services performed for investigators. The new program has led to increased revenue, customer requests for services have grown, and our technicians have increased their knowledge and value to our customers. The Technical Services Team is also being used to supplement the unit's other research services, such as blood and serum testing, necropsy, and rodent health surveillance assistance. Efforts put forward in the redesign of technical services has already shown great promise; the number of technical service hours recorded for the first quarter of 2011 (254 h) has already surpassed the total number of service hours for all of 2010 (191 h).

P74 Weight Management Program for Adult Cynomolgus Macaques

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Our institution has a stable population of breeding male cynomologus macaques (*Macaca fascicularis*). To maintain the animals at an optimal weight, we have developed a weight management program (WMP). Animals identified as being grossly obese, after a hands-on veterinary assessment, are enrolled in the program. We initially identified one obese male to enroll in the program as a pilot study. Normal feeding consisted of 20 to 40 biscuits (4.14 kcal/g or 26.5 kcal per biscuit) daily divided between 2 feedings. The first approach was to reduce the number of biscuits given. The reduction was based on the lowest normal biscuit amount given (20 biscuits). Reduction of feed by 10% saw no weight loss over 1 mo. Reduction of feed by 35% resulted in an increase in weight over 1 mo. A reduction of feed by 45% accompanied by dividing the feed between 3 feedings resulted in a steady decrease in weight each month. A twice a day feeding of a high-fiber (3.72 kcal/g or 67 kcal per biscuit) diet was tried. Nineteen biscuits daily resulted in an increase in weight over a 1-wk period. Reducing the daily high fiber-biscuit number to 10 with twice a day feeding, then to 3 times a day still saw an increase in weight over a 2-mo period. We tried 30% reduced feed (of regular biscuits) with a 3 times a day feeding, which saw a steady decrease in weight over several months. This became our WMP for obese animals. Over a 7-mo period 11 males, between the ages 8.3 to 16.1 y, have responded with similar results as the initial male. In conclusion a 30% reduction of the lowest normal biscuit amount with 3 times a day feeding showed the best results for a steady reduction of weight.

P75 Neurodegenerative Models and the Use of Gel Diet to Improve Survivability

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Neurodegenerative models create unique challenges for husbandry staff. Variations in daily care need to be applied based on phenotypic presentation to ensure viability of these strains. Of interest at our institution is the pale tremor model. Laboratory personnel approached our husbandry care unit with a need to increase survivability in this line. It was hypothesized that the expected phenotypic traits of pale tremor mice—stunted growth, ataxia, decreased motor function, and progressive muscular degeneration—could inhibit their ability to consume standard chow pellets. An alternative food source then could increase survivability in this model. Animals were given both standard chow (pellets) and a gel diet, fed ad libitum, in separate containers on the cage floor. Each food was weighed 3 d/wk to determine which food the animals were consuming. Mortality was also recorded to compare animals given the gel diet with previous lab reports of a 28- to 30-d average lifespan. To ensure accuracy in food consumption measurements, prestudy data was gathered to assess moisture loss in the gel diet over time. Autoventilated housing conditions were replicated and 0.50 serving of the gel diet (1.10 oz) was added to empty caging 3 d/wk (Mondays, Wednesdays, and Fridays) for 2 wk with measurements taken at each interval. Data showed that the gel diet experienced an average of 0.18-oz weight depreciation due to moisture loss per day. This data was used in correlation with on-study food consumption measurements to give a formula of $((x-y)-(0.18z))/n$ with x = preweight, y = postweight, z = number of days, and n = number of cage mates. Data gathered supported our hypothesis, showing pale tremor mice consumed the gel diet almost exclusively

over chow pellets at a rate of 0.08 to.02 oz, respectively, over the course of the study. Mortality rate decreased in pale tremor weanlings after the implementation of the gel diet feeding regimen, satisfying laboratory personnel's need for up to 6 wk (endpoint) of survivability. Based on these findings we would recommend the use of a gel diet for neurodegenerative mouse strains demonstrating phenotypic traits similar to the pale tremor model.

P76 Assessment of the Maternal Reproductive Traits of BALB/cByJNarl

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Studies have indicated that offspring body size is related inversely with litter size (LS) of the dam. In mice, LS is influenced deeply by dam breed (long-term reproduction), and affected by genetic and environmental factors. We collected 10 reproductive traits parameters of BALB/cByJNarl mating dams and further analyzed the reproductive performance, for example, additive distribution of parity, of the MC2 population mating dams in our institution from 2007 to 2009 to establish a high-yielding reproductive pattern. The results showed that postpartum breast-feeding of BALB/cByJNarl dams leads to difficulties in estrus cycle and mating performance, thus lengthening the distance between parities, and lowering the week average litter size significantly (compared with other strains, $P < 0.05$). We adjusted the litter size (adjusted litter size, ALS = 8 to 11) in April and May 2008, and found reproduction traits were lower compared with those in 2007 and 2009 (ALS = 9). The reasons may due to adjusting litter size and training of animal caretakers. In addition, the high peak of litter size was in the seventh parity; afterwards in the eigth parity, the litter size had a down trend. We concluded that adjusting litter size in the early parities can be beneficial to increase economic value of BALB/cByJNarl production, and analyzing the parameters can be beneficial to create a complete reproductive system in the future.

P77 Gnotobiotic Manipulation of Germfree Mice in Class 2 Flow Hoods

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Germfree mice are normally housed in sterile soft-sided bubble isolators which effectively prevent bacterial contamination, but limit access and hinder complex manipulations, such as gavage, tail vein injection, retroorbital bleeding, collection of urine and feces, among others. For this reason, our facility uses Class II Type A laminar flow biologic safety cabinets to house germfree mice for short term experiments. We have found that mice transferred aseptically to sterile microisolation cages can be maintained in the germfree state for up to 14 d in these hoods, and that such housing facilitates complex or repeated manipulations. Maintenance of sterility in this environment requires specific aseptic handling and the coordinated efforts of a minimum of 2 staff members, as described below. To transfer mice from the bubble isolator to the sterile hood, we introduce a sterilized polypropylene solid top and 11.5 × 7.25 in. box via the 18-in. isolator port. The mice are placed in the box and contained by a solid top. The box is aseptically removed from the isolator via isolator port and immediately transferred to a sterilized Class II Type A laminar flow biologic safety cabinet that contains sterilized water and sterile cages containing food and bedding. The solid box and surrounding area are then immediately sprayed with a liquid sterilant. The assistant helps the handler apply sterile PPE (gown, gloves, bonnet, and face mask) using surgical technique. The handler then transfers the mice into the appropriate groups. Sterile items (for example, needles, pens, transfer box) are aseptically introduced into the hood by the assistant. All manipulations in the hoods are performed by an operator wearing a surgical gown, gloves, mask, hair bonnet, with an assistant to provide sterilized items as needed. Using this technique, we are able to perform complex tasks and data collection without risking high cost, loss of time and most importantly, the germfree status of our colony.

P78 Improving Onboarding of New Husbandry Staff via Lean Management Principles

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Prior to creation of a formal onboarding program, new animal care staff in our department were placed directly in facilities and received on-the-job training. This resulted in variability in training and quality. To tackle this problem we used lean management principles to 1) analyze how new employees were trained, 2) develop countermeasures for initial testing, and, if proven successful, 3) apply those improvements to the new employee training process. We began by involving stakeholders responsible for training or supervising new employees. The training program staff, workforce and outreach manager, team leaders, and facility managers worked in collaboration to develop a standard plan for didactic classroom and facility hands-on instruction. As a result, after initial training is completed, new staff move into their assigned facilities and are paired with a team leader to coach them in performing daily tasks. Training continues for approximately 4 wk until the new employees are ready to be assessed for proficiency. Since 2009, we have onboarded 25 new rodent husbandry technicians. The average time needed before new staff could perform basic facility work proficiently and independently has been 72 calendar days. During this period, new employees have a posted training schedule and measurable assessments of progress and proficiency. In addition, facility teams have standardized tools and instructions on how to incorporate and train new team members. Using this program as a baseline, we further refined and improved our onboarding process. We are now able to train new staff, with little or no prior experience, to be fully functional team members that meet all required proficiency standards, in less than 40 calendar days.

P79 Crystals in the Water: A Systematic Approach to Determining the Cause

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In one of our buildings on campus, crystals were forming consistently in 16-oz, glass water bottles after being autoclaved at a standard cycle (121 °C, 17 lb/in.^{2}, for 30 min). These crystals could be described as "translucent flakes." The water in this building was Ann Arbor city water that was filtered to 0.2 µm, and the bottles were filled via bottle filler. After reviewing a paper, our initial hypothesis was that the rubber stoppers were causing this phenomenon so we substituted the rubber stoppers for neoprene stoppers and autoclaved them. Using the neoprene stoppers with the glass bottles did not stop the crystals from forming. Water samples were sent to an independent lab to have the crystals analyzed. We then tried several different scenarios to eliminate the crystal formation: filling and autoclaving the bottles in another building, filling the bottles in another building but then autoclaving them in the original building, filling them with water from a different municipal water source then autoclaving, autoclaving the bottles without stoppers, and increasing the cycle time from 30 to 45 min. All of the aforementioned scenarios still produced crystals. The results on the crystals from the independent laboratory were as follows: dominant presence of chlorine, sodium, magnesium, and oxygen, and moderate presence of calcium, silicon, minerals, and metallic salts. We expected the silicon to be a dominant factor in the sample because silicon was prevalent in the crystals in the referenced paper. An analysis of Ann Arbor city water was done and we were able to discuss our situation with an industry water expert. His hypothesis was that when the water is being autoclaved, there is enough water loss to create a super-saturated solution so that when the water cools the chloride ions are attaching to the silicon (among other ions) to form the crystals. In an effort to reduce water loss we covered the bottles with aluminum foil, but this still produced crystal formation. However, we filled and autoclaved a polysulfone bottle and this produced no crystal formation. We intend to have a working hypothesis for presentation at the meeting.

P80 The Use of Enrichment to Facilitate Data Collection in a Pig Study

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Recently a researcher approached our large animal facility manager about housing 19, 2-mo-old piglets and assisting with data collection. Part of the protocol required that the piglets be fed an individually measured high-sugar/high-fat diet over a period of 16 wk. The piglets

would also need to be weighed once weekly, a duty that he reported took him 3 to 4 h/wk. We were already short staffed and wondering how we would be able to take care of the piglets and collect the data needed for the study. The use of positive reinforcement training to reduce stress and facilitate voluntary cooperation in laboratory animals is well documented. Knowing that pigs are very intelligent animals, we decided to train the pigs to walk about 45 ft from their holding room, down the hall and get on the platform scale. After identifying a palatable treat that would not interfere with the study, we offered the piglets a sugar wafer in their runs daily for 3 d. For the next 2 wk we led each pig down the hall until they reached the scale while enticing them with the sugar wafer. After just 2 wk of training, all 19 pigs run down the hall and stand on the scale where they enjoy about half of a sugar wafer while their weights are being recorded. The positive reinforcement training was a stress free, positive experience for the pigs and the technicians, as well. This task is now accomplished in less than 30 min and the fast-growing piglets seem to enjoy their run down the hall.

P81 Animal Care Training in the Face of Colony Expansion

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Our animal health team comprised of veterinarians, technologists, and husbandry staff has consistently provided a high level of animal care in our facility. Challenged by program expansion primarily driven by an increase in animal population, a modification of the roles and responsibilities of all levels of staff became necessary. In response to these demands, we developed a training program to educate all staff levels as to new expectations, responsibilities, and a case-management algorithm. Staff were trained within a framework of 3 stages involving group training, individual training, and a combination. Within this framework, a case-management algorithm was created to serve as a training and assessment tool. Upon completion of the training program, technologists transitioned from the basic identification of health issues to making the actual decisions for the treatment and euthanasia of rodents. In addition, husbandry staff was trained to uniformly identify health issues. Instead of waiting for the veterinarian to assess animals, treatments were administered immediately upon examination by the technologists resulting in improved response to therapy. Animals requiring euthanasia were identified and euthanized more efficiently, improving animal welfare. Thus, as a result of the training program, all levels of the animal care team were empowered and the quality of animal health care was advanced with improvements in efficiency and a reduction in animal losses.

P82 Communicating with the Principal Investigator: How Templates Reformed Our Work

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There is a growing demand of disseminating vital animal husbandry information such as treatments, health status, mortalities, and others from the veterinary staff to the principal investigator (PI) using a simplified means for all involved. This information is critical and can affect costly studies. Clear communication, both verbally and written, is critical and must be completed in a timely and concise matter. In our institution, veterinary technologists must effectively communicate with PI on a daily basis, whether it is in person, on the phone, or via email. Past instances of communication often produced grammatical errors, incomplete information and questions from individuals about veterinary jargon. An immediate need arrived to streamline this process in order to reduce errors and simplify communication. An extensive template system and corresponding PI communications SOP was created for the veterinary technologist staff to use as a guide when communicating both written and verbally with PI. We will demonstrate how this easy new template has benefited our program tremendously by ensuring important information is relayed to the PI quickly and efficiently thereby reducing the need for follow-up communication.

P83 The Impact of Bedding Type on Cage Changeout Frequency

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The frequency of cage changes varies among institutions as a result of several considerations: animal stress, allergen exposure to personnel, experimental interference, and costs associated with bedding and sterilization procedures. The goal of this study was to evaluate the effectiveness of a new sanitized corncob bedding material as compared with standard corncob in a ventilated mouse rack system. Intracage ammonia levels, bacterial growth and absorptive capacity of bedding were measured for cages of female C57BL/6 mice under standard and autoclaved conditions on static and ventilated racks in a barrier facility. Intracage ammonia concentration was measured daily, and cages were removed when measurements were equal to or greater than 25 ppm. Quantity of bacterial growth and bacterial species in bedding were determined at the time of cage removal. Bedding absorptive capacity and bacterial load were also evaluated in all conditions without the addition of mice. Cages with nonautoclaved sanitized corncob bedding took significantly longer to reach ammonia concentrations of 25 ppm than standard corncob. Autoclaved sanitized corncob bedding did not differ significantly from nonautoclaved standard corncob in length of time required to measure 25 ppm ammonia. All nonautoclaved sanitized corncob cages remained in the study a minimum of 3 wk. No significant differences were noted on bacterial load at the conclusion of mouse housing. Standard corncob was significantly more absorbent than sanitized corncob bedding, and autoclaved sanitized corncob bedding was significantly more absorbent than autoclaved corncob. This study demonstrated that mouse cages with nonautoclaved sanitized corncob bedding on ventilated racks may be used with a cage change interval of 3 wk.

P84 Reduce, Reuse, Recycle: How an Animal Resource Program is Going Green

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Our institution produced over 15,000 tons of waste in 2010, which amounts to 41 tons of waste produced daily. We have a very active recycling program and although the national average for the recycling of solid waste is 38%, our program recycles almost 60% of its solid waste. This percentage equates to 23 tons of daily solid waste recycled instead of heading to the local landfills. The Animal Resource Program (ARP) has long participated in the standard recycling program consisting of office paper, aluminum cans, plastic bottles, and newspaper. However, ARP decided to reevaluate its recycling program to determine if more of the solid waste produced by animal operations could be recycled. In consultation with the university solid waste representatives, the Environmental Health and Safety program and management at the Animal Resource Program, more items were identified that could be recycled. Besides dirty bedding, which is all recycled through the universities composting site, the Animal Resource Program set up recycling of plastic syringe casings, glass bottles, shrink wrap, feed bags, and other items. ARP management plans to periodically review its recycling program to ensure that the program continues its success.

P85 Ensuring Animal Welfare through Effective Cage Checks

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According to the *Guide for the Care and Use of Laboratory Animals*, all animals should be observed for signs of illness, injury, or abnormal behavior by a person trained to recognize such signs. Consistent with the *Guide* and as an essential component of our institution's animal care and use program, we require these cage checks to be performed on a daily basis by members of the Vivarium Operations (VO) team within the Laboratory Animal Services (LAS) department. However, in 2008, the adequacy and effectiveness of cage checks were identified as areas of concern at our institution. Specifically, there was not a standardized process for completing these mandated checks adequately, the staff did not fully understand the importance of cage checks, and there were general concerns about the welfare of animals due to the inadequacies of cage checks. Therefore, members of VO Management and LAS Training Services created a standardized cage check process, and incorporated the associated Cage check training module into our animal use training, instruction, and certification system. Cage check training provides comprehensive species-specific information, microenvironment requirements, and standardizes the

cage check process. In order to "keep it short and simple" (KISS), the cage check process was distilled down to the following 5 observations, which are performed in a specific order and manner: water, food, animal, cage, and enrichment (WFACE). Cage check training is now a standard component in the training regimen for all new employees and has enhanced the welfare of the animals at our institution.

P86 Incidence of Clinical Calls in African Clawed Frogs (*Xenopus laevis*) before and after Changes in Watering System

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Until 2007 our institution maintained its colony of African clawed frogs (*Xenopus laevis*) in large tanks (114 L, maximum 15 frogs per tank) arranged in a flow-through drip water supply system. In February of that year, the majority of the colony was moved to smaller tanks (50 L, maximum 5 frogs per tank) arranged in a recirculating water supply system. A survey of clinical calls, including for animals found dead in their enclosure, from before and after that time revealed a decreased incidence of morbidity and mortality after the switch. Many of the clinical calls were placed due to signs of red leg, bloat, and the shedding of their skin. Necropsy results showed the most common results of deaths were due to gram-negative sepsis, with *Aeromonas* spp., the most commonly associated pathogen, mycobacteriosis, and idiopathic chronic renal disease, including nephritis and nephrosis. Prior to the switch, there were 0.118 clinical calls and 0.144 nonexperimental deaths per animal per year. Afterwards, there were 0.090 clinical calls and 0.056 nonexperimental deaths per animal per year. This is in contrast to the purported drawbacks of a recirculating water system, including the potential for cross contamination between animals. Potential causes for this discrepancy include temperature fluctuations within larger tanks, as well as, the lower housing density in the smaller tanks, with 10.67 L per frog in the smaller tanks verses the 8.49 L per frog in the larger tanks.

P87 Eradication of *Helicobacter* spp. from Multiple Mouse Strains: Results of Cross-Fostering

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We have successfully eradicated *Helicobacter* spp. (*H. bilis*, *H. hepaticus*, and nonspeciated *Helicobacter*) in multiple infected mouse strains by fostering pups up to 10 d of age from *Helicobacter*-positive parents onto *Helicobacter*-free foster mothers in a nonbarrier environment. Infected parents were fed a medicated diet for a minimum of 1 mo. Pups are removed from parents (1 male and 2 females) generally within 48 ± 24 h after birth and cross-fostered onto Swiss Webster *Helicobacter*-free mice after removing their pups from the cage. No special manipulations or cleaning of foster pups was performed prior to placement. Foster mothers were fed a regular, nonmedicated rodent diet. Fostered pups were *Helicobacter* PCR-tested using pooled fecal pellets—one pooled sample per cage at weaning and 4 wk later. Over a 4-y period, 2300 pups of multiple mutant mouse strains were cross-fostered. To date 100% of pups remain *Helicobacter*-negative. Fostered pups to be used as self-propagating foundation colony stock were moved to the barrier facility, which remains *Helicobacter* and murine pathogen free. Our results are the first to describe continuous, large-scale successful cross-fostering of pups from *Helicobacter*-positive parents. In contrast to other reported labor-intensive techniques, or barrier maintenance, our fostering method is simple and does not interrupt breeding of mice. Foster pups can be raised in a nonbarrier room before confirmation of their *Helicobacter*-negative status and relocation to the barrier facility. Cross-fostering took place between 1 and 10 d after birth and 19% (n = 436 pups) were cross-fostered as late as 10 d after birth and still remained *Helicobacter* negative.

P88 Auto Water: An Easy Way to Deliver Medicated Water to Cages without the Hassle of Switching to Water Bottles

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Our institution has over 10,000 cages of rodents housed in individually ventilated cage racks outfitted with manifolds to deliver drinking water. Some investigators needed to provide medicated water to several hundred cages of rodents. Our only solution at that time was to discontinue automatic water and provide the medicated water via water bottles. This posed a few issues for our staff. They did not like handling the heavy cases of water bottles or changing the bottles in the cages because of ergonomic issues. The cage changing process took much longer with the water bottles and the addition of the medication to each bottle also took a lot of time. We developed a way to deliver water without using bottles. After disconnecting the rack from the automatic water, we secured a sterile 5-gal carboy to the top of the rack with silicone tubing, stop cock and stainless steel connector on the end of the tubing. We then added medicated water to the carboy, connected it to the rack manifold, opened the valves on the rack to let the water fill the rack completely and checked that all drinking valves were working properly. The cap was loosened slightly to allow water to flow freely. The water flow and level was checked daily to assure proper function. Fresh medicated water was added as necessary. The entire carboy and tubing was replaced with sterile parts at least once a month and an SOP was developed for care and maintenance of the system. We currently have between 700 to 1000 cages on racks outfitted with carboy water systems. We have used this type of carboy water system successfully for over 10 y.

P89 Maintaining Proper Humidity Range in Fluctuating Climates

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Many facilities face the issue of low humidity during the cold, dry winter months and high humidity during the warm humid summers. Although the *Guide* offers an acceptable range for humidity in animal rooms of 30% to 70%, many facilities struggle to maintain this range on extremely warm or cold days in the summer and winter seasons. With minor modifications to our facility's approach to humidity control, we have been able to stay within the acceptable humidity range and reduce adverse conditions for our animals. Our air handling units (AHU) are controlled by a system with adjustable set points based on real-time feedback of outdoor temperature and humidity (span blocks). Span blocks maintain the desired humidity level by adjusting on a linear scale, the heating and cooling settings of the AHU. Thus outside air temperature controls the preheat valve and chilled water valve. Adding moisture (humidity) into warm air is the key to maintaining animal room humidity above 30%. Discharge air from air handling units can be as high as 60 to 62 °F in cold, dry weather, and as low as 52 to 55 °F in hot humid weather. We are now able to maintain our humidity levels within the range with the help of the span blocks. It is important to know the type of air handling units and how they are controlled to maintain proper humidity levels at your facility, in variable outdoor conditions.

P90 Specialized Husbandry Improves Welfare in a Mouse Model of Spinal Muscular Atrophy

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Neonatal models require special husbandry and nursing to assure humane care and allow proper evaluation of complex therapeutic strategies. Spinal muscular atrophy (SMA) is a devastating hereditary neuromuscular disease that often results in death from muscular atrophy and respiratory failure by 2 y of age in severe type 1 patients. The SMA (SMN-/-) mouse model recapitulates the human disease. Affected pups exhibit severe muscle weakness and growth retardation within 5 d of birth and generally die or require euthanasia for humane reasons by 14 d. We have recently reported marked improvement in neurologic function and survival in P0 pups treated with gene therapy or antisense oligonucleotides that increase SMN levels in the CNS. Although treatment may prevent neurodegeneration, vascular necrosis of the tail, digits, and ears often complicates recovery beginning around 21 d. An analogous condition affects some severe type I SMA patients with endstage disease. Without intensive support, SMA pups with necrosis require euthanasia because of impaired feeding ability and secondary infection of necrotic tissue. Skilled nursing care now allows us to maintain animals in an acceptable welfare state for 1 y or longer. The regimen provides nutritional supplementation, with antibiotic and analgesic medication as required. Animal care technicians score animals using easily applied criteria to allow rapid identification of animals which require intensive care or euthanasia for humane reasons. With specialized care, mice continue to grow

and perform well on weight bearing tests. Indeed recent research has also demonstrated possible therapeutic interventions that may control vascular necrosis. The care we provide keeps treated SMA mice in an acceptable welfare state, allowing investigators to develop therapeutic approaches that might significantly improve the prognosis and quality of life of patients with this severe debilitating disease.

P91 Turning "Trash" into Treasures: Cost-Saving Methods for Animal Resource Management

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The current economic climate has been stormy. A disastrous financial period that has persisted for years makes every operation reassess where money is spent. The reality is that our institution's research and instruction missions must continue, but the way the tasks are accomplished have to change. Our department has used new methods and inexpensive materials to provide educational and research services at a low cost of time and/or resources. Cost of the materials used for various projects was minimized by researching the availability of supplies and finding the least cost item that would accomplish the task. Materials were procured from thrift and large chain stores, borrowed for short term use, or recycled. For example, a stuffed animal was purchased from a local thrift store and modified with a discarded syringe case and tubing to simulate a trachea and esophagus to demonstrate the anatomy pertinent to intubation of animals. Chenille sticks (that is, pipe cleaners) are used to designate rows on ventilated racks that are being sampled for health surveillance. Other examples of innovative uses for common items will be detailed in this poster. Recycling and repurposing supplies used for teaching and research has provided the opportunity to use management skills learned through CMAR studies and the creative talents of our department's staff. Thrifty resource management has the potential to be used as a motivational tool for staff by providing incentives for innovative resource management. Creativity and fiscal management training can be implemented in all aspects of animal resources management to provide education and services in an economical and novel manner.

P92 Innovative Approaches to Managing Odor and Moisture Levels in Diabetic Rats

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Rats are routinely used as a model for diabetes mellitus, which can result in frequent urination. This results in the need for frequent cage changes, markedly increasing the time and labor required to maintain humane conditions. Our static rats are housed on 1/8-in. corncob bedding, which becomes saturated with urine quickly, requiring daily cage changes. This may increase stress on the animals, and may adversely affect study results. In addition to bedding saturation, the urine of the diabetic rats has a distinct smell that permeates the cages and the housing room. We hypothesize that using a more absorbent bedding in place of the corncob may reduce the cage changing frequency and diabetic aroma. This could decrease the stress level of the animal, improve the cage conditions, and reduce the olfactory impact on staff. Long–Evans rats were injected with streptozotocin to induce diabetes and labeled "excessive urinator" by the technician. All were housed in static caging with corncob bedding and a 12:12-h ambient light: dark cycle. Bedding was weighed prior to placing animals in caging and then again 24 h later to assess approximate urine output. Water intake was recorded and wetness of the bedding was subjectively scored. After 4 wk, the cages were assigned into 2 groups so that each group had similar average urine output. Room temperature and air pressure were logged daily. In week 1, group A received 1250 mL of bedding no. 1, consisting of 0.25-in. paper squares. Group B received 1250 mL of bedding no. 2, consisting of a combination of 0.25-in. paper squares and corncob bedding. In week 2, group A received 1250 mL bedding no. 3, consisting of pelletized recycled paper. Group B received 1250 mL bedding no. 4, consisting of a different manufacturer's 0.25-in. paper squares. In week 3, group A received 1250 mL of bedding no. 5, consisting of pelletized hardwood sawdust. Group B received 1250 mL of bedding no. 3. Cages were observed daily Monday through Friday and scored for wetness level, being changed if 0.25 wet or more. Cage changing, based on observed wetness, decreased by 50% using beddings no. 3 and 5 when compared with corncob bedding. There was only a small advantage in using bedding no.1, 2, and 4. This study indicates that using alternative bedding no. 3 or 5 may reduce the cage changing frequency of diabetic rats.

P93 Management of a Rabbit Model of Osteomyelitis Using Probiotics and Objective Tracking Measurements to Maintain Normal Gastrointestinal Function

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Therapeutic and experimental use of antibiotics in rabbits can be challenging due to the sensitivity of essential microorganisms in the cecum required for breakdown of indigestible fiber. Any disruption of the normal intestinal flora can potentially lead to fatal microbial dysbiosis. Significant gastrointestinal (GI) distress, indicated by varying degrees of diarrhea, was observed in a New Zealand white rabbit model of osteomyelitis following antibiotic administration. Per standard operating procedures, rabbits were singly housed and provided with automatic water. Rabbits received a pelleted diet, supplemental hay, and dietary enrichment. Pain management for osteomyelitis was provided via opioid analgesia, which may have contributed to some inappetence. In order to maintain normal GI flora and prevent diarrhea, prophylactic treatment included nutritional supplements such as probiotic oral gel mixed with flavored Greek yogurt, acidophilus tablets, and commercial critical care diet. Presentation of the supplements ranged from free choice to syringe-feeding depending on the degree of weight loss. Initiating prophylactic treatment of probiotics resulted in a significant decrease in the incidence of diarrhea over 5 trials. However, the frequency and severity of diarrhea was greatly dependent on the antibiotic used in each trial. In order to provide a consistent and objective assessment of lameness, food intake, fecal output and consistency, visual guides were created and used as training tools for the research staff. Over time, we developed a successful method in which to manage the colony using nutritional support and probiotics to maintain healthy microorganisms in the intestinal tract as well as an objective and clear assessment of clinical signs. In conclusion, rabbits can maintain normal GI function with antibiotic administration when probiotics in a palatable form are made available.

P94 Evolving Towards a Cohesive Animal Care and Use Program

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Animal care and use programs are typically divided into 2 functional entities, the IACUC members and support staff and the animal husbandry program, which may result in 2 groups that are at odds with each other. IACUC tend to focus on the overall regulatory compliance and animal welfare while the animal husbandry program focuses on the day-to-day animal care and welfare. To establish a quality animal care and use program, it is essential that these 2 factions work together beginning with a solid foundation of similar missions and goals. These similarities may be actualized through a variety of methods including building cross-functional teams, creating comprehensive training programs, jointly developing post approval monitoring programs, formalizing open communication pathways and colocating office areas. We have built cross-functional teams by including members of the IACUC, the IACUC support staff, the husbandry staff, and the veterinary staff, with members of the research community on several different committees: the Safety Committee, the Communication Committee, SOP Subcommittees, and a Training Committee. Our Training Committee oversees a comprehensive training program where members of the IACUC support, husbandry, and veterinary staff work together to create and implement IACUC-approved online and wet-lab training sessions. The basis of our Post Approval Monitoring Program is to use members of the IACUC, research, and husbandry staff as a team to monitor research projects and keep the university in compliance. While each of these activities enhance communication, we augment it by holding quarterly Small and Large Animal Advisory Committee meetings and Animal Care and Use Program Informational meetings that are open to all personnel. The major challenge that we have faced has been colocating offices, and while this would greatly assist with the cohesion of the program, at this time, due to space constraints, it is not possible. The

benefits of a cohesive animal care and use program is reflected in the improved overall care and welfare of the animals, a higher quality of research projects, a commitment to institutional compliance and improved staff morale.

P95 Establishing a Mouse Quarantine and Rederivation Service Core

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Mouse health and data integrity can be compromised with the presence of pathogens. To protect these, physical and functional barriers that redirect mice from unapproved sources (regardless of their written health assurances) to a process of quarantine, evaluation, treatment, and/or rederivation prior to facility entry, were instituted. A separate, autoclave-out quarantine facility was constructed; and a unidirectional process of standard procedures, from receipt to characterization-rederivation to release, was implemented. Two isolated housing rooms, one for uncharacterized mice and another for mice established as specific pathogen-free, lead to an intervening laboratory and equipment decontamination area. This room is equipped with 2 pass-through, prevacuum sterilizers with bioseals that are assessed daily for efficacy; and all ventilated caging and equipment are autoclaved here prior to sanitation. Mouse receipt into quarantine and release to the facility occurs through separate, dual-access Class II biosafety cabinets. Uncharacterized mice are treated for potential parasites using an antiparastic topical application on days 1 and 10 of their quarantine. During the first, third, and fifth weeks an antihelmintic medicated diet is given and evaluation by gross magnification of the pelage, cellophane tape test of the perineum, and pooled fecal floatation are also completed. A confirmation of health status is derived via comprehensive panels of serological and polymerase chain reaction tests. For this, at least one mouse from each cage and no less than 25% of the population is evaluated to achieve a 95% confidence such that the results reflect the population's health status, assuming an infectivity rate of 15%. In the event that positive results occur, services of embryo transfer, cesarean rederivation, ovarian transplant, and cross-fostering to surrogates are provided. Cross fostering, our most used method, has been an effective process with a 97.4% success rate in the last 14 mo. All mice, original and rederived, which test negative, are released into the vivarium for use. Establishing a mouse quarantine and rederivation core permits more diverse and unique mouse genotypes to be acquired from unapproved vendors for research.

P96 Development of a Multipurpose Inhouse Animal Data Management System

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Managing information in an animal facility is important for regulatory requirements, cost accounting and space and staff planning. Automated systems should improve the workflow of your current functions, be user friendly and collect data efficiently. To support the IACUC and Laboratory Animal Resources (LAR) functions, an internal web-based Research Management System (RMS) was developed. The system consists of an IACUC portal that allows input of IACUC protocols, collection of review comments, status tracking and approval. The InVivo Experiments portal allows principal investigators to submit an experimental protocol, which describes the technical details of an individual animal experiment, along with the animal order for that experiment. The system tracks orders against the number of animals approved in the IACUC protocol and overall animal usage automatically. Because the system is web-based, researchers, veterinary and LAR staff are able to readily review records of ongoing or past studies from any networked computer. LAR management is able to track trends of animal usage and verify that procedures performed on experimental protocols have been approved in the IACUC protocol. This system has improved efficiency by decreasing input of redundant data and reduced excessive paper generated for purchase orders and protocols. Although inhouse development of animal resource software may be time consuming and costly up front, work efficiency and positive end-user feedback make the venture a worthwhile investment.

P97 Improving Animal Care through Proper Reporting Procedures

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This training session had a number of objectives. Animal care staff were trained to improve their observation skills and correctly document and describe the health condition on our health cards. Additionally, they were trained on which situations are considered emergencies and which are not, and how to properly relay emergency situations to the appropriate member of the veterinary staff. The Chief Diagnostic Veterinarian gave a presentation to the animal care staff in each of our 7 animal facilities. This presentation consisted of information regarding emergent and nonemergent cases, as well as a number of photographs of cases seen in our facility. When presented with a photograph, the staff was asked how they would document it on the health card. There were plenty of opportunities for interaction, as the presentation had over 60 images of different health conditions, as well as some that were marked as normal. A veterinary technician assigned to the area was often present for the sessions, and added information regarding specific animal health concerns that are seen in that building. In all 7 facilities, there was an increase in the correct diagnosis being documented on the health cards. In 6 of the 7 facilities, the number of cases reported to veterinary technicians increased. Although there are more cases being brought to the attention of the veterinary technicians (one building had a 25% increase in reports), the more specific diagnosis made on the card ensured that veterinary technicians were more prepared to properly treat the animal, and did not have to return to the office to get the proper supplies. This training seemed most beneficial to buildings that had a higher number of inexperienced animal technicians. All technicians now have more of a dialogue with the veterinary technicians, verifying that they have made the correct diagnosis, and continuing to learn more to improve their ability to diagnose the issues.

P98 Double Decker Caging for Ferrets

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There are no specific USDA or NIH *Guide* housing space requirements for ferrets beyond providing for "normal postural and social adjustments with adequate freedom of movement." Ferrets are usually housed in rabbit cages or large tubs, both of which are unsuitable for infectious disease research work. In order to provide containment for ABSL2 influenza research work in ferrets, we had been housing them in large-sized guinea pig internally ventilated caging (IVC). The guinea pig caging had sufficient floor space but did not allow ferrets to express activities such as rearing up and stretching. While attending the 2008 National AALAS meeting, we identified a new IVC caging system that contained a second tier or shelf ("double decker") within the main cage body, designed to be enriched housing for rats. This taller IVC sparked the idea that it might be useful for ferrets on ABSL2 studies. Using a sample "tester" cage, we discovered that the cage height was perfect but that there were 2 flaws that required modification to be ferret-suitable. First, the ferrets were unable to use the shelf since the solid plastic was too slippery for them to "get a grip" and pull themselves onto it effectively. Second, the feeder was designed for rats which meant that the far more curious, tenacious and stronger ferret was able to detach the feeder. We worked with the vendor to find solutions to both issues: the shelf was modified from solid plastic to wire bar, and the feeder became a hopper suspended from the shelf by a more robust attachment. We have been successfully using this modified IVC caging over the past 18 mo and have found that the "double decker" allows for full postural adjustments of the ferret while providing containment compatible with ABSL2 research work.

P99 Improving Efficiency and Productivity by Implementing a Web-Based Laboratory Animal Management Systems Combined with RFID Technology

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While Laboratory Animal Management Systems (LAMS) are commonly used to facilitate research animal facility operations, many systems are limited in scope and accessibility and often are

simply data collection tools leaving operations to continue to rely on manual, redundant, paper-based processes. This leads to inefficient use of personnel, is not scalable and makes tracking for auditing and compliance difficult. In order to better manage the operations of a multisite, academic, research animal facility, it was determined that a web-based LAMS, which could be integrated with internal systems (IACUC and financial) and combined with an automated census process, would deliver the functionality needed to meet the objectives of a paperless, streamlined operation and allow timely data delivery. Commercial vendors were evaluated and internal teams were established to detail functional and technical requirements. After vendor selection, refinements were made to the system and included using radio frequency identification (RFID) technology to conduct census. Implementation of the system was done in a phased manner, allowing time to identify and resolve technical and functional issues. The new system included online submission of procurement requests, direct verification of financial information through an electronic interface, email notifications, and reporting on housing and billing. Challenges faced included educating researchers about the proper use of RFID, obtaining adequate staffing during development and creating interfaces between several systems. Factors contributing to the successful implementation were institutional commitment, dedicated project management staff and documenting of baseline processes for requirements development. Initial assessment of the new system indicates that there has been a 50% reduction in data entry requirements, fewer questions regarding census and billing, faster identification of census exceptions and an increase in the availability of data. Overall, this project demonstrates that through the implementation of a web-based LAMS and RFID technology animal facilities can create a nearly paperless environment, eliminate redundant processes, improve census and tracking, and provide timely access to information.

P100 A National Assessment of Occupational Health Programs for Animal Care and Research Personnel in the United States

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A national survey was conducted to assess occupational health and safety (OHS) programs and practices for immunoprophylaxis protocols and tuberculosis screening across US biomedical research setting. ACLAM & ASLAP veterinarians were surveyed (n = 953) via a web-based logic-based questionnaire. Responses describing institutional OHS programs were received from 621 veterinarians, of whom 311 were self-designated as "Attending Veterinarians"; a total of 308 completed surveys were analyzed. Respondents represented universities, pharmaceutical/biotechnology companies, contract laboratories, nonprofit organizations and hospitals, civil and uniformed services, vendors, and others. Results showed that programs were well-developed at the majority of institutions, enrolling veterinary, husbandry, and research staff at rates exceeding 90% and involving multiple modalities of health assessments and risk communication for vaccine-preventable diseases. Most (72.7%) institutions did not store serum samples from animal research personnel. Over half of surveyed institutions housed nonhuman primates of one or more species and confirmed tuberculosis screening programs. Methods for screening of personnel for tuberculosis exposures were highly varied. Not surprisingly, immunoprophylaxis protocols included a range of recommended or required vaccines that differed depending upon job duties, type of institution, and nature of scientific programs. A single case of an identified vaccine-preventable illness in a laboratory worker was reported. Tetanus was the predominant vaccine administered (91.7%) to animal care and research personnel, followed by hepatitis B (54.8%), influenza (39.9%), and rabies (38.3%), among others; for some vaccination protocols, inconsistent rationale for administration was evident. Our comprehensive survey did not identify extensive programmatic deviations from current OHS guidelines which would increase the risk of workplace exposure to vaccine-preventable illnesses associated with animal research. We recommend continued investment by institutions, with intellectual capital and multidisciplinary input, to rationalize approaches to vaccination and tuberculosis screening practices relative to hazard-based health objectives and outcomes.

P101 Investigation into the Integrity of Disposable Nitrile Gloves Sprayed with Chlorine Dioxide-Based Sterilant in Rodent Facilities

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In contemporary laboratory animal medicine, a common practice involving personal protective equipment (PPE) selection is the use of disposable gloves and application of disinfectant before manipulating laboratory species. This practice varies between facilities and was developed through personal habit and history of the institutional PPE culture than on scientific evidence. At our institution, a reported chemical burn of a researcher resulted in an investigation into current practices for use of chlorine dioxide-based sterilant. An informal survey of use of gloves with chlorine dioxide-based sterilant amongst our veterinary, animal care, and research staff identified a dysregulation of individualized glove practices resulting from unique work efforts, personal preferences, and observation of others. Nitrile glove permeability studies contracted by University Laboratory Animal Resources at our institution identified differences in relative permeability of both nationally and internationally manufactured nitrile gloves (4 to 5 mm thickness) to chlorine dioxide-based sterilant at a 1:5:1 concentration for freshly prepared solution, compared with solution that was 1 wk old. American Society for Testing and Materials F739-07 standard test methods were used to demonstrate variability in relative permeability. A disruption in permeability for certain nitrile gloves types was noted within 60 min of 1:5:1 exposure between the selected nitrile glove types (n = 4) tested over a 2-h exposure period. The investigation into best practices involved veterinary, research, and husbandry staff, as well as experts in environmental health, distributors, vendors, and international and national manufacturers of nitrile gloves. The review of our PPE process has resulted in a renewed training effort, including expectations that gloves will be changed within 30 min of chemical exposure, and updates to relevant SOP. The use of an evidence-based approach to PPE practices has had important implications for maintaining worker occupational health and safety and assisting with the rationale for financial investments in PPE and facility operations.

P102 Transporting Rodents between Buildings within Walking Distance: A New Approach

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During rodent transport, it is critical to keep the microisolation unit intact to ensure animal wellbeing, minimize human and animal exposures, and minimize research impact. Safe transport should prevent escape, injury, and exposure to pathogens, and minimize stressors like temperature and humidity extremes, odors, and excessive noises. Our institution's Office of Laboratory Animal Resources (OLAR) has transported rodents in microisolation shoebox cages inside a wheeled cooler (used every day for picnics) between buildings. The cooler was large enough to accommodate 2 mouse cages housing up to 5 mice per cage or one rat cage housing up to 2 rats per cage. Data will be presented to demonstrate that temperature and relative humidity were maintained within the parameters for rodents set by the *Guide for the Care and Use of Laboratory Animals*. By using the wheeled cooler; OLAR has been able to successfully transport rodents safely in simulated extreme weather between buildings, without exceeding temperature and relative humidity parameters. There was minimal stress to the animals because they remain in their cages during the transport and the transport was less than 20 min from point of origin to the point of destination.

P103 Punch List: A General Purpose Facility Management and Animal Welfare Tool

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Animal facility construction projects are complex and require close oversight by institutional animal resource groups, from initial planning-design through final acceptance of the building when the certificate of occupancy (CO) is issued. If not well managed, project delays may result in additional cost, both financial and scientific. We will present a punch list (PL) that was developed to help manage final

acceptance of a major vivarium construction project. A PL is a to do list of repairs or incomplete work. It is an essential tool commonly used by engineers to help manage and document completion of building systems and finishes per design specifications. Developing a useful punch list can be performed on a project basis, customized to specific needs. Determining essential tools and time-efficient PL practices are important considerations, especially for larger projects. At project turnover, baseline data collected will serve as a resource for animal resource groups who may need to manage these activities, especially in an era of diminishing resources. Punch-listing, is readily learned by animal resource staff with minimal training and knowledge gained will be useful for other normal facility operations, such as completing future building renovations, implementing predictive failure-maintenance programs or for budget forecasting. Equally important, baseline PL data and data accumulated after CO, serve as cornerstones for managing vivariums to ensure quality animal welfare and care.

P104 Incorporating the 3Rs into a Training Program

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Training is an essential component of any reserach program; the need for trained personnel is not only a regulatory requirement, in some instances, it is also vital to the success of research. How can we incoproate the 3Rs into a training program that still allows personnel to gain the necessary skills and competencies required to perform their job? Repurposing of unused animals or animals that have undergone minimally invasive procedures is one way to address the reduction component of the 3Rs. Using video or classroom presentations can be a form of replacement but can often leave personnel without any practical experience. Use of models or simulated tissues is another replacement technique that is growing in popularity with the availability of better commercially made models. And last but not least refinement; having knowledgeable, skilled trainers who are continually learning and staying on top of advancements in veterinary medicine and science to improve upon the techniques we use in research. In our institution's Comparative Medicine (CM) department we have taken this multimodal approach of incorporating these various tools into a robust and comprehensive training program for research staff and CM staff with great success. Using all of these tools and training approaches has allowed us to use fewer animals, and yet, get personnel who are more knowledgable and better skilled at performing technical procedures.

P105 A Simple and Safe Handling Technique of African Grass Rats

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African grass rats are very excitable animals and when changing cages it can be rather difficult and stressful for both the rats and the technician. Originally we used metal forceps to grasp the rats when transferring them to a new cage. Some of the disadvantages of using the forceps were that many times they would cause injury to the tail. The rats would also try and climb up the forceps and bite at them while it was grasping their tail. This frantic behavior indicated they were quite stressed, which in turn made the technicians stressed. After exploring a number of different options in handling the rats, one of our technicians came up with the idea of using salad tongs to scoop up the rats and transfer them to the clean cage. The size of the salad tongs and the size of the rats seemed very compatible. The ends of the tongs are scissor like, so you open them, gently scoop up the rats, and close the tongs. The rats are cupped inside the tongs comfortably. This eliminates the pinching of the tail and appears to be causing a lot less stress on the animal. Inside the tongs, the animal is unable to climb and bite, as they did with the forceps. The technicians are extremely happy with this new technique and it seems to be working very well. We strongly recommend any other institutions/facilities that are housing Nile rats to try the salad tongs when changing cages.

P106 A Model to Evaluate Software Options for Billing and Compliance

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Much emphasis has been placed upon automating census management and billing along with the tracking of animal numbers and related IACUC compliance issues through advanced software applications and databases. Deciding whether to purchase software from a vendor or developing an application inhouse requires careful deliberation. From our experiences at our institution's Health Science Center, we devised a model that provides steps in the decision process to ensure a careful and analytical approach. Certain preliminary decisions must first be determined: who is in charge of the process, what resources are available, what is the timeline for implementation, what will be the interactions with existing programs, what is the ability to import and verify the integration of the legacy data. When considering commercial software, critical information must be obtained from the prospective vendors in tandem with a feasibility analysis of developing one's own software. For either process, a detailed cost–benefit analysis is essential and must include not only the development and implementation costs, but recurring (maintenance) costs as well. Once the options have been clearly identified and analyzed along with fixed and recurring costs, timelines for implementation, and additional resources (both human and hardware/infrastructure) determined, a decision matrix can be developed to guide the selection process. It is extremely important to track both direct and indirect costs as the work progresses. With completion of the application and beta testing initiated, legacy data may need to be imported to populate the database. Consideration must also be given to how the system is presented to stakeholders and how it will impact other operational areas of the institutional animal care and use program. The final challenge will be identification and training of users. The decision process for purchasing commercial software or developing an application inhouse must be planned for all stages of the process: procurement, development, implementation, training, and support. A model to guide the process can be a valuable tool in determining an optimal solution while maximizing time and resources.

P107 Validation of an Autoclave Program for Sterilization of Mouse Carcasses

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The sterilization of potentially infectious animal carcasses is an important biologic safety issue in animal facilities operating infection or quarantine units. Autoclaving is a common method to sterilize small rodents' carcasses. However, only few autoclave programs have been validated using dead mice. The aim of this study was to develop and validate a safe procedure for efficient sterilization of mouse cadavers. In the context of the commissioning of a new ABSL2 and ABSL3 facility, several standard and modified disposal programs for solid material and liquids were assessed putting mouse cadavers in a large pass-through autoclave using various temperatures and sterilization times. To validate these processes, heat sensors and vials containing *Geobacillus stearothermophilus* spores were inserted in the peritoneal cavity of dead mice. The autoclave chamber was loaded with several individually ventilated cages containing about 5 mouse carcasses each and alternatively with 10 kg of densely packed cadavers in metal boxes. Heat sensors revealed significant temperature differences between autoclave chamber and mouse bodies. The time to achieve the sterilization temperature inside the mouse bodies was considerably prolonged compared with other waste material. Moreover, temperature was not distributed homogenously throughout the material. We could show that a modification of a "dirty cage program" which is run at 134 °C for 20 min is efficient to sterilize a small number of carcasses. For a larger amount, it is essential to run a disposal program for liquids at 121 °C for 40 min. In conclusion, we emphasize that it is absolutely necessary to validate the cadaver program of an autoclave using spore vials in mouse bodies. All other test material is not suitable to simulate the particular characteristics of mouse cadavers due to the temperature isolation of the fur. Inadequate validation leads to inefficient, and therefore, unsafe procedures in daily routine.

P108 Eradication of *Helicobacter* and Mouse Norovirus Using Cross Fostering Following Hypochlorous Acid Disinfection

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Several methods are widely used to eliminate the newly recognized mouse pathogens, such as *Helicobacter* spp. and mouse norovirus

(MNV). Fostering of neonatal mice by the pathogen free dam is an easy procedure to eliminate multiple enteric pathogens. Hypochlorous acid is effective against a broad range of microorganisms and widely used as a disinfectant. In our laboratory animal facility, we use hypochlorous acid (50 ppm HOCl) to control the environmental microorganisms. *Helicobacter* spp. were detected by fecal PCR with genus-specific primers, and confirmed by DNA sequencing. MNV were detected the fecal RT-PCR or ELISA. About 60% to 70% sentinel mice were infected with *Helicobacter* and MNV. We tried to use cross fostering and HOCl to eliminate the 2 pathogens in our facility. For eradication, pathogen-free dams were prepared and maintained in a clean room. Within 24 h of birth, the contaminated pups were rinsed with HOCl solution several times to reduce pathogens on the surface, and then transferred to the foster dams. After 4 wk of fostering, the foster dams were tested for the absence of pathogens. Until now, 108 litters were transferred to foster dams, and 85 litters survived. Among the survivors, *Helicobacter* but no MNV was detected in only 2 litters. With screening all of fostered litters, no recurrence occurred in the following up survey for more than 8 mo. After moving into an empty redisinfected room, *Helicobacter* and MNV were not detected in the rederived mice by sentinel mice according to our monitoring program. Thus, cross fostering after HOCl disinfection is simple, inexpensive, and effective to eliminate *Helicobacter* and MNV at the same time.

P109 Impact Evaluation of Various Individually Ventilated Caging Systems on Mouse Phenotyping Studies

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With the growing use of genetically engineered mice in phenotyping studies, a wealth of functional genetic information is being generated. In order to generate consistent data within and across labs, mouse phenotyping studies require standardization of protocols and mice husbandry conditions. Housing conditions are well known to influence mouse phenotypes such as behavior. Individually ventilated cage (IVC) housing is becoming popular among animal facilities as this caging system is very effective for animal bioconfinement and improves microenvironemental conditions. Nowadays, various IVC systems with their own specific features (such as ventilating and air flow system, size, structure, and functionality) are commercially available. While several studies have been done to compare the IVC housing with the conventional housing (open cages), no information is available regarding how different IVC system may influence mouse phenotypes. We investigated here the impact of 3 different IVC housing systems on mouse phenotypes. For this study, a systemic phenotyping screen covering the behavior, clinical chemistry and hematology, energy metabolism, and cardiovascular functions was performed, using the European standard workflow. Our results show that most parameters recorded were not affected by the caging system. Nevertheless, some differences were observed depending on the caging system used. However, such effects are not likely to occlude potential phenotypes associated with genetic manipulations. These results also argues that phenotyping data obtained under standardized protocol using various existing IVC housing system may be confidently shared or replicated across or within laboratories. This is particularly important for the present and ongoing integrated international phenotyping programs designed to better understand the functions of the genome.

P110 Using An Automated Euthanasia System For Neonatal and Adult Mice

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Euthanizing neonatal mice using an automated system that was preset by the manufacturer failed to euthanize all neonatal mice in the chambers. The factory presets on the automated euthanasia machine are set for 5 min of CO_2 at 50 L/min followed by 5 min of CO_2 at 25 L/min and then a 45 min hold time. In order to euthanize all mice humanely, it was necessary to determine the exact age at which a neonatal mouse would expire in an automated euthanasia machine at the factory preset neonatal time and CO_2 levels. Using 5-, 9-, 11-, 15-, and 16-d-old neonatal mice, the automated euthanasia machine was first run with the original factory presets, but these were insufficient to euthanize all of the neonatal mice in each of the 20 cage chambers. The presets were adjusted to 10 min of CO_2 at 50 L/min followed by 15 min of CO_2 at 50 L/min, and then a 59 min and 59 s hold time. After making these adjustments to CO_2 levels and times, all neonatal mice aged 9, 5, and 3 d old were successfully euthanized in the automated euthanasia machine in all cage chambers. The conclusion reached is that the factory preset neonatal cycle on the automated euthanasia machine is inadequate for euthanizing all ages of neonatal mice. Therefore, in order to ensure stress-free euthanasia for all neonatal mice, the amount of CO_2 and the amount of time the mice are exposed to the CO_2 must be increased, and the hold time must be increased to the maximum setting.

P111 Protecting Rodent Barrier Integrity: High-Pressure Hydrogen Compressors/Tote Sanitizing

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The purpose of a rodent barrier facility is to maintain animals free from adventitious pathogens. Following specific barrier guidelines helps protect the health of the rodent colonies and validity of each investigator's research. Identification of materials that are high risk for crossover contamination is important to protect a rodent barrier. Plastic received in rolls used to make water pouches for rodent caging have been identified as one such concern. This study had 2 aims; 1) determine if the plastic film was a cross-contamination risk, and 2) identify a practical and effective means of chemical disinfection of these water pouches after production and during tote storage to ensure microbial control. Two independent experiments were conducted using contact plates as a means of microbial analysis of surface contaminants of the plastic used for water pouch production via the high-pressure hydrogen compressors system. Routine handling of water pouches was conducted in both experiments. These 2 mirrored experiments were conducted using 2 different sources of contact plates. In addition, 3 water pouch/tote disinfectant treatments were employed to assess the effectiveness of chemically treated, stored water pouches. Totes storing water pouches were sampled with contact plates before and after disinfectant treatments. Of the 96 total contact plates collected between the 2 experiments and read after 48-h incubation, 7 plates resulted in minimal levels of microbial activity. Five of the 7 positive plates were from predisinfectant-treated samples and 2 of the 7 plates were from postdisinfectant-treated samples. Given the minimal bacterial contamination observed, comparing the effectiveness of the 3 disinfectant regimens was not possible. Our results suggest that the use of plastic received in rolls does not pose a risk to barrier integrity. Management of the low microbial activity reported could be easily controlled by any of the 3 disinfectant treatments employed in this study.

P112 Blood Collection from Göttingen Minipigs: A Peripheral Approach

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Many techniques have been described to collect blood from swine. Due to their anatomy, such techniques typically involve a 'blind stick' of a deep vessel such as the jugular vein or cranial vena cava. Due to the nature of the studies being carried out at our institution, the ability to adequately hold off the vessel after venipuncture, assure hemostasis and visualize the vessel for complications was critical. In larger breed pigs, collection from the ear vein is often possible. However, our studies use the Göttingen minipig, and their small ear veins are not well suited for serial blood collection. After exploring other peripheral vessels for blood sampling, the cephalic veins and cranial epigastric veins were used. Greatest success was obtained with the cephalic vein as it crossed the chest toward the thoracic inlet. The minipigs were bled daily under midazolam sedation (0.1 to 0.5 mg/kg SC) for greater than 30 d obtaining volumes of 0.5 to 2.0 mL/d (larger volumes were not attempted). Some pigs did develop bruising or hematomas at the venipuncture site. The cranial epigastric veins were a viable option for sampling if there was bruising over the cephalic vein, but had more of a tendency to collapse during collection. While not typical sites for blood collection in pigs, peripheral vessels can be used for daily collections of small volumes with excellent results.

P113 Database of Mice Phenomes: A Valuable Tool for Model Selection

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The database of mice phenomes (DMP) is a powerful, growing collection of baseline data generated by the worldwide scientific community. This resource helps researchers identify the most relevant inbred mouse models for their research, or background for their mutant strains, and can help reduce the number of mice needed to complete a study. DMP contains extensive characterization data for over 600 strains with focus on the 35 most commonly used and genetically diverse inbred stains in research today. DMP provides practical information for anyone working with mice. Researchers can access data on reproductive performance, disease onset and susceptibility, age-related phenotypes, sensory deficits, and much more. Tools are provided to discover new models of human diseases, to identify genetic backgrounds which may alter the presence or progression of a particular knockout or transgenic phenotype, and for choosing optimal strains for QTL analysis and identification of new genes. Detailed characterization is available across all research areas including cancer, development, neurobiology, toxicology, cardiovascular, diabetes and obesity, immunology, hematology, autoimmunity, and aging. DMP is a freely available resource that contains phenotypic, genotypic, and gene expression data. Each dataset has a detailed protocol, summary statistics, and outlier identification (highs/lows). Web-based analysis tools are available for data mining. DMP enables in silico hypothesis testing and validation, which diminishes the need for de novo generation of baseline data, ultimately decreasing the number of mice needed for research.

P114 A Strategy for Developing and Auditing Training Programs in a Rapidly Expanding Global Environment

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Our institution recognizes the need for global standards in the care and use of laboratory animals, and promotes training as critical to ensuring animal welfare and quality science. To that end, a comprehensive audit strategy can serve as the foundation for assessing and enhancing training and development programs wherever animal research is conducted. A team of highly experienced training professionals from the laboratory animal research community worked with our institution to develop an audit strategy that facilitates assessment of an institution's top-level commitment to sustaining a culture of care through training. The audit strategy provides a framework of targeted questions that enable an auditor to evaluate the breath and depth of an institution's training program. The audit framework is built on over-arching principles that support and promote the highest possible standards of animal welfare and science.

P115 Introducing a Standardized, Web-Based Approach to Transferring Rodents between Noncommercial Institutions

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Not long after the creation of the first transgenic mouse, when collaborating researchers began sharing these valuable research models globally, research institutions were thrust into the unfamiliar role of rodent 'vendor.' The process of transferring rodents between institutions is complicated by numerous factors including the provision of incomplete or inaccurate information, the effort required to search for and contact an ever-changing cast of veterinarians and shipping coordinators, and the lack of standardized animal health reports and other required information shared between institutions. To address these inefficiencies we gathered and studied information collected from shipping coordinators and veterinarians at over 50 research institutions. With their feedback, we developed a computer software system including a secure website, database and set of flexible web-based, standardized forms and processes. The features of this system include: A web-based interface and registry where users can easily and securely register and update their institution's contact information and search for and contact other veterinarians, shipping coordinators, and researchers; a collaborative step between researchers at both importing and exporting institutions at the beginning of the process to ensure a complete transfer request; standardized web-based forms and documents to facilitate the timely exchange and review of needed information; automated email alerts to users when their attention to a transfer request is required; and system-generated emails to nonusers such as requests to export or import animals, a transfer approval form and a summary of shipment information. Here we describe our attempt to address the difficulties inherent in the current process of transferring rodents between research institutions via the development and promotion of standardized web-based transfer forms, reports, and processes.

P116 A Simple Method to Determine the Maximum Cage Change Interval for Mice Housed on Individually Ventilated Caging Racks

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To provide optimal care for mice, the cage change interval for Balb/c mice housed in individually ventilated cages (IVC) was established after evaluating ammonia levels in the cages for a period of time. The maximum interval for cage change was set when 30% of the cages registered ≥20 ppm. CO_2 levels were also checked for several days in a small population of cages and did not exceed the 5000 ppm. Each of the 10 cages contained 5 female Balb/c mice, 6 to 8 wk old, and housed on corncob bedding. The animals were fed standard rodent chow. The animal room and IVC parameters were maintained at 72 °F and 50% relative humididty and recorded in a log daily. The blower was set at 70 air changes per hour and the cages were negative to the room. Ammonia levels were measured using a gas detector pump and ammonia gas detector tubes. Daily room, rack parameters and ammonia results were recorded on a spreadsheet. The cages were removed from the rack daily and placed in a biologic safety cabinet to check ammonia levels in the cages. The ammonia probe was inserted into the cage via the automatic watering port. The probe is approximately 1in. above the bedding while taking the reading. Once 30% of the cages reach ≥ 20 ppm the bedding will be changed. On day 8, 40% of the cages had ammonia readings of ≥20 ppm; therefore, it was determined that 8 d was the maximum change interval for Balb/c mice housed under the same conditions. In conclusion, measuring ammonia levels in individual IVC can be used as a simple method to establish maximum cage change intervals for a strain of mice under given conditions.

P117 Comparative Perception and Effects of Construction-Induced Vibration on Humans, Rats, and Mice

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Vibration is considered to be an environmental influence that can have detrimental effects on research integrity as well as animal wellbeing. Because the effects of vibration on an object depend on the mass of the object and its inherent stiffness, an identical vibration source is likely to be perceived differently by the human, rat, and mouse. Resonance frequency range (RFR) is the frequency range at which an object is most susceptible to vibration and at which vibration can be amplified by the object. Sensitivity frequency range (SFR) is the frequency range at which a human or animal may perceive vibration and is typically wider than the RFR. The current work was performed to determine the potential effects of vibration caused by common construction equipment on the human, rat, and mouse as well as on the abdomen, thorax, and head of these species. Studies were conducted where vibration was measured inside of a ventilated mouse cage when various implements of construction equipment were used 3 ft from the cage and at various distances and locations in the facility when a large jackhammer was employed. Vibration caused by various items of construction equipment at 3 ft from the cage tended to be higher in the RFR of the rat thorax and head as well as the mouse abdomen, thorax, and head relative to humans. Vibration 3 ft from the cage in the SFR is highest in the rat thorax and mouse head, but there is also a trend upward for the mouse abdomen and thorax as well as the rat head relative to the human levels. The vibration levels in the RFR and SFR that resulted from the use of a large jackhammer and measured at various locations and distances in the facility tended to be higher for the rat and mouse than the human, especially for the rat thorax and head and for the mouse abdomen and head. Taken together these data indicate that vibration levels in the frequency ranges that are most likely to cause adverse effects are higher in rats and mice relative to

humans for the same vibration source.

P118 Refinement of Restraint Procedures on Short-Term Rodent Intravenous Infusion

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As part of our facilities ongoing efforts to refine restraint procedures to increase dosing efficiency while still ensuring the animal's wellbeing, we have developed and implemented an improved restraint method for rodent infusion studies. The improved restraint method is designed to be cost effective, increase dosing efficiency and prevent contamination during periods of restraint (which range from 2 to 90 min). Previously, rodent intravenous infusion studies were dosed using commercially available rigid plastic restrainers which were expensive, required more space, and were time consuming to clean between animals. The refined restraint method uses a disposable plastic bag which the animals readily enter and remain calm in throughout the restraint period, with no apparent signs of distress. The restraint bags are secured at the back of the bag leaving the animal's tail exposed. A cage card with the unique animal identification and dose level can be attached directly to the bag and the restrained animal can be secured to the table using a piece of packing tape. The bags are inexpensive and readily available in several sizes to fit most rodents. After each use the bags are disposed of, eliminating labor required to clean the restraint devices and preventing possible contamination of subsequent animals.

P119 A Novel Approach to Increasing Efficiency in Sterilizing Rodent Water Bottles

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Most animal facility employees would agree that preparing and autoclaving water bottles is time consuming and can present challenges such as limited storage space, time constraints, and limited autoclave capacity. Achieving a short processing and turnaround time for essential equipment within an animal facility is an important and challenging daily task. The ability to increase the number of water bottle baskets per autoclave cycle is a simple way to increase efficiency. The goal is to fully use the chamber space of the autoclave without compromising sterility. Standard commercially available water bottle baskets usually have an additional spacer built in, which maintains each stacked row of baskets elevated above the row below. This wasted space reduces efficiency by limiting the number of baskets that can be autoclaved per cycle, as well as increasing the storage space required for water bottles. To eliminate this waste, each water bottle basket was modified to remove this spacer. This approach reduces the amount of empty space in each autoclave load, and allows a greater number of baskets to be stacked in the same amount of space. After contacting the manufacturer, and consulting with the inhouse facilities department, it was confirmed that the added density per load should have no effect on the sterility of the product. The following sterilizing indicators are used during standard operating procedures: 4 steam sterilizer control tubes inside the water bottles at all 4 corners per autoclave cycle (top left, bottom left, top right, bottom right) and one steam emulating indicator at the center of autoclave rack per autoclave cycle. Both indicators revealed that the cycle reached 121°C for a minimum of 15 min exposure by changing the color of the indicators. The initial test trials showed only minor increases on overall cycle time without compromising the capabilities of the autoclave for sterility. The printouts from the autoclave were reviewed before and after the water bottle basket modification, and showed no significant difference. The cycle time ranged from about 60 min to 75 min before and after the modifications, respectively. No changes to indicator results have been detected after modifying the water bottle baskets. By altering the water bottle baskets, the number of water bottles per cycle was increased from 144 bottles per cycle to 432 bottles per cycle, a threefold increase. This was a tremendous gain for the facility; reducing the time spent sterilizing water bottles, and freeing up usable man power. By altering the water bottle baskets storage space requirements were also drastically reduced. Baskets were reduced from 20.5 cm each when stacked to 14.0 cm. This resulted in a gain of 6.5 cm per basket, ultimately increasing the number of baskets that can be loaded on a single push cart to be stored in animal rooms, storage rooms, and other spaces.

P120 Energy Reallocation to Breeding Performance through Improved Behavioral Thermoregulation

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Mice are housed at temperatures which increase their basal metabolic rates and impose high energy demands to maintain core temperatures. Thus, energy may be reallocated from other biologic processes to increase heat production. We hypothesized that nesting material will allow for behavioral thermoregulation by reducing heat loss. We predict this reduction will improve feed conversion as well as breeding performance. We housed naïve C57BL/6NCrl, BALB/cAnNCrl, and Crl:CD1(ICR) breeding pairs (30 cages per strain) at 20 °C with a nesting treatment: no additional material, 8 g of paper nesting material, or 8 g cotton fiber nesting material for 6 mo. Feed was weighed when added and at the end of the experiment, and fresh nesting material provided weekly. Pups were counted at birth and weighed and sexed at weaning. Analyses used GLM with post hoc contrasts. Nesting material improved feed efficiency per pup weaned ($P = 0.02$). However, there were no differences in the total feed consumed ($P = 0.49$). The breeding index (pups weaned per dam per week) was higher when either nesting materials were provided ($P = 0.02$). Thus, the energy conserved by nesting material was reallocated from heat generation to improved breeding performance.

P121 Comparison of Palatability and Preference of 3 Novel Gel Supplements in Dutch Belt Rabbits

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Nutritionally balanced gel supplements have been available for rodents for several years. Gel supplements have been used for providing water and nutrients during transport as well as for animals that require extra care, for example, postoperative animals. Recently a gel supplement was made available for rabbits. The purpose of this study was to evaluate the palatability and preference for this product in rabbits during the initial quarantine/acclimation period upon arrival, and to evaluate if use of this gel product as a supplement during acclimation made a difference in their adjustment to the facility and to their wellbeing. Twenty Dutch belted female rabbits were divided into 4 groups of 5. All rabbits received water ad libitum and 160 g of rabbit chow once a day. Groups 1 to 3 received one 4-oz gel supplement each day for 5 d. The 3 gel groups were 1) regular gel, 2) gel with sucralose, and 3) gel with molasses. Group 4 was the control group and received no additional supplements. Body weights were collected on day 1 and on day 6. Rabbits in all groups ate between 82% to 89% of their regular chow with no significant difference among groups. Group 1 rabbits ate an average of 36% of the gel supplements over the 5-d period. Group 2 ate 59% and group 3 ate 75% of the gel supplements. There was a significant difference in the amount of gel supplement consumed for the molasses compared with the sucrose or regular gel (ANOVA single factor, $P < 0.05$). All groups of rabbits increased in body weight during the 5-d period (3.6% to 5.4%). The rabbits supplemented with gel with molasses increased the most body weight (5.4%); however, there was no significant difference in weight gain between the groups (ANOVA single factor and *t* test).

P122 Unsterilized Feed: The Probable Cause of Sporadic Mouse Parvovirus Outbreaks

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In early 2009 we experienced a widespread outbreak of mouse parvoviruses 1 and 2 (MPV) in our parvovirus-negative colonies, which consisted of over 40,000 cages located in 7 campus vivaria. During a single 4-mo sentinel testing rotation, 62 separate rack sentinels (out of 1377) tested serologically positive for MPV1, MPV2, or both, with each positive rack containing between 0 and 10 infected colony cages. Fecal samples from several index cases tested positive for MPV by PCR. Positives were randomly distributed, although several small facilities escaped infection. How could this infection have penetrated our defenses? All rodent facilities on campus had been parvovirus-free. Mice were maintained in individually

ventilated cages and handled in biosafety cabinets using chlorine dioxide-based disinfectants. Cages, cage furniture, and bedding were autoclaved, feed was sterilized, and water was treated by reverse osmosis and hyperchlorination or acidification. Incoming mice from other institutions were quarantined and tested before release, and health reports from vendors and our transgenic core (where mice entered colonies directly) remained negative throughout. Loose mice occasionally captured in live traps were parvovirus-free. The only widespread change in the 3 mo preceding the first positive test was that every cage had been treated for 12 wk with an unsterilized fenbendazole-medicated diet. At completion of the fenbendazole treatment, sterilized feed was reinstituted. To eliminate the infection, all cages on racks testing positive were tested serologically (one mouse per cage) for parvoviruses at 1-mo intervals and positive cages removed. Once all cages had tested negative twice, new rack sentinels were placed and exposed for 1 mo before being tested and replaced. Two negative sentinel tests at monthly intervals resulted in rack quarantine being lifted. Racks that had not initially tested positive were tested monthly; however, no new unrelated cases were detected at that time, and all facilities have remained parvovirus-free in the 2.5 y since the outbreak. The timing and extent of the outbreak (widely distributed throughout several buildings immediately following the change in feed) together with the complete absence of new cases once sterilized feed was reinstituted, strongly implicates unsterilized feed as the source of this outbreak.

P123 Enhanced Canine Enrichment and Its Effects on Staff Morale

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The use of dogs in research has been under increasing public scrutiny and criticism over the past several years, and our institution has been no exception. Of particular concern is the use of Class B dogs. In order to investigate potential implications of public perceptions for our animal care staff, we surveyed our husbandry and veterinary technician staff regarding issues specific to the use of dogs in research and teaching. The survey focused on worker attitudes and morale with regard to canine research studies and recent public criticism of such studies at our institution. The survey also addressed issues of environmental enrichment. The survey response showed a trend between the importance of animal welfare and overall job satisfaction among the staff. The importance of an organized, institution-supported enrichment program for research dogs was also indicated. However, negative media attention did not appear to influence their feelings on the use of dogs in research. Following the survey, a volunteer Animal Enrichment Committee (AEC) was formed and consisted of husbandry staff, veterinary technicians, and veterinarians. The AEC then instituted an enhanced canine enrichment program to benefit both canine welfare and human morale. The program consisted of 2 main components: positive reinforcement training of basic commands to facilitate routine husbandry duties and experimental procedures; and play-based exercise sessions. Throughout all interactions, participants were encouraged to show general affection to the dogs. Staff participation was encouraged using a shared time policy in which staff time was provided equally through institutional expense and staff volunteers' time contribution. Overall, the program has been a success, improving both animal welfare and staff morale. Future efforts will include identifying and implementing ways to maintain staff participation over time and further strengthening the program to improve animal welfare and staff morale alike.

P124 Rederivation 101: Eliminating Murine MNV and *Helicobacter* Effectively

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Management decided to eliminate murine norovirus (MNV) and *Helicobacter* spp. from barrier mice by cross-foster rederivation. Mice were relocated to a temporary barrier location. This isolated area was divided between dirty rooms for housing barrier mice and clean rooms for cross-foster housing. CD1 females were bred to serve as foster mothers. Specific procedures based on current literature for cross-foster rederivation were implemented, including removal of transgenic males from the transgenic female cage on gestational days 14 to 16, daily changing of the transgenic female cage after the male was removed, and excluding nesting material from the cages. Husbandry personnel maintained clean/dirty roles during the process. Female foster mothers were transferred after parturition to a designated clean room in the temporary barrier. Strict aseptic technique was followed during the exchange of pups from the transgenic to the foster mother. Transgenic pups were kept with the foster mother until the pups could be weaned. Pups were ear tagged, genotyped, and tested for MNV and *Helicobacter* spp. at weaning. Positive litters were culled. Negative litters were retested at 12 wk of age. Over the course of 1 y, 25 transgenic or knockout strains were cross-fostered. Out of 64 cross-fostering attempts, 43 were negative and 3 were positive for both agents at the 3-wk test. The other 18 were not successful for various reasons, including pup death or cannibalization (5), a foster mother was not available (4), and the investigator decided not to test the litter (9). All litters that tested negative at 3 wk also remained negative at 12 wk. All barrier sentinels have since tested negative for MNV on a quarterly basis and *Helicobacter* spp. on an annual basis. This successful outcome depended on attention to detail, adherence to strict aseptic technique, and effective technician teamwork.

P125 How to Protect a Water Heating Blanket from Needle Damage

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A common standard practice in the veterinary field is the maintenance of equipment used in diagnostic and surgical procedures. The use of this equipment in laboratory animal medicine is no exception. Maintenance of equipment is important as it allows continued use at an uninterrupted pace during critical time points and prevents the unnecessary and costly purchase of new equipment. At our institution, each rodent operating room is equipped with a heating water blanket to maintain an animals' body temperature when anesthetized. Often after use, we have found punctures in water blankets that were caused by incorrect control of uncapped needles. Replacement of these water blankets was expensive due to equipment replacement and labor when cleaning puddles of water. To combat the penetration of needles through the water blanket while maintaining the integrity of heat administration to the patient and continued accessibility to cleaning the water blanket after use, a thin plastic cutting board was placed on top of the water heating blanket. To sustain placement of the cutting board during use, Fabric hook-and-loop fasteners was used to affix it to the top surface of the water blanket. We found that allowing additional time for conductivity was helpful. To examine the integrity of temperature conductivity to the cutting board, 2 temperatures of the water pumps were assessed, a low setting of 35 °C (95 °F) and a high setting at 42 °C (107 °F). We found that allowing additional time of 10 min with the cutting board benefits conductivity with a reading of 30 °C (86 °F) on low setting and 31 °C (88 °F) on a high setting. In conclusion, this simple modification has resulted in a reduction of water blanket replacement, thus saving money and time.

P126 Withdrawn

P127 Design and Implementation of a Database of Institutionally Maintained Genetically Engineered Mouse Strains

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The use of genetically engineered mice (GEM) in research has greatly increased our understanding of the role that specific genes play in development, cellular function, and disease. Thousands of strains of GEM are available from centralized resources and commercial vendors. However, acquisition of strains can be hampered because of limited availability, legal restrictions on distribution, and processing of material transfer agreements (MTA). We undertook a project to develop a database of locally maintained GEM at our institution. The initial step involved an assessment of the project scope. From biweekly census data, we estimated that GEM breeding colonies occupied 12,500 cages. An analysis of IACUC records revealed that 288 out of 538 (54%) principal investigators (PI) had approval to breed mice. Our centralized breeding colony service maintained 261 colonies of mice for 60 PI (average of 4.4 colonies per PI). This would suggest a total of 1267 breeding colonies (4.4 colonies per PI × 288 PI) on campus. Further analysis identified 23 redundant colonies (9%) (identical colonies maintained by 2 or more PI). Redundant colonies belonged to 17 different investigators and included 10 unique GEM

strains. Collectively, the scope analysis indicated a large number of cages dedicated to GEM colonies. An informal poll indicated broad PI support for development of a central database of GEM strains on campus. An online survey is being used to obtain GEM specific information (PI name, strain nomenclature, genotype, mouse genome number (MGN), and MTA requirements) on any strains maintained at our institution. Data will be transferred to a central database that will allow investigators to search for GEM strains available on campus. Listed strains will be linked to the MGN database to provide PI with ready access to strain specific data. The database will also contain informational links on correct nomenclature, institutional animal transfer procedures, and MTA requirements. The completed project will reduce animal use through of GEM colonies, improve access to unique strains, reduce investigator cost, and reduce overall cage inventory.

P128 Factors Influencing the Preferred Nesting Location of Laboratory Mice

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Understanding the preferred nesting location of laboratory mice within the cage may serve as a useful, objective, behavioral assessment tool. To determine if caging ventilation rates influenced nesting location, we observed nesting site (front, middle, or rear) within the cage, in static, low velocity (30 ACH), and moderate velocity (70 ACH) airflow caging. We observed that 60.66% (n = 244 boxes) of mice housed in static caging preferred to nest in the rear of the cage, compared with 48.66% (n = 187 boxes) of mice in low ventilation caging, and only 14.02% (n = 635 boxes) in moderate ventilation caging. These data suggest that mice tend to nest distally from the sourced of forced air. To determine if we could mitigate the mice's nesting preference in ventilated caging we placed nesting material in the rear of the cage and observed the nesting site 24 h later. Placement of the nesting material in the rear of the cage did not significantly increase the proportion of mice that nested in the rear of the cage, in either low ventilation caging ($Z = 0.148$, $P = 0.882$) or moderate ventilation caging ($Z = 0.253$; $P = 0.800$). Breeding conditions may, however, mitigate nesting preference. In moderate ventilation caging, we observed that mice with newborn pups nested significantly more often in the rear of the cage ($Z = 5.53\%$, $P < 0.01$), when compared with mice without litters. The data suggest that some environmental factors do influence nest site location, and that future studies should evaluate the effects of other macroenvironmental stressors on nesting behavior.

P129 Using Surveys to Identify Employee Injury Risks and Effective Engineering Controls

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Animal facility technicians are exposed to numerous hazards in the work environment, often resulting in injury. At our institution, a retrospective review of formal injury reports indicated that of nonanimal related injuries, the majority of other injuries were contusions (50%) and muscle strains (28.50%) associated with moving heavy equipment. Suspecting that many injuries are not formally reported, we surveyed technicians to determine which injuries were most common, and which could be minimized through the implementation of novel engineering controls. Of 14 cagewash technicians surveyed, 86.67% reported that they had been injured while moving equipment. To mitigate this hazard, we installed ergonomic handles on nonhuman primate and rabbit caging, and on shelving units. The handles were mounted vertically, and with sufficient margin from the exterior edges of the equipment to prevent hand crush and contusion during transport. We also identified repetitive motion tasks that resulted in pain and discomfort: 80% of cagewash technicians reported the need to take a break from scraping rodent boxes during the work day, due to hand pain. As a result, some technicians had modified scrapers to increase surface area contact with the palm of the hand, reducing discomfort. Of rodent husbandry technicians surveyed (n = 32), 62% reported leg or foot pain. The frequency of pain reports were most prevalent in those who stood in one location for greater than 3 h. In response to employee request, we will implement the use of antifatigue mats across our program in the upcoming fiscal year. Using employee feedback and suggestions to enhance employee safety empowers technicians, encourages them to take ownership of their safety, and ultimately contributes to a more safety-conscientious workforce.

P130 Using Total Quality Management Techniques to Establish an Effective Rodent Husbandry Training Program

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Technology and regulatory changes have generated the need for progressively skilled husbandry technicians. In response, we used total quality management (TQM) principles, such as decentralizing authority, encouraging employee input, and striving for continual improvement, to implement a successful training program, improve staff performance, and increase retention. A training committee, comprised of managers from multiple facilities, developed the framework, and met routinely to review and modify the training program. A team of trainers comprised of husbandry technicians, facilitated employees' hands on training within the animal facilities and provided progress updates to the managers. Over a 6-wk period, new employees reviewed job tasks with a husbandry manager, shadowed a trainer in the animal facility, and before performing the task unsupervised, completed 2 competency exams. Employees who had completed the training program were surveyed. Of the respondents, 82.35% reported satisfaction with the amount of hands on training, but 76.47% (n = 18) reported dissatisfaction with the amount of classroom training. Interestingly, 50% of respondents reported that they desired more performance feedback, both from supervisors and trainers. Survey results also identified inconsistencies in husbandry tasks, such as sentinel and microisolation technique procedures. Since implementing our program, we increased the rate of employees who successfully completed the probationary period, from 77.78% (n = 24) in 2008, to 100% (n = 26) in the past year. Additionally, we greatly reduced the number of involuntary terminations. Qualitatively, we have observed greater group cohesiveness, and employees who purport "quality at the source", or a personal responsibility for individual performance. Our findings suggest that a well-managed training program that promotes trainee buy-in, empowers technicians, and is routinely reviewed and modified, can reduce costs associated with employee attrition, and may improve overall job satisfaction.

P131 Development of a Pictoral Guide for Standardization of Tissue Trimming in a Core Comparative Pathology Laboratory

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Our institution's Unit for Laboratory Animal Medicine Pathology Cores for Animal Research (PCAR) provides fee-for-service pathology support to laboratory investigators. Requests range from general necropsies and tissue harvests to more unique organ preparations such as isolated mesenteric arteries for toxicology studies. We noticed a lack of consistency in tissue trimming and excessive pathologist time devoted to providing assistance in this area. With this in mind, a pictoral guide to tissue trimming was created for PCAR technicians. Existing tissue trimming guides from other sources were too specialized or in an inconvenient form for daily use. The guide contains steps for trimming all major organ systems and includes special procedures such as mesenteric artery preparation. Pictures and brief written descriptions were created to illustrate the desired tissue sections and their arrangement into cassettes. Flexibility was incorporated into the guide to accommodate a wide variety of project types. The pictoral guide has increased consistency and efficiency among PCAR technicians and provided higher quality samples for pathologic evaluation. This guide can also be distributed to students and investigators performing their own tissue trimming.

P132 Refinement of a Contract Research Organization Training Program and the Importance of Time-Based Training

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A contract research organization (CRO) serves many industries, each with their own exposure, observation, and testing requirements. As such, a CRO must master a variety of technical competencies to

attract business. A well-structured training program lends to building a biologist's confidence and abilities through organization and appropriately timed training. In addition, such a program prioritizes the training of staff in highly sought after skills and minimizes training time spent in areas of lesser demand. Management at our facility recognized that our training program must be flexible and easily adaptable to the evolving laboratory environment, including ever-changing client demands. Our training program was already designed to take newly hired employees with varied animal experience and develop their handling, observational, and technical skills in an efficient and informative manner. We refined this program by developing a plan for employees to follow, which prioritized the learning of frequently requested technical skills and set timelines to meet these goals. The department was divided into teams, each specializing in an area of expertise. A list of skills relative to team assignment, experience level, and skill difficulty was created. Each employee received a team-specific list to work from, which was broken down into time intervals (30-, 60-, 90-d, 6-m, 1-, and 2-y lists) for expected completion of the skills. The 'skill lists' are reviewed periodically by management and refinements are made based on current industry demands. By implementing this program, we have been able to increase our department's skill-base and ensured that we have a staff trained to perform standard and unique study designs. It has also allowed for biologists to focus on a choice of career paths while maintaining the flexibility necessary to gain a broad spectrum of technical capabilities.

P133 The Development and Implementation of an Enrichment Committee

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The Division of Laboratory Animal Resources (DLAR) at our institution recognized the need to form a committee composed of individuals from several functional areas within our research community to examine the currently accepted enrichment standards, assess the feasibility of novel ideas, and design and implement an integrated enrichment program. The overarching goal is to enhance the wellbeing of the laboratory animals housed at our institution. The aim of the committee is to meet or exceed the current enrichment regulations and expectations as set by oversight, regulatory, and accreditation agencies. The committee strives to stay abreast of the current environmental enrichment standards and to evaluate, adapt, and apply novel ideas to our own colonies of laboratory animals. Since inception, the committee has improved housing conditions by evaluating and implementing numerous novel devices and practices. The IACUC was assisted by the enrichment committee in developing enrichment policies for all laboratory animals. The expectation is that all animals will be provided enrichment as outlined in these policies, or alternatively, that an IACUC-approved exemption will be required. The committee played an active role in the development and implementation of the university's adoption and disposition policy. Numerous animal species have been adopted, donated, transferred, or retired under this university-wide policy. The Enrichment Committee has proved to be a successful component of the overall laboratory animal program. The committee, in cooperation with the DLAR, the Office of Animal Welfare Assurance, the IACUC and the research community, works to enhance the social, psychologic, and physical wellbeing the laboratory animals in our care.

P134 Temporary Trainer Positions for Animal Husbandry Technicians

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In the world of animal research, experienced and knowledgeable husbandry technicians are a valuable asset. Providing opportunities for technicians to learn and develop new skills can be challenging in busy animal facilities. However, fostering the increase in technicians' knowledge and experience not only allows for growth of the individual husbandry technicians but also supports the growth and flexibility of the entire staff. It creates a sense of purpose to the individual technician and encourages an excitement for learning, and provides work experiences that are valuable when seeking promotion within the department. Offering temporary assignments for technicians to train in specialized areas is a creative way to provide and foster new opportunities for staff. The Training Core at our institution created a temporary training position which allowed an animal technician to work as a trainer for a 9-mo period. The temporary position provides the staff member with experience in developing and administering training courses for a variety of tasks including: animal husbandry and technical procedures such as mouse and rat injections. Additionally, the staff member gained experience with administrative and regulatory issues such as updating SOP, and husbandry/IACUC inspections. This opportunity proved to be an excellent way to allow the technicians to gain new experience and knowledge and increased the diversity of skills available to the husbandry staff members. At our institution, 2 technicians have had the opportunity to experience this position and they both describe it as a great opportunity to learn a lot in a short period of time. It gave them more insight into how management runs, the steps involved in writing SOP, and other areas within the department that they would not have otherwise experienced.

P135 Withdrawn

P136 2011 Book of Normal Data on Selected Lineages of Miniature Swine

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In an effort to further support biomedical research, we recently updated a comprehensive dataset on normal data (reference intervals or ranges) for our 4 lineages of miniature swine. Included are Yucatan, Hanford, Sinclair S1, and microYucatan lineages. This effort collates and summarizes normal biologic and physiologic data collected over many years. Data categories include: uses in biomedical research, body measurements (biometrics), growth, clinical pathology (hematology, chemistry, coagulation, urinalysis), organ weights, background histopathology findings, blood glucose, ocular, diet/feeding, cardiovascular, ECG, dermal, reproduction data, and references. Over 145 tables of data are presented. These data are offered to veterinarians, biomedical investigators, preclinical clients, and university staff to facilitate research when using our miniature swine animal models. This poster will outline the contents of this 'book' and present representative data tables. Copies of the full pdf on CDROM will be available by request.

P137 Development of a Comprehensive Management System for Standard Operating Procedures

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SOP are a vital part of any animal research program. Writing SOP is a challenge, but managing SOP is as challenging and not often addressed. Complexities include topics such as who will write what SOP, who will check the SOP, who will approve the SOP, and how will all of this be done in a timely and organized fashion. In an effort to streamline this process, we developed a comprehensive management system for SOP creation, review, and approval. The SOP Comprehensive Management System includes an SOP planning committee, an SOP review committee (consisting of 3 to 4 people), a table of contents (TOC) using a spreadsheet, many authors, an approval process, and a team to create/update quizzes as needed for certain SOP. It is a collective process requiring all individuals involved to play active parts in this massive process. All areas where SOP were in existence or needed were entered into a detailed password protected TOC spreadsheet. Each writer was provided with a table listing the SOP they were responsible for writing or revising, along with 2 due dates; first due date is for submission to the SOP review committee and the second due date is to secure approval from the Animal Program Director. We also designated SOP as primary, secondary, and tertiary in degree of importance. This helped to focus our attention on the most pertinent SOP first. We put all SOP on our shared access drive. Each SOP was given its own designated folder on our shared access drive in the folder designated "working on." Once approved, the SOP was moved to the folder designated "final." One person was designated to update the TOC as SOP were approved or new SOP were added. Many new SOP were found to be needed and were added as we progressed. This designated person was also responsible for updating the "final" SOP folder. Another person was responsible for assigning each SOP to the writer(s) most qualified. By having designated individuals for the

multiple, yet necessary, tasks needed afforded us the opportunity to stay focused and motivated in moving the project forward, remaining organized and sticking, as close as possible, to our proposed timeline of completion for this massive project. We intend to give further details of our SOP Comprehensive Management System and expand on the efficiencies gained within the process.

P138 A Simplified Way To Compare Vendors' Health Reports To Your Facility's Health Status Requirements For Incoming Animals

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Health status requirements for animals entering a research facility are critical to preventive medicine and biosecurity. Assuring that data provided in vendors' health monitoring reports meets facility's health status requirements is challenging for several reasons. Facility requirements and vendor reports may use different synonyms or abbreviations for the same infection, for example, ectoparasites instead of fleas, fur mites, lice, or follicle mites; or names of specific species such as *Myobia*, *Mycopetes*, *Psorergates*, and *Radfordia*. Each vendor uses a different format for their reports. Personnel placing animal orders with vendors or receiving animals into the facility may not have the in depth knowledge to assure animals meet the facility's requirements. We have developed visual aids that overlay our health status entry requirements onto a vendor's health report. Health report formats used by each of our approved vendors for rodents and rabbits are obtained. A veterinarian compares infections or infestations listed against our requirements, and assigns 'Accept', 'Reject' or 'Ask Veterinarian' status to each item. 'Ask Veterinarian' indicates opportunistic pathogens that veterinarians want to restrict to specific locations or that may not be acceptable for animals on specific studies. 'Accept' is indicated by green background and a green star. 'Reject' is indicated by red background and a red prohibition symbol. 'Ask Veterinarian' is indicated by a blue background and a blue question mark. If a vendor-specific note is required, the note is typed on an adhesive address label that is then adhered to a blank spot in that report. Completed templates are scanned and saved as pdf files. The combination of color, symbols, and a key on each template assures readability if the receiving technician has color impaired vision or the template needs to be copied in black and white. Copies are given to the individuals who process animal orders. A copy of each template in a protective sleeve is placed in a binder that stays on the loading dock for easy reference when animals arrive. This system has proven effective since implementation.

P139 The Toy Menu: Photo Chart to Concisely Communicate Critical Details When Using a Large Number of Diverse Enrichment Devices Across Multiple Species and Multiple Facilities

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A successful enrichment program requires the management of a diverse assortment of items to enhance the captive environment and promote species typical behaviors. It is necessary to provide a variety of manipulanda, structural enhancements, destructible items, and devices for the promotion of sensory stimulation and foraging activities. However, safety and sanitization can become a concern with such a large diversity of items. Also, an item that provides valuable enrichment for one species may be hazardous for others. Therefore, effective communication concerning the intended species and the use of individual items is essential. We have developed a system consisting of color-coded charts for the purpose of communicating special considerations for individual enrichment items. Separate charts were developed for each species to identify the specific items approved for those species. Each chart displays the item's name and picture along with columns highlighting special safety considerations, sanitization methods, suggested uses, and the proposed enrichment benefits. They have been laminated and placed in applicable toy and cage wash areas, and also electronically distributed to research groups, husbandry supervisors, and vet services staff. They have proved to be useful for identifying and sorting items in multispecies facilities, and have provided staff with a reference for important information regarding safety and sanitation. Use has also broadened staff understanding of why specific items are used and the specific behaviors we are attempting to promote. The implementation of a pictorial guide to enrichment devices has greatly enhanced our internal communication by allowing us to more precisely define items for the purposes of managing usage, collaborating about new ideas, and for relaying concerns about how to best avoid endangering animals with inappropriate use.

P140 Genetic Quality Control and Quality Assurance Methods for Neurologic Mouse Models

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Maintaining mouse colonies with neurologic diseases can be challenging. Mice with neurologic deficits often have compromised breeding performances and in many cases, demonstrate severe phenotypes that require strict attention to animal care and use protocols. In addition to these physiologic challenges, data generated using neurologic mouse models can vary significantly if the genetic background of the model is segregating or if the method used to engineer the model is genetically unstable. The Mouse Repository at our instittution maintains one of the largest collections of live mouse colonies in the world with a mission to provide the biomedical research community with a large variety of stable genetic mouse resources, so that data can be reliably reproduced across labs. To that end, our quality assurance (QA) program has developed a number of standard operating procedures (SOP) for the husbandry and maintenance of neurologic models. When implemented correctly, well-designed SOP allow animal care technicians to efficiently maintain mouse colonies, even for strains with challenging disease phenotypes. Information presented here, outlines some key SOP used at our institution for the housing of some commonly used disease models, including monitoring transgene copy number and moribund analysis in amyotrophic lateral sclerosis (ALS) mouse models, monitoring trinucleotide repeat expansions in Huntington and Friedreich ataxia mouse models, and the monitoring of affects of genetic background on disease progression in spinal muscular atrophy (SMA) and ALS mouse models. The development and utilization of solid animal husbandry and quality assurance programs will ensure genetic stability in live mouse colonies and will result in reduced variability in phenotype expression and experimental findings, allowing for a more productive and efficient use of animals with reproducible results.

P141 Effective Cage Wash Disinfection at Lower Final Rinse Temperatures

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The *Guide* recommends an acceptable water temperature range of 143 to 180 °F for equipment disinfection, but often laboratory animal programs choose the traditional higher end to ensure compliance and disinfection efficacy. Most cage wash equipment has an option to guarantee operation only when the final rinse temperature reaches 180 °F. Building utilities as well as malfunctioning equipment at times prevents the final rinse from reaching this 'mystical' temperature, thus leading to cage wash shut downs. This can seriously affect throughput and productivity by creating bottlenecks and disrupted workflows. We evaluated disinfection efficacy by testing microbial growth on a variety of equipment materials using different water temperatures in the final rinse, while maintaining consistent concentration levels. Nonreversible, cage surface temperature-recording labels were used on plastic, stainless steel caging equipment such as feed lids, cage tops and bottoms. These labels have an indicator that activates at 160, 170, and 180 °F. After washes of equipment at these 3 temperatures, equipment was swabbed with ATP swabs to test for the presence of organic material. Items washed at all 3 temperatures passed, with little to no microbial growth, regardless of the equipment material. Although higher standards may be advantageous in many cases, validation of practices can yield beneficial results that can be used to improve facility throughput and operation without impacting quality. As a result we were able to decrease the number of tests performed as well as no longer needing to guarantee the final rinse temperature of 180 °F. We continue to use the labels and only increase microbial monitoring if final rinse temperature does not reach at least 160 °F. Thus, we no longer halt operations, which result in no downtime while maximizing overall productivity.

P142 A Novel Delivery Device for Rabbit Hay

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Rabbits require additional roughage when housed in laboratory conditions for long periods. Hay is often given as a supplement and also for enrichment. In cages with slatted flooring, hay often falls through the bars, or becomes soiled with urine and feces when added to the regular feed container or put directly on the cage floor. We researched different commercially available hay holders, all of which did not fully meet our criteria for a cost-effective stainless steel autoclavable holder capable of being hung in a rabbit cage. Autoclavable stainless steel wire whisks available in a local supermarket, fit all our criteria. Whisks are filled with autoclaved hay daily. Animal Care techs find whisks easy to fill and sanitize because they are left hanging in the cage when it is sanitized. Rabbits readily eat the hay from the whisk, and the hay stays clean and dry until consumed while also providing enrichment. Some hay loss still occurs, but the bulk remains clean, dry and accessible.

P143 A New Strain of Micropigs for Research

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A new strain of selectively bred micropigs has been developed for a variety of biomedical research applications. Exceptionally small foundation animals from an original Yucatan miniature swine colony were crossed with selected Vietnamese miniature swine in 1990 resulting in hybrid F-1 progeny. Subsequent later generation matings used the following selection criteria: structural soundness, reproductive performance, mothering ability, milk quality, docile temperament, and small size. The new strain known as Panepinto micro/minipigs is significantly smaller than the original Yucatan micropigs reaching sexual maturity at a weight of only 8 to 10 kg. Adults at the age of 5 y weigh 20 to 25 kg, making them ideal for chronic studies or studies requiring the use of mature older adults. Their exceptionally calm disposition facilitates the conduct of studies requiring extensive human contact with minimal stress to the pigs or their handlers. When housed in environmentally enriched facilities optimally designed for humane handling and restraint, these unique swine provide an economical, user friendly alternative to traditional farm or miniature swine.

P144 Characterization of Prairie Vole (*Microtus ochrogaster*) Pup Development

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Prairie voles (*M. ochrogaster*) serve as a valuable research model for behavioral studies, in particular, alcohol addiction. One important aspect of understanding alcohol addiction is characterizing the social impact and influences involved in alcohol consumption. Since prairie voles remain pair-bonded throughout their lives, this makes them an important and unique model in behavioral studies. When philopatric (those remaining near their point of origin) voles are exposed to a new litter, they express parental care such as huddling over their younger siblings and grooming the pups. This phenomenon, called alloparenting, affects future reproductive behavior of the juvenile voles, and can confound behavioral research results. Therefore, it is important to determine exact birth dates and wean the pups in a timely manner (typically, 20 d after parturition). At our institution's Veterinary Medical Unit (VMU) we have created a reference chart with photographs and descriptions of the vole pup in order to characterize their daily development. This was created in order to assist technicians working with breeding voles to determine precise ages and birth dates, which can be challenging due to the fact that pups stay attached to females and remain concealed beneath the dam's body; furthermore, the voles prefer to burrow in dense nesting material making observation challenging. By determining these dates more precisely, we can wean the animals more safely and avoid an alloparenting experience. We have also provided pictures and illustrations on the chart to identify the sex of the vole pup as this can be challenging. By creating a reference chart for sexing and aging the voles, we have improved our breeding program.

P145 Facility Maintenance: The Ultimate Game of Chess in Facility Repairs

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Older animal facilities require maintenance and repairs to keep them in good working condition. Ideally, swing space would be available within the vivarium to temporarily relocate rodents while work is conducted. However, when facilities are close to capacity, it is difficult to vacate housing rooms for repair work. Our institution has investigated the following ways to have work completed, using short-term room evacuations or leaving rodents in the rooms. For maintenance work with rodents remaining in housing rooms, maintenance staff must be educated about procedures to be followed for the safety of the animals and research integrity. It may be possible to schedule repairs after husbandry shifts end and before lights turn off to reduce disruptions to daily rodent room work. Floors in the housing rooms can begin to fail after years of use. When swing space is unavailable, floors can be repaired over the course of 2 nights. The first night, the floors are sanded for short periods of time to reduce the noise exposure to the rodents. Equipment is shifted from one side of the room to the other, with the rodents staying in the room. The second night, epoxy is applied to half of the room, allowed to dry, then applied to the other half of the room, shifting equipment as needed. When painting is needed, low fume paint can be used as well as increased air exchanges to reduce the olfactory impact to the animals. Large covers can be placed on racks and the racks temporarily placed in the hall to allow more space for maintenance to occur. Automatic watering racks covered in the hall can have carboys of water added so the rodents still have access to water. Short periods of drilling can be conducted with animals present, if research staff is alerted to the need for the repair work and they have the opportunity to remove any noise sensitive rodents from the facility. Several of our older facilities have been updated applying these methods. Research staff has been receptive to animals remaining in housing rooms during maintenance so they still have access to their animals. Throughout this process, there has been no reduction in breeding, no visible display of stress by the rodents when the work was being completed, and no known impact on research being conducted.

P146 Establishing a Community Outreach Program within Your Animal Care Program

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Being involved in the community surrounding your facility is very important for building a relationship with the community. The more involved you are in the community, the more you will be able to increase the community's familiarity with your facility as well as allow them to have a positive perception regarding the research being conducted. There are numerous ways to set up a community outreach program. For any program to survive, you must have institutional or company support. Also, a mission statement should be established and goals should be set. Then, determine which type of volunteer work you would like to focus on. Animal welfare, awareness benefits or campaigns, and scientific experience can all be used in the community. At our institution, we really focused on being involved with the animal community and wanted to provide assistance to the veterinary and animal care community. We worked with the Animal Care Shelter (SCAA) to provide food, adoption assistance, and veterinary support as needed. Also, we attended benefits such as trivia competitions and other events to support the local causes. Our staff provides seminars and training at the local schools and often serves as judges for the science fairs. Also, we provide laboratory animal and research methods training at several local college and universities. Overall, establishing a community outreach program within your animal care program will provide your staff the opportunity for positive interactions within your community.

P147 Integrating Aromatherapy into the Nonhuman Primate Enrichment Program

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Aromatherapy has been used as a method for reducing anxiety and increasing relaxation throughout history. The Chinese were credited with using aromatic plants for wellbeing. They would burn incense to help create balance and harmony. The Egyptians used distillation

and infused oil as methods to reduce stress and memory fatigue. The use of aromatherapy in laboratory animals has been limited to food items. The use of aromatherapy methods has not been popular in the laboratory animal community because some critics feel that the facility is trying to hide undesirable odors in the animal rooms. We decided to expand our aromatherapy program at our facility. With our current program we are making popcorn in the nonhuman primate rooms and providing sound and smell stimulation. With the response we noticed during the popcorn enrichment session, we decided to expand the different smells that would stimulate nonhuman primates. We decided to introduce one new fragrance for 1 h up to 3 times per week. We used cynomologus macacaques, 3 to 5 y of age and 2.3 to 4.2 kg body weight. During behavioral observations the cynomologus demonstrated increased awareness and curiosity, and they also presented to the front of the cage and remained calm. There were no significant changes noticed in their CBC or in their serum chemistries to indicate that the aromatherapy enrichment caused a stress response or toxic effects. Overall, the benefits of introducing aromatherapy into the nonhuman primate enrichment program include stimulating their sensory response, providing increased awareness to their surroundings, and a calming effect. Improvements could be made for better delivery systems and other methods to stimulate their sensory responses.

P148 Refining Housing and Husbandry Techniques in Transgenic Mice through Environmental Enrichment

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Dynamic changes in housing and husbandry techniques based on the concept of 3Rs should be included as routine practice in breeding and experimental animal facilities tending to improve animal welfare. Immunosupressed mice are susceptible to diseases and have special requirements for husbandry and maintenance. In this case we tried to improve welfare and optimize the reproduction of a transgenic colony through environmental enrichment. The effects were evaluated by progeny production and frequency of species-specific conduct as behavioral indicators. The design consisted on the combination and alternation of materials for social and physical enrichment (environment complexity, nesting material, sensory and nutritional enhancement) every week within the cage changing in routine husbandry to the experimental group (n = 13) and no enrichment to the control group (n = 13), during 3 mo. Number of pups born alive, pups per female and survival rate at third week of age were evaluated and analyzed by χ^2 tests. Results showed that 58.6 % of pups born alive belonged to the experimental group ($P < 0.05$), with a higher number of pups per female (9.1 compared with 6.4) and a higher survival rate than the control group (94% compared with 70%; $P < 0.05$). An initial and final ethogram showed an increase in frequency of exploratory behavior in the experimental group. With this experience, a refinement was made through environmental enrichment implementation. Fewer breeders could be used based on the increased number of pups and survival rate, and an improvement in animal wellbeing maximizing species-specific behavior could be achieved. Moreover, collecting data and performing new refinement protocols are important for the establishment of new guidelines on housing and husbandry techniques based on a responsible and rational use of laboratory animals.

P149 Nonhuman Primate Positive Reinforcement Training: Flexible Resource Approach

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In a review of current literature on positive reinforcement training, the majority of sources suggest that in order to produce results worthy of the time investment, training sessions must be held 2 to 3 times per week at regular intervals. We investigated the possibility of training animals at irregular intervals and frequency so that training could be accomplished while not impacting other time commitments and responsibilities. We trained one room of 12 singly housed, nonnaïve, adult male cynomolgus macaques (*Macaca fascicularis*), investing 1.5 to 2.5 h per animal over a period of 21 mo. Training sessions lasted 5 to 10 min per animal, at varying times of day, from 0 to 3 times per week. All training took place in the animals' home cages. The initial work involved conditioning the secondary reinforcer (a clicker device), taking an average 5 to 10 min per animal, and desensitizing the animals to the trainer (an individual the monkeys were unfamiliar with), which took 0 to 30 min per animal. Animals were then trained to station on a perch at the front of the cage when the trainer approached, move toward and follow a laser dot presented at locations inside their cage, move toward and follow the trainers' hand presented at locations outside their cage, present body parts to the cage front, present hands and feet for initial nail trimming training, and to calmly present their collar at the cage door opening and allow pole attachment. Results of training included increased willingness to participate, decreased time to acquire new behaviors, improved quality and duration of learned behaviors, and less aggressive gestures directed toward the trainer and other monkeys. Our results show that positive reinforcement training can be successfully accomplished using an irregular training schedule.

P150 Hematologic and Serum Cytokine Values in 2 Strains of Laboratory Mice Shipped to High Altitude

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Little is known of the effects of altitude exposure in mice. Since the major vendors of laboratory rodents exist at or near sea level, the length of time required to acclimatize mice to high altitude should be a concern for all researchers. Known changes in other species include increases in erythrocyte production and certain cytokine levels. As these changes have the potential to significantly affect biologic research, we investigated their presence, magnitude, and duration in ICR and C57BL/6J mice, 2 commonly studied strains. Our goal was to ascertain exactly how many days after arrival at altitude hematologic and cytokine parameters would take to become "normalized". Our first aim was to identify any hematologic changes present. We hypothesized that altitude would induce reticulocytosis and a gradually increasing hematocrit, with reticulocyte counts decreasing and hematocrit stabilizing by 3 wk after arrival from sea level. Our second aim was to identify the degree and duration of changes in IL6, TNF, CRP, IL10, and EPO during acclimatization; with our hypothesis being that all factors would increase and then level off or decrease (by 3 wk) during acclimatization. Although no evidence of erythropoiesis was found over the 3-wk study period, significant elevations in lymphocyte and neutrophil counts were observed in both strains. CRP values, likely representative of normal basal values, remained constant over time but differed significantly between strains. TNFα, IL6, and IL10 levels in the majority of mice were below detectable limits. Investigation into the cause of the observed leukocyte changes is ongoing. Based on these findings, we recommend a minimum 3-wk acclimatization period before using mice shipped from low to high altitudes.

P151 Post Approval Monitoring Program: A De Novo Review

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The latest method to be implemented in the continued monitoring of animal use protocols is the idea of post approval monitoring (PAM). While, not required by law, first mention of PAM by a guiding document is in the eighth edition of the *Guide for the Care and Use of Laboratory Animals*. At our institution, PAM is considered imperative after the initial approval. Since 2003, we have incorporated a formal policy for the monitoring of approved protocols but it was not until 2008 that a formal PAM program was implemented. With approximately 1000 active protocols, 4 regulatory compliance associates (RCA) meet with principal investigators or laboratory managers. Any items that affect the health or welfare of animals or personnel is brought to the UCUCA for review. All other items are corrected with the PI through meetings, emails, or phone calls. The RCA play the role of enforcer, but also of educator which helps form a relationship of mutual respect with laboratory personnel. A review of other secondary institutions with PAM programs shows that PAM are performed based on risk. PAM at our institution are performed annually for all active animal use protocols regardless of risk. The visit consists of discussing the animal use protocols in detail, updating investigators on recently revised policies and/or guidelines which may affect their protocols, and answering questions from the investigative personnel. The feedback and metrics have uncovered many areas of improvement for investigators and for the UCUCA to evaluate the effectiveness of policies and guidelines they have in place. By conducting our PAM on an annual basis we can show an increase in the number of self-reported

incidents and a greater improvement in compliance with animal use protocols, policies, guidelines, and a better working relationship with investigative personnel.

P152 Undergraduate Classes in Laboratory Animal Environmental Enrichment: Benefits to Animals and Students

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An environmental enrichment program for research animals is recommended by the *Guide for the Care and Use of Laboratory Animals*. Environmental enrichment techniques such as novel presentation of food items, rotating the supply of manipulable objects in the cage, and positive interaction with personnel can benefit animal welfare, but may be time-consuming for animal care staff to provide on a frequent basis. In order to expand the environmental enrichment program at our academic institution, we have developed a class in which undergraduate students participate in the creation and application of enrichment methods for a variety of species in the animal facility such as swine, rats, and rabbits. Four to 6 students who have shown a strong interest in veterinary medicine and have actively participated in preveterinary club activities are selected for the class per quarter, with a preference for upperclassmen (juniors and seniors). Students attend a classroom lecture or discussion once a week to learn about principles of environmental enrichment, animal behavior, and basic research methods. Students are then broken up into smaller groups in order to deliver established methods of enrichment to the animals. During these sessions, they also gain practical experience in animal handling and behavior. In the final phase of the class, students develop a new enrichment method, offer it to several individual animals, and observe the resulting animal behavior in order to evaluate the efficacy of the enrichment. All classroom and hands-on activities are supervised by veterinarians and veterinary technicians, who also evaluate all new enrichment methods for potential adverse effects on animal health and research studies prior to use. To date, 14 undergraduate preveterinary students have participated in the program over a 3-y period and have given the class high ratings. Overall, animals benefit from the increased frequency of environmental enrichment, while students gain unique animal and research experience.

P153 Developing Online Training Classes for Researchers and Staff

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For years we offered lecture style training. Trainers use presentations to train large groups in a classroom setting. Participant feedback indicated we could convey the same information online, and therefore, save time since they could take the class outside of work and day college courses. An additional concern was consistency between trainers when creating presentation classes. The training core decided to developed online modules to meet our participants' needs and to also maintain consistent training. To make a smooth transition, we created a template for the online modules. This template outlined all the performance and learning objectives. Following the template, we developed the training material and placed photos, videos, and links to standard operating procedures and guidelines throughout. Once finished and the material met approval by our supervisor, the material was placed into an online module by a computer specialist. Each trainer updates each modules at least twice a year or as needed by taking the course and sending in changes to computer specialist. To date we have been able to implement 2 classes online. This has expedited training and the times we offer it is now 24 h a day, 365 d a year instead of only offering this class once a week at limited times. This has allowed participants to not have to skip other duties such as lab work and college course work. With our training process streamlined, we do as much training as possible online and then follow up with hands-on training. Additionally, participants get quality customer service. Our training summary for the last 6 mo indicates that in 1 mo alone we increased classes offered from 4 per month to 47 per month. The average participant cuts required class time by 3 h, and the trainers now have 12 additional hours each month to offer hands on training classes.

P154 Managing and Maintenance of *Cercopithecine herpesvirus* 1 (B Virus) Exposure Kits

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Maintaining nonhuman primate (NHP) B virus exposure kits is an essential requirement for the health and safety of personnel. In a facility with multiple NHP functional use areas it becomes challenging when regular maintenance of exposure kits is required. Exposure kits must be available at all times, clearly visible (that is, red box), self contained in a portable container, and well stocked. All personnel dealing with NHP must have training on first response and the proper use of the kit. We have developed an efficient process to streamline management of kits using a spreadsheet to inventory supplies. Contents were determined in consultation with the Institute Occupational Health and Safety Department, Veterinary Services Department, and the health consultant at a university's Center for Occupational and Environmental Medicine. The spreadsheet uses a tracking log to identify the location, standardize supplies, and monitor expiration date(s). Kits contain the following items: eye wash, povidine scrub brushes, additional bottle of povidine scrub, 0.9% sterile sodium chloride irrigation, squeeze bottle for saline irrigation, valacyclovir caplets, drinking water, mouthwash, gloves, sterile gauze and nonstick dressing pads, band aids, permanent marker, timer, a copy of the *Guidelines for Prevention and Management of Potential B virus Exposure*, and an instructional flow chart. Animal husbandry technicians set up and maintain the kits throughout campus, reviewing the tracking log monthly for expiration dates. Due to open accessibility, kits are checked weekly for missing or damaged items. Proper maintenance of NHP exposure kits can be done by updating the tracking log to ensure all necessary supplies are available for the initial treatment. Efficient and effective management of exposure kits is essential to support the health and safety of personnel and accomplished by maintaining an updated spreadsheet and routine kit inspections.

P155 Nontraditional Methods of Vivarium Equipment Procurement

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During periods of shrinking operating budgets institutions are always searching for methods of making budgets stretch. Even with substantial economic pressures, environmental concerns play a significant role in decision making. There are a number of commercial companies that allow you to keep both points in mind by offering the ability to recycling used equipment. In the past our institution has used several of these options for targeted, minor equipment purchases. Recently, we took part in an online auction and significantly expanded the scope of this style of equipment recycling. Our institution was able to purchase nearly $1 million dollars of equipment for about $40,000. Similar to most Canadian universities, limited budgets exist to purchase these items from commercial suppliers at full price. However, a minor budget became available when we could purchase items for $0.04 on the dollar. Researchers played a crucial role by committing two-thirds of the capital from existing operational and equipment grants. We have begun to expand our budget by selling our surplus equipment in a similar manner. During challenging economic times innovation can be invigorated in an environmentally friendly way.

P156 Modifying Dog Kennels: From a Wash Down to a Dry-Clean Environment

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In order to meet global standards, reduce water usage, and improve husbandry practices, we decided to house our dogs in kennels with solid floors and loose bedding. This meant that we would have to retrofit our kennels from plastic coated wire grate bottoms and figure out a type of bedding that was cost effective, easy to clean, and safe for the animals. We tested several designs of flooring, both shape and materials, and different bedding substrates before finding solutions that satisfied our needs. We chose 2-piece bent aluminum pans with a diamond-plate pattern and XM aspen shavings. We also installed a vacuum system to help with removal of shavings instead of using shovels and bags. While slightly increasing the time for weekly cleanup this change has greatly decreased the time to complete the daily husbandry tasks as well as being more comfortable for the animals. In addition, water usage for sanitation is down over 90%. This changeover has been a remarkable success and with consultation we would recommend this to any other facility looking to house their

animals on solid floors without expensive renovation.

P157 The Effects of Sanded (Bottom Only) Perches on Finch Feet

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Birds that are kept in captivity often require nail trims, presumably due to the lack of opportunity to wear the nails down on natural substrates. For many birds, restraint for the nail trim may result in increased stress. In an attempt to reduce the need for nail trims, and thus potentially reducing stress, we evaluated the effect of using a perch with sand attached to the bottom half. By applying sand only to the bottom, we hypothesized it would reduce the need for nail trimming and also prevent any abrasion to the bottom of the foot. We evaluated the perches for 3 mo, by placing a sanded perch in each of 3 cages and comparing those cages with 3 control cages. Husbandry staff continued to trim the nails as needed, and recorded this on data sheets. At the end of 3 mo, all birds remained free of soft tissue abrasions. Birds in cages with experimental sanded-bottom perches required significantly fewer nail trims than birds in control cages. Sanded-bottom perches may reduce stressful handling for nail trims, improving animal welfare and the birds' validity as research models.

P158 Using Training, Teamwork, and Work Team Projects to Incorporate the Principles of Lean Management

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Lean management (LM) tools offer the opportunity to increase efficiency and productivity through a streamlined organizational structure and refined work practices. To take advantage of LM benefits, the Division of Laboratory Animal Resources (DLAR) at our institution underwent organizational changes and incorporated the concepts of LM using the Toyota Production System as a foundation for staff training. To facilitate reorganization and training DLAR conferred with colleagues who successfully implemented LM programs at other institutions before determining what aspects of LM were important for DLAR's mission and the best way to accomplish the needed changes. We then worked to gain acceptance of the new organizational structure and lean practices among staff members and support from our administration. Finally, we developed a training program to teach and promote the concept of a lean organization. Rather than limiting the training to managers and leaders or to specific functional areas, we took a different approach by including all employees in DLAR. Our training goal was to teach staff members the tools that could help them get to the root causes of work problems, make their work processes more efficient, and eradicate waste within their immediate work areas. Training classes included instruction on communication, leadership, teamwork, and employing various LM tools. Articles and case studies featuring businesses that successfully used LM practices within their organizations were used to stimulate discussion in class. Participants attended training classes with other members of their immediate work group to foster teamwork and team problem-solving skills. When the training courses were concluded, each team was required to complete and present a project that involved using the lean tools introduced in class to address and resolve a problem in the team's immediate work area. To date, all of the class participants have completed a team project and some of the teams have initiated new projects on their own, demonstrating that we have successfully trained staff members to use LM concepts. As a result, DLAR has benefitted through better teamwork and more efficient work practices.

P159 The Cost of Clean: Establishing and Maintaining a No Tolerance Standard in an Laboratory Animal Facility

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Because disease outbreaks cost institutions unnecessary financial resources, animal loss, and research delays, we established a "no tolerance" health standard for all animals housed at this institution. The multiyear transition process used a "3 W" approach. Why should it be established? Financially, scientifically, and ethically this is a reasonable, realistic, responsible, reliable, and respectable standard. When should it be implemented? It was considered in past outbreak response, established during present clean-up processes, and continued for clean status maintenance. What would it take to achieve? It required consideration, commitment, cooperation, containment, compliance, continuity, control, consequences, and contingencies. Since establishing the standard, we have maintained mouse and rat parvovirus-negative rodent colonies, survived a worldwide mouse hepatitis virus outbreak, dealt with the mouse norovirus dilemma, and prevented *Helicobacter* introduction. Currently, all rodent colonies at this facility are seronegative for 22 mouse and 15 rat infectious agents (including MNV), PCR negative for *Helicobacter*, and negative for ecto- and endoparasites. Health status is monitored by quarantine procedures and health surveillance. Respiratory and intestinal bacterial flora have been classified in mice, rats, guinea pigs, and rabbits. Viral, bacterial, and parasitic agents in conventionally housed rabbits have been identified and the process to establish the "no tolerance" health standard for these animals is in progress. Cats, dogs, pigs, and nonhuman primates are screened prior to arrival, purchased from reputable vendors, quarantined for a designated period, treated for parasites, and are monitored by ongoing health surveillance. All gross and histologic lesions observed during necropsy are classified to identify strain-related lesions, characterize clinical and subclinical infections, and associate experimentally/technically induced causes of death to improve animal health and reliability of research data. "No tolerance" is a high health standard to achieve that requires diligence to establish and vigilance to maintain. If this was an industry-wide standard there would be fewer disease outbreaks, healthier animals, lower research costs, and better research data.

P160 Environmental Enrichment Program in Transgenic Mouse Breeding Colonies

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Results of studies on the benefits of environmental enrichment in group-housed mice and breeding colonies are often inconsistent and conflicting. After observing a significant drop in some of the genetically engineered mice (GEM) breeding colonies during a major construction project in an adjacent building, an environmental enrichment and nutritional supplementation program was implemented in order to determine if these changes could salvage colonies at risk. During the construction, many of the 12 strains monitored experienced a decrease in reproductive performance (average litter size, average number of litters born per female, and survival). In order to determine if enrichment was effective, 10 types of nutritional supplements, 4 types of nesting material, and 3 types of huts were tried in every breeding cage. After an 8-mo trial period we empirically determined that 3 types of nutritional supplements were consumed more readily, one type of nesting created the best nesting instinct among the mice and plastic huts were most useful in that they did not block the water valves. Approximately 4 mo after the program was initiated a 2-fold increase in production for the 3 most affected colonies was documented. The program was also simultaneously implemented in 4 colonies that had stable reproductive performance during the construction period and no change in this status was observed. The results of this practical approach to transgenic colony management suggests that when risks of losing valuable mouse strains are encountered, implementation of an environmental enrichment with nutritional supplements program may be highly beneficial.

P161 Effect of Environmental Enrichment after the Occurrence of Wet Bedding Created by Mice and Abnormal Fur in Mice

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In management of laboratory animals, wet bedding created by mice and abnormal fur in mice cause problems on housing, leading to negative effects both on the wellbeing of mice and on the validity of experimental results. Most studies have investigated whether environmental enrichment (EE) prevents those problems by adding toys in advance. However, strategies for EE after the occurrence of those problems have not yet been established. In this study, we investigated the effect of giving toys after the occurrence of wet bedding created by mice and abnormal fur in mice. The cage was judged as containing wet bedding when more than 10% of the bedding was saturated with water for 14 consecutive days. The wet bedding was considered to have been created by some behavior of mice, because there was no

equipment failure of automatic watering system valves provided outside the cage. To determine the effectiveness of giving toys, a piece of wood and a mouse house were added as EE to 62 cages judged as containing wet bedding after the cages were changed. After adding toys, the cages were changed at least once a week and bedding conditions were checked once a week on the standards of judgment. Fourteen days after adding toys, the number of cages containing wet bedding was reduced to 42 (66.1 %). The mice housed in the cages were as follows: female or male C57BL/6, ICR, BDF1, and genetic recombination mice. There were some obvious differences among sexes ($P < 0.01$) and strains ($P < 0.01$) in reduction of the frequency. The frequency decreased dramatically for male mice (42.1%) compared with that for female mice (76.7%). The most effective reduction was found in male ICR mice (0%). We also investigated the effectiveness of adding toys after the occurrence of abnormal fur (that is, loss of hair along with scratches). The toys were placed in 8 cages in which the mice were judged as having abnormal fur on scoring by visual assessment. The abnormal fur in mice improved after giving toys in all of the cages (100%, $P < 0.01$). Statistical analyses were carried out by analysis of nonparametric test. These results suggest that adding toys for EE after the occurrence of wet bedding and abnormal fur is effective for reducing the frequency of those problems.

P162 Project Management in the Regulated Laboratory Environment

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When it comes to buying a piece of laboratory equipment or upgrading software we often overlook the importance of project management in the regulated laboratory environment. Strict FDA and GLP regulations place added demands on facilities, IT, and business personnel. What seems like a typical software upgrade can become an arduous task without the proper knowledge of specialized regulations, such as CFR 21 Part 11. The project management lifecycle separates the project into manageable and measurable project phases. The number of project phases or, sometimes called, process groups can vary within different organizations. There are typically between 4 to 5 phases that occur within the project lifecycle. The first phase is the initiating phase. This phase is where the project gets formally authorized and the core project team is formed. In the second phase, planning, you define the project objectives and scope. The project plan is refined and you are ready to begin the executing phase. The third phase, executing, is where the project plan is carried out. It is critical throughout the executing phase that you are monitoring and controlling the project milestones. Milestones are significant events or major deliverables within a project. Measurable milestones guarantee successful transition through each phase of the project lifecycle. Often times, despite meticulous planning, new Sponsor requirements or revised FDA guidelines can jeopardize a project timeline. Comprehensive monitoring and controlling of changes and trends in regulations will enable a timely and effective response. Project close out is the last phase the project where it is formally completed and closed. Understanding the regulatory laws and guidelines like the FDA, GLP, and 21 CFR Part 11 are critical to a project's successful completion. Navigating through the project lifecycle can be difficult but having a foundation of project management knowledge and regulations can make a project a success.

P163 Evaluation of Several Hemostatic Agents to Control Bleeding in Factor VIII Knockout Mice

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Some articles describing Factor VIII knockout mice recommend application of silver nitrate sticks (caustic pencil) to the cut end of tail or other appendage to control bleeding when tissue is excised for genotyping or to conduct bleeding time tests. Material safety data sheets for silver nitrate sticks characterized silver nitrate as a caustic and as an irritant. The objective of this project was to find a less irritating, topically applied product that effectively controlled bleeding after collection of tissues for genotyping from young (7 to 10 d old) pups from the Factor VIII knockout breeding colony. Six products sold for use as topical hemostatic agents during dental or surgical procedures were evaluated. These products contained 1) ferric sulfate, 2) aluminum sulfate, 3) aluminum chloride and ferric subsulfate, or 4) regenerated etherized and oxidized natural fiber cellulose. An individual product was either powder, liquid, gel, or a cellulose pad. The regenerated and oxidized natural fiber cellulose pad required time to cut the pad into small pieces and even small pieces could not be easily applied. The powder containing aluminum chloride and ferric subsulfate was difficult to apply. The liquid formulations of ferric sulfate or aluminum sulfate were also difficult to apply. The gel formulation of ferric sulfate was supplied in a 1-mL syringe with a small flat-tipped applicator. The combination of gel formulation and applicator design made this product very easy to apply, and it was chosen for a more extended study. During a 2-mo period, tissue was collected from 1319 pups at 10 d of age for routine genotyping. Approximately 25% of these pups (approximately 330) were expected to be hemizygous males with hemophilia. An occasional pup required a second application of the ferric sulfate gel within a few minutes of tissue excision. No subsequent bleeding was observed, and the site of excision healed rapidly.

P164 Effects of Different Caging System on Behavioral Testing in Mice

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Depending on the way the air is supplied within individually ventilated cages, motor-free and forced air ventilated systems were developed. The aim of the present study was to compare the influence of the forced air ventilated caging system with the motor-free ventilated ones on different behavioral tests related to general locomotor activity and anxiety. A total of 20 C57BL/6J male mice at the age of 14 to 15 wk were divided into 2 groups. Group A ($n = 10$) consisted of animals that were born and housed in a forced air ventilated system with 60 ACH, while group B ($n = 10$) consisted on mice that were born and housed in a motor-free ventilation system with approximately 25 ACH. Separate groups of mice were subjected to locomotor activity observation for a 30-min period in an open field, 5 min in the elevated plus maze and 10 min in the light-dark box. Locomotor activity was assessed by measuring distance travelled and vertical counts, separate indices of anxiety were taken from time spent in the center of the open field, time spent in the open and closed arms of the elevated plus maze and time spent in the light and dark compartments of the light-dark box as well as light-dark transitions. In terms of locomotor activity, group A revealed increased spontaneous motor activity and similar habituated motor activity to group B. When considering indices of anxiety, time spent in the center of an open field and transitions in the light-dark box were increased in group A compared with group B. Based on our findings, we can conclude that the different caging systems have differential effects on locomotor activity and indices of anxiety in the specific behavioral tests carried out. Such factors must be taken into account when planning behavioral experiments.

P165 No Mouse Left Behind

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Reaching the rodent health monitoring goal of "no mouse left behind" in a large medical research institution requires the daily cooperation of animal care personnel on all levels and a mechanism for open communication with the researchers. Therefore, our animal health monitoring system consists of the following steps. First, the animal care staff notifies the veterinary technical staff of any ill animals. After an assessment of sick animals by the veterinary technical staff, the research lab is contacted to discuss the case. A clinical agenda is implemented for the case, including treatments and follow-up procedures. Optimal communication channels among the involved parties are necessary to avoid losing track of any clinical case, especially when working in a high volume (over 200,000) mouse facility. To better track the status of mouse health issues, a color cage-tagging system has been implemented that allows us to quickly target rodents with clinical problems and identifying new cases as opposed to those already under treatment. In addition, a sick animal report sheet correlates numerically with the colored tags, making it easy to keep permanent track of the case until it is resolved. With the efficiency gained by this clinical record system, it has been possible to rank the relative morbidity and most common health related issues of over 1000 sick reports that span 1 y. Our data demonstrates that the the leading causes for clinical animal morbidity were bite wounds and pruritus, with 29% and 27% of all cases, respectively. Research-induced disease

accounts for an 11%, with excessive tumor burden being the most prevalent (7% of all cases). Thirty-eight percent of all clinical cases were successfully treated and 58% of sick animals were ultimately euthanized due to unsuccessful treatments or for experimental purposes. Veterinary technicians perform follow-up treatments for approximately 40 animals a month, averaging 2 treatments per case. Trough this record system, we are able to determine the most common problems affecting our mouse colony, allowing us to adjust treatment strategies to provide the best outcomes for research and animal wellbeing, thus demonstrating our commitment to the 3Rs, the hallmark of laboratory animal welfare.

P166 Vibration Inside of a Mouse Cage Caused by Construction Equipment

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Vibration produced in a laboratory animal facility whether stemming from daily operations, facility renovation or new construction is a concern in regard to altering research outcomes, affecting animal physiology, and inducing stress in laboratory animals. This concern has been reflected in the eighth edition of the *Guide for the Care and Use of Laboratory Animals* where new emphasis has been placed on controlling vibration in animal facilities. Because there is minimal information in the literature concerning the levels of vibration that are induced in the laboratory animal environment, the current study was performed to determine the levels of vibration that occur inside of a mouse ventilated cage due to common construction equipment. Vibration levels were measured within the cage when various implements of construction equipment were used 3 ft from the cage and at various distances and locations from the use of a large jackhammer. Vibration produced 3 ft from the cage was increased from ambient vibration (0.023 m/s^2) by all equipment evaluated and ranged from 0.136 m/s^2 for the vacuum to 1.092 m/s^2 for the shotblaster (without shot). Although vibration from the large and small jack hammers remained at relatively constant levels over the measured frequency range (approximately 12.5 kHz), vibration from the other implements decreased as the frequency increased. Vibration caused by the shotblaster tended to be greater at the lower frequencies (<1.5 kHz) than the other equipment, which contributed to the overall high level of vibration for this implement. As the distance between the large jackhammer and cage increased, vibration was lower in amplitude particularly at frequencies above approximately 300 Hz. The total level of vibration generated by the large jackhammer increased from the vibration of the ventilated rack alone at 0.024 m/s^2 to 0.475 m/s^2 at 15 ft and 0.065 m/s^2 at 50 ft on the same floor as the jackhammer. Vibration from the large jackhammer was 0.162 m/s^2 on the second floor and 0.082 m/s^2 on the third floor directly above the jackhammer. This work provides data for reference when considering vibration levels produced by construction implements in an animal facility.

P167 Assessment of Variability in Rodent Husbandry Practices Using Disposable Gloves with Chlorine Dioxide-Based Sterilant Application

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Consistency in husbandry practices of animal care staff in a laboratory animal setting is critical for the successful operation of the facility. Within rodent facilities, a primary objective is to prevent potential pathogen dissemination between animal cages and colonies. At our institution, expectations of husbandry practice include the use of disposable gloves sprayed with topical chlorine dioxide-based sterilant to maintain disinfection during cage and animal handling. To emphasize the importance of consistent training amongst staff and therefore assess relative consistency of glove use with chemicals, we observed animal care technicians (n = 5) for 5 1-h periods, for a total of 5 h of recorded cage-changing per person and then surveyed on individual practices. The survey did not permit collection of personal information and was reviewed by the Institutional Review Board. Results found that 1 of 5 staff elected to double-glove for more 'safe' work, and that others selected nitrile or latex depending on personal preference and comfort. Breakdown of glove materials due to intensity of use was not reported; instead, rationale for glove changes correlated to the need to remove dirtied caging from rooms or to bring in additional clean supplies (cages, food, etc). With respect to spraying gloves before handling each cage, 4 of 5 had almost perfect practice, with less than 10 skipped sprays noted over the course of the 5 h observed. Of the 5 individuals, only 2 had been trained on husbandry practices by the same trainer and 1 staff member did not recall any formal training offered. Despite the variability in husbandry practices, the facility has had no murine viral outbreaks since October 2008. As a result of this project, use of gloves for rodent husbandry will be reviewed with the full complement of staff to best achieve a more consistent level of practice throughout the facility.

P168 Solving University Budget Woes: Maximizing Budget Dollars By Purchasing Used Equipment and Supplies

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Every director or administrator at some point in their career has had to reduce or cut their operational budget. In difficult economic times, budget strategy is needed in order to determine where reductions, potential savings and operational efficiencies can occur in the budget cycle. At our institution's Medical School, we are constantly trying new methods to stretch those operational dollars. Typically, we purchase new equipment and supplies annually and plan these costs within the budget. During operations of that budget year, personnel determine what refurbished or used equipment and supplies could be purchased rather than procuring untouched commodities. Personnel will research the quality and availability of used merchandise and purchase accordingly. A good relationship between the vendor and customer is important with these transactions. If stock is unavailable the vendor can accommodate on future availability of the merchandise as well as locating other source of used commodities that will meet the needs of the customer (university). As result of purchasing used equipment over a 1-y period the university saved approximately US$203,328 in much needed operational dollars and costs of items averaged 43% lower than buying virgin products. In tough economic times our department has been able to absorb budget reductions that have impacted our budget subsidy.

P169 Double-Sided Biologic Safety Cabinet Improves Productivity in a Vivarium

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Productivity improvement was needed in a 5000-cage vivarium. A design team was assembled that was composed of engineers, technicians, and staff of the vivarium, and engineers and designers of biologic safety cabinets. This team developed a specification for a double-sided type A2 biologic safety cabinet that would meet the following criteria: be controllable from both sides, improve productivity, include ergonomic features that would reduce fatigue and help repetitive motion injuries, these should include a bottle uncapper, a drain for the recyclable bottles, storage for both cage changing and procedures, and allow for the separation of recycling and composting materials. A prototype cabinet was built and tested. Modifications to the design were made and 6 cabinets were built, tested, and installed in a 5000-cage vivarium and have been in service for over 9 mo with the following results: productivity improvement has been over 50%, there is no more moving of tables or cage racks because all cages are within 8 ft of the cabinet, isle space stays clear, removing bottle caps with the uncapper is much easier than doing manually, shelving for the bottles and storage under cabinet make it more orderly to work, water drains in the cabinet make it more efficient for draining water bottles, the double-bagged waste chute makes it possible for removing recycled and composted material, and the convertible work surface allows more flexibility for the cage changing.

P170 Synopsis: Protocol Information at your Fingertips

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The IACUC-approved animal protocol is an essential tool used by animal technicians everywhere. However, not only are they long, some can be about 30 to 40 pages, they can also be very difficult to understand to the lay person. In our facility, approved protocols are

available only on the IACUC website and are not easily accessible to the technicians. As an animal care technician it is very important to have some of the information in the protocol at your fingertips when you are working in the animal housing room. Animal care technicians should know information such as: what are the humane endpoints of this project; are these animals expected to have tumors and if so, at what size should I report them; are these animals expected to show any clinical signs of illness, etc. Unfortunately, it is just not practical to have to read or search a 30- to 40-page document for specific details while you are trying to care for animals. That is how we came up with the synopsis. The synopsis is a compilation of specific information from the protocol, is only about a paragraph long, and is posted at the animal room. This information is easily available for anyone working with the animals. The synopsis will include a short summary of the research project, humane endpoints, approved procedures, contact information, and/or any other information that may be needed for that particular room. Having the synopsis at the room where the technician can easily access the important information helps keep our technicians informed about the research projects their animals are involved in. It helps them perform their job better by understanding what to expect of the animals they care for. This poster is targeted towards managers, supervisors, and technicians as a suggestion for providing important information for technicians regarding the care of the animals with whom they have daily contact.

P171 Evaluation of Nesting Materials for Use in Nude Mouse Colonies

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Nesting material has been reported to be beneficial to mice by providing environmental enrichment as well as a means of thermoregulation. Cotton nesting material has been reported to cause conjunctivitis in athymic nude mice. We evaluated 3 different paper-based nesting materials to determine the optimal nesting material for our nude mice housed in individually ventilated cages with wood chip bedding. Tissue paper nesting sheets, twisted white paper bedding, and shredded brown paper were evaluated. Cages were provided with either 4 nesting sheets, 8 g of twisted white paper bedding, or 8 g of shredded brown paper. One hundred and twenty-five CD1 Nu/Nu mice (housed 5 per cage) were housed with each product for 2 wk, using a crossover study design. Ammonia levels were monitored in all cages on days 0, 7, and 14 of the cage change cycle. Nesting material was evaluated daily for usage and any nests built were scored using a 5-point naturalistic scoring scheme. The mice were examined daily for the development of any clinical health complications including conjunctivitis and fight wounds. Each product was also preevaluated for low dust production and compatibility with our vacuum bedding disposal system. Nests made with shredded brown paper scored highest (average nesting score: 2.8 ± 0.37), followed by the tissue paper nesting sheets (2.3 ± 0.41) and the twisted white paper bedding (2.0 ± 0.08). All animals interacted with each of the nesting materials to some extent. No nesting material produced a measurable amount of ammonia. No adverse health effects were noted during the study. A few other criteria for our final selection were realized during the study. The tissue paper nesting sheets, for a large scale operation, would be the easiest to sterilize and dispense, in comparison to the other nesting materials. The smaller space required to store the tissue paper nesting sheets would be beneficial in our animal facility. The nests created with shredded brown paper had high, thick walls making cage-side health observations by the veterinary staff difficult to conduct. Taking all factors into consideration, the tissue paper nesting sheets are the most suitable nesting material for use with nude mice in our animal facility. Tissue paper nesting sheets appear to strike the best balance between animal use, ease of visual observations, and ease of storing and husbandry duties.

P172 An Institutional Program for Safety and Efficacy When Using Inhalant Anesthesia Machines for Rodents

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A large centralized animal care program faces many educational challenges with a continual influx of new researchers. Our program uses more than 50 rodent anesthesia machines in 9 animal facilities housing approximately 30, 000 cages of mice and approximately 800 cages of rats. Safe and effective use of general anesthesia for rodent models presents some unique conceptual and practical difficulties for both new and experienced scientists working with animals. The most common problems faced are implementing safe use of inhalant anesthesia, assuring the proper level of anesthesia, and coordinating supplies and equipment maintenance with a large number of users. Difficulties with program implementation may adversely impact animal welfare. We have recently initiated a centralized program to overcome these difficulties. This program consists of training individuals, maintaining equipment, and keeping accurate records of all activities. In addition to trouble-shooting and consulting assistance on an as needed basis, the program also serves to strengthen direct communication between the animal care program and the research lab. Since the centralized anesthesia machine program has been implemented, fewer concerns have arisen about scheduling, supplies, equipment malfunctions, and personnel safety. The improved communication and more efficient use of animal care resources has resulted in improved animal welfare and more accurate and reproducible research results.

P173 Using an Online Application to Streamline Animal Transfers

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The transfer of animals between protocols and holding rooms is a regular occurrence in animal research. A transfer submission, approval, and completion system is needed between the husbandry management and research staff to ensure that all regulations, entry orders, space assignment, and census are properly maintained. Manually submitted paper transfers routinely resulted in long delays between transitions and human error tendencies, increasing the time and workload needed to gather the correct data and track the process. In order to improve the procedure, an automated online transfer system was created and implemented. The system is made of 2 parts: 1) a website where the transfer requests are submitted by the research staff and 2) an administration application for the management of processing and reporting. The online transfer system extracts information off of IACUC database and the Procurement, Receiving, and Census Office database. Upon login, the user is limited to only submitting transfers for protocols which they hold IACUC approval. All portions of the request are completed using filterable dropdown menus and a note section is provided at the end for special comments. Research staff members are also able to check the status of their submissions online. All labs were notified and trained before implementation of the system and further training is available as needed. Through the use of this system, the margin for human error has been greatly reduced. The submission and turnaround times have shown marked improvements as well, going from 3 to 4 to 1 to 2 d. This has resulted in an increase in efficiency and productivity for all parts of the research community.

P174 Nylon Cylinder: Safe and Cost-Effective Chewable Rat Enrichment

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Offering enrichment to stimulate chewing behavior in rats has been found to promote psychologic and physiologic wellbeing. While there are many commercially produced nylon products available for use as chewable enrichment, these items are costly, especially with current economic constraints. FDA-certified 8-ft lengths of 1-in. thick nylon cylinders cut into 3-in. pieces were identified as a possible cost effective alternative. To determine safety and efficacy, a 4-wk study was conducted on male and female CD rats. Six animals per sex received no chewing enrichment (control group), 6 animals per sex received nylon chew toys (positive control group), and 6 animals per sex received the 3-in. nylon cylinder pieces (test article group). Clinical observation, food consumption, body weight, clinical chemistry, hematology, and microscopic observation data were collected from all animals. Additionally, the enrichment items themselves were weighed to measure usage. No test article-related effects or differences in overall usage were noted. The nylon cylinder proved to be an acceptable alternative to the more costly nylon products. We have also demonstrated that chewable nylon enrichment devices can be safely used on drug safety studies without adversely affecting commonly assessed endpoints.

P175 Standardizing Nonhuman Primate Dietary Instructions with Visual Cues

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Fulfilling the various nutritional requirements of nonhuman primates (NHP) in a research setting is a continuing challenge for husbandry staff. NHP at our institution may be fed different diets and amounts based on study and health requirements, perhaps differing even daily for the same animal based on changing experimental or veterinary needs. And not providing the proper food may in some instances interrupt or cause delays in a study as well as possibly compromise the animal's health. It is well established that simple visual cues are effective aids in the workplace to facilitate correct performance and improve productivity by eliminating confusion and uncertainty. Thus, a colored tag system was developed to make individual NHP dietary instructions more clear. Large plastic tags to be attached to cages were matched by colors to different diet types, while letters and/or numbers written on tags designated the number of chow biscuits to be given or special instructions for each feeding. Two rooms with a total of 40 macaques were used to test this system for 3 mo, involving 3 feedings per day of varying diets, feed quantities, and feeding documentation requirements. Parameters assessed included ease and reliability of use, plus time spent during each feeding with and without the tag system. Tags were easy to work with and understand, and held up to multiple sanitation cycles as well as handling in the animal rooms. Preliminary productivity results showed a 10% reduction in the time involved for each feeding, for an annual labor savings of over 80 h. No feeding errors occurred. In summary, a simple visual tool eliminated guesswork or memorization and reduced the time involved to reliably serve complex dietary needs for laboratory NHP.

P176 Evaluation the Most Efficient Mating Strategy of BALB/cAnN. Cg-*Foxn1^{nu}*/CrlNarl in Individually Ventilated Caging System

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BALB/cAnN.Cg-*Foxn1^{nu}*/CrlNarl (BALB/c-nu), one of the immunodeficient mouse models, should be housed in the high-level barrier system. The individual ventilated cage system (IVC system) is recommended for housing BALB/c-nu but it has a high setup cost. It cost about US$50,738 per set of IVC (including 112 IVC), but only US$2834 per set of open rack (including 112 open cages). In this project, we would like to evaluate the cost-effectiveness by analyzing the reproductive capability and supplies consumption of different mating strategies in IVC system. SPF BALB/c-nu colony was maintained in the IVC units with a consistent condition (double-sides = one unit of 112 cages, 23 ± 1 °C, RH = 55% ± 5%, ACH = 70, and 12:12-h light:dark cycle) and breeders kept mating for 24 wk until retiring. Three groups of BALB/c-nu mice were assessed: group A of monogamy mating system were housed in 8 units of IVC system (n = 8), group B of harem mating system with pregnant female removed-and-replaced mating model were housed in 11 units of IVC system (n = 11), and group C of harem mating system with changing breeding female weekly mating model were housed in 5 unit of IVC system (n = 5). After normalization, 3 groups were assayed with ANOVA, and statistical differences between each group were examined by Student t test. The reproductive capability in groups A, B, and C was 14.26 litters per week per IVC unit (SE = 0.65, SD = 1.83, and 95% CI = 1.53), 22.22 litters per week per IVC unit (SE = 0.62, SD=2.04, and 95% CI = 1.37) and 21.89 litters per week per IVC unit (SE = 0.85, SD = 1.91, and 95% CI = 2.37) separately and group B was significantly higher than group A ($P < 0.01$) while no difference between groups B and C; the average supplies usage per week of each IVC unit in group A, B, and C was 109 cages per week (the maximum capacity of an unit of IVC in monogamy mating system; basis standard of normalization), 85.82 cages per week (SE = 2.11, SD = 6.99, and 95% CI = 4.70), and 95.56 cages per week (SE = 1.11, SD = 2.49, and 95% CI = 3.09), respectively. The cost of supplies usage and manpower within 24 wk (not including IVC system setting up cost and depreciated cost) for groups A, B, and C was US$2,933.66, US$2,309.78, and US$2,571.93, respectively. Both groups A and C were significantly more costly than group B ($P < 0.01$). These results showed that group B, the harem mating system with pregnant female removed-and-replaced mating model, would be an effective and economic way for BALB/c-nu colony breeding system.

P177 Chutes: Multiple Applications to Improve the Daily Lives of Group-Housed Macaques

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There are many benefits to group housing nonhuman primates, but there are also some difficulties that can arise due to this type of housing. By adding chutes to a group housing unit, it is possible to address and even eliminate some of these issues. There are approximately 1200 to 1500 macaques (*Macaca fasicularis* and *Macaca mulatta*) in groups of 6 to 30 animals at our institution. Chutes were designed to address the following issues: removing animals during daily cleaning, providing a means of closer observation of animals, and allowing easy capture of animals in transport boxes inside the chutes without the use of nets and catch gloves. Once the chutes were installed, it was apparent that they provided and improved not only what they were intended for but also a few other applications were discovered. By leaving the chute doors open during new group formations, aggression between conspecifics has decreased. Animals have also been easily trained in the pole and collar restraint from inside of the chutes to participate in procedures in the restraint chair or to relocate to a nearby housing unit. The simple addition of purposely designed chutes has made significant improvements to various tasks at this primate facility.

P178 A Comprehensive, Modular-Based Training Program for Laboratory Animal Facilities

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The pharmaceutical and biotechnology industry are under increasing pressure to reduce costs and improve the time-to-market. Furthermore, the ability to conduct a range of preclinical studies, deliver high quality data while continually refining the technical and animal welfare practices employed, is a fundamental expectation. Training departments have a pivotal role in this process and recognize the need to employ a strategy that delivers and retains a competent and professional body of staff, capable of producing high quality work as a matter of routine. The Training and Compliance Group was established to complement operational management and ensure that quality improvement practices and enhancements in animal welfare are properly assimilated and enshrined within the day-to-day thinking of the division. The group was selected in light of their technical ability as trainers, their inherent regard for animal welfare, and the quality of their data. New employees participate in an 8-wk modular based program that provides classroom and on-the-job training for all core skills. Individuals who complete the program are released to the home department with a solid understanding of regulatory requirements, animal husbandry, and technical skills relevant to their job function.

P179 Determination of Organic Matter Buildup in Rodent Water Bottles

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Water bottles are commonly employed to provide animal drinking water to research rodents. The eighth edition of the *Guide for the Care and Use of Laboratory Animals* recommends that water bottles should be replaced rather than refilled to reduce risk of microbiologic cross-contamination. Additionally, the *Guide* recommends that water bottles and sipper tubes usually require sanitation at least once per week. However, scientific justification for the recommendation is not provided. In the absence of scientific data supporting a specific recommendation in the *Guide*, professional judgment may be used in development of performance standards in achieving a particular outcome, such as determination of appropriate sanitation intervals. This study was designed to evaluate how fast, and to what extent, organic matter accumulates within animal drinking water. Such information is necessary when challenged with developing guidance on the required frequency that rodent water bottles should be sanitized to preserve animal health and wellbeing. This study was designed to measure the rate of organic matter buildup in rodent water bottles over a 14-d period in a production barrier facility. Thirty 16-oz glass bottles were used on group-housed mouse cages. An ATP swab sample was collected from each water bottle daily over a 14-d period, at which time bottles were refilled (topped off) with fresh water as needed. Swab samples were then analyzed with an ATP luminator.

The result of the study indicated that organic matter accumulated rapidly, at approximately 200% per day, for the first 48 h, followed by a reduced rate of 13% per day for the following 48 h. Following day 4, the amount of organic matter plateaued and remained relatively stable through completion of the evaluation on day 14. These results suggest that replacing water bottles every 7 d provided little benefit to water quality compared with topping off water bottles every 14 d. Consequently, it may be appropriate to change rodent drinking water bottles every 14 d, and refill bottles as needed, while not compromising animal welfare.

P180 High Throughput Identification of Animals Using Miniature ID Digital Ear Tags

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Herein we report the results of an evaluation of miniature ID digital ear tags for the rapid and efficient identification of standard laboratory rodent species. Identification via metal ear tags, RFID (microchip), tattoos, ear punch, toe notch, and other methods are common, but these methods are not compatible, nor scalable, for automated data entry into a research database. These methods also pose various challenges for biomedical imaging, MRI, and CT scans. The digital ear tags system has numerous advantages, including their small size, weight, and their ability to withstand autoclaving. In our studies we found the tags to be easy to apply, scan, and importantly can be readily incorporated into our existing database for higher throughput digital data entry and automated label printing. We also found it to be readily used in MRI studies, when the tag is placed at the side of the head, representing a clear advantage over the other methods of identification (for example, metal ear tags, which interfere with the magnetic field). In summary, the digital ear tag system is a viable, robust, safe, and humane system that can be readily used for high throughput identification of laboratory animals.

P181 Acclimation of Cynomolgus Monkeys to Pole and Collar Chair Restraint: Creating Efficiency and Improving Quality

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Cynomolgus monkeys are acclimated inhouse during their 4- to 5-wk quarantine period to pole and collar chair restraint to facilitate safe handling and adequate immobility for study procedures like oral gavage and blood collection. Acclimation is both human resource and time intensive (to provide animals just in time for study use). In order to gain efficiency (more animals pass acclimation) and decrease full-time employee (FTE) resources, we looked into the effectiveness of outsourcing part of the acclimation procedure and evaluated the results inhouse. In a pilot study, 25 of 44 animals received 20 acclimation sessions at the contracted holding facility. This acclimation occurred less than 1 wk prior to shipment to our site. Acclimation focused on using the pole and collar system to remove the animal from the cage. Upon arrival at our site, animals were acclimated per our standard acclimation process. Animals were scored on each step in the acclimation process at 3 time points (weeks 1, 2, and 4) during quarantine by a technician blinded to the 2 groups. Outsourcing acclimation resulted in an initial increase in the number of pretrained animals passing acclimation but the 2 groups scored around 65% by the end of the quarantine period. However, we identified the FTE metrics for acclimation, uncovered the rate limiting steps in the acclimation process, and created an acclimation program that eliminated the nonvalue added procedures. In addition, we discovered the value of doing acclimation evaluations during the acclimation process to identify individual animal behavior and to customize training for each animal. Implementation of the new program for pole and collar acclimation resulted in additional training sessions for animals with no increase in FTE requirements and a greater percentage (35% increase; 100% pass) of animals passing during the 4-wk quarantine period.

P182 Use of Semirigid Isolators as Part of a Rodent Quarantine Program

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Investigative requests to the Comparative Medicine department for the use of mice from nonapproved vendors and academic collaborators are very common. Historically, these requests were denied for reasons of biosecurity. The health status of such animals is routinely not acceptable for entry into the conventional facility and, with a lack of a proper quarantine space, the risk to the vivarium was too great. However, with the demands of project timelines and the increased collaborations with academic institutions and biotechs, these requests had become increasingly more frequent. It was evident that there was a need to find a way to house animals originating from high risk sources without jeopardizing the health of the entire rodent colony. Our resolution to this challenge was the introduction of semirigid isolators to house rodents with an undesirable or unknown health status for a quarantine period lasting a minimum of 6 wk and depending on the health status of the sentinel animals, potentially for the lifetime of the project. The implementation of this program was complex and challenging. It entailed engineering almost every facet of a standalone animal facility; including purchasing and ultimately altering the isolators, modifying the air and electrical supply in the room, drafting SOP, and finding creative solutions to hurdles we encountered throughout the process. All supplies introduced to the isolator were sterile when possible. Bedded cages, water bottles, and other supplies were autoclaved. Items that could not be autoclaved were sprayed into the isolator with chlorine dioxide-based sterilant. Procedures for maintaining the integrity of the isolator were outlined in SOP. Although challenging at times, the team was able to deliver a functional isolator housing program in less than 9 mo, allowing us to provide a high quality quarantine service to our investigators.

P183 Effects of Time of Day and Handler on the Response of Laboratory Rats to Playful Handling

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Rats can be playfully handled (tickled) in a manner that mimics playful social contact with conspecifics. However, response of rats to tickling varies between individuals. We hypothesized that the time of day and identity of the tickler would affect the response of rats to tickling. We assessed the effect of time of day and tickler identity on 16 male and female Long–Evans rats (*Rattus norvegicus*; HsdBlu:LE; found free of internal/external parasites and disease via quarterly serology and parasitology, and yearly necropsy evaluations). Rats were tickled for 2 min twice daily (morning and afternoon, corresponding to the beginning and end of the light phase) by 2 different handlers (ticklers A and B). Tickling was administered daily for 3 d starting at 22 d of age. The preference of rats for the tickler was assessed in a 5-min choice test on day 4. We measured the rate of frequency-modulated 50-kHz ultrasonic vocalizations (FM-USV) emitted during tickling, and the time spent near the hand of each tickler in the choice test. Rats emitted similar numbers of 50-kHz FM-USV, which are associated with positive affective states, both during the morning compared with the afternoon tickling sessions, and when tickled by tickler A compared with tickler B ($P > 0.05$). They spent an equal amount of time near the hand of each tickler ($P > 0.05$). Although rats can discriminate between familiar and unfamiliar humans, they did not show any preference between 2 familiar ticklers. These findings suggest that the positive, beneficial effects of tickling on rats are not affected by time of day or handlers. These results could be useful in integrating tickling in the handling of laboratory rats, especially when handlers and convenient times of tickling administration may vary.

P184 The Development and Maintenance of a Rodent Anesthesia Machine Rental Program in a Large University Setting

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It is frequently a requirement to anesthetize laboratory animals during the conduct of biomedical research. While many research projects require the frequent use of animal anesthesia for the duration of the project, in certain cases the frequency of animal anesthesia is not great enough for a principal investigator (PI) to consider purchasing and maintaining a rodent anesthesia machine. To help PI provide the best animal care possible while limiting costs, we recently developed and implemented an anesthesia machine rental program. The program is coordinated by a member of the veterinary technician team and is

available to all PI. The machines are fully equipped with isoflurane, oxygen, custom-made induction boxes, rebreathing tubes, scavenging system, and appropriate nose cones. The PI is charged an hourly rate that takes into account the materials and labor associated with the program. The coordinating technician is responsible for the daily and yearly maintenance of these machines. Currently, 3 single-station and one multistation fully-equipped rodent anesthesia machines are available for rental. Each rental transaction requires approximately 40 min of technician time and includes initial communications with the lab, personnel training, and preparation and cleaning of the machine. PI learn about the program through our Unit's website, our animal care staff, or from other PI that have used the service. The machines are rented approximately 130 times per year. This program has benefited labs by providing a reliable source of fully functioning machines at limited expense.

P185 A Measurement of Stress Levels in Mice Housed in the Same Room with Rats

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Current standards set by the *Guide for the Care and Use of Laboratory Animals* recommend separate housing for rats and mice to minimize stress and behavioral changes in mice due to interspecies conflict. Policies prohibiting shared-room housing may be justified based on increased levels of stress experienced by mice (prey species) when exposed to rats (predator species); however, the effects of exposure to visual and olfactory stimuli have not been investigated. Furthermore, the effects of shared housing rooms on murine stress and behavior are equivocal, as some research suggests cohousing has no effect on reproductive success or growth in mice. To determine whether exposure to rat visual and olfactory stimuli would increase stress in mice, we evaluated measures of physiologic and behavioral stress in mice during a 2-wk period of cohousing with rats. Adult, male C57/Bl6 mice (n = 8 per condition) were randomly assigned control (no exposure), positive control (exposure to predator urine), visual, olfactory, and visual/olfactory exposure conditions. Body weight and food consumption did not differ between groups. There was no significant difference in anxiety-like behavior in open field testing or adrenal weight at the conclusion of the cohousing period. Altogether, cohousing with rats and exposure to olfactory or visual stimuli failed to produce changes in measurements of physiologic stress response or anxiety-like behavior in this sample. These results suggest that stress effects of cohousing on mice are negligible and have no implications for housing rats and mice in shared rooms, thereby using research resources more efficiently.

P186 Department of Comparative Medicine Mouse Husbandry Process Improvement Project

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Mice account for 96% of the warm-blooded animals used at our institution. Because of this, mouse husbandry activities account for the majority of our departments full-time equivalents. Current mouse husbandry practices were increasing the risk of repetitive motion injury, decreasing teamwork and morale, and creating process bottlenecks. An internal collaboration between the Department of Comparative Medicine, the Office of Research Quality Management Services, and Systems and Procedures set forth to improve safety, increase teamwork, and balance daily and weekly employee workloads, and decrease risk for noncompliance. The mouse husbandry process project used the DMAIC Roadmap and employed Lean Six-Sigma tools, including: Value Stream Maps, SIPOC+R, 5S, Spaghetti Diagram, Work Balance Chart, Circle of Work, Benchmarking, Kaizen Teams, SPC and Change Management. With additional consultation with ergonomics and human resources, a 5-d staffing model with rotated tasks, defined breaks, and leveled workload was developed. Standard operating procedures were created to support the new schedule and activities. Overall, safety was improved by reducing repetitive motion risks and balancing hourly and daily workloads. Teamwork and staff satisfaction were improved by standardizing, documenting and communicating caretaker, cage wash, autoclave, and other support activities. Process flow was improved by relieving bottlenecks, designating supply locations and identifying additional value-add activities. The likelihood of a noncompliant event was decreased by defining job duties, scheduling activities, and improving metrics. The project ultimately provided a tool for future management of mouse husbandry processes and determination of appropriate staffing.

P187 Novel Outreach Activity Demonstrates the Importance of Environmental Enrichment and Promotes Discussion

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Outreach activities play an important role in fostering positive public perceptions of animal research. Our institution hosts local high school tour groups multiple times each year. During the planning for an outreach event, we observed that the usual didactic approach was not engaging the students. With the understanding that a forum for active discussion is the key to helping students resolve conflicts between prior knowledge and new information, we developed an activity that promoted student involvement in discussion. The theme of the discussion is the importance of enrichment for all laboratory animals. This theme sends a positive message about the quality of care provided to research animals. During the activity, the students are initially provided some basic information about the concept of enrichment and asked to describe some normal species behaviors. Various enrichment items that have been filled with varying amounts of candy are distributed to all students. The items have been prepared purposefully to provide access to the candy that is too easy or too difficult. Some student will receive items that do not contain candy at all. The students are asked to interact with their "enrichment" and report their experiences and the kind of animal that might appreciate the enrichment item. Participants who did not receive candy with their item are rewarded by the instructor (with candy, or other things) for helping to demonstrating the error in the enrichment design. Through discussion, the instructor helps the participants discover the importance of learning about species-specific behavior when planning environmental enrichment. This exercise has been successfully implemented during 4 programs so far. Planning learning activities that get students involved goes a long way toward changing negative perceptions of the use of animals in research.

P188 Maintain A Parasite-Free Animal Facility

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Maintaining a parasite-free facility is essential for any animal research program. We have had some colony outbreaks of pinworms and mites over the past few years, even though we have a quality assurance program and quarantine room for noncommercial vendor animals coming into the facility. Before we initiated our new policy, we would quarantine incoming noncommercial vendor animals for 30 d using a quality assurance sentinel program. At the end of the 30 d we would collect serum and lymph nodes from the sentinel animals to be tested. If the test came back negative the animals were released from the quarantine room. We have found that with extra testing and preventative measures we have a way to increase the chances of keeping a parasite-free facility. Any animals that are from noncommercial vendors, going into our quarantine room are now given preventative treatment while in the quarantine room in the form of gel packs impregnated with fenbendazole in lieu of water bottles, and the animals are also treated with a commercial permethrin product to kill mites. We are also asking for extra colony mice, when possible, to use for parasite testing as soon as the animals arrive. Now with our new policy, when animals from noncommercial vendors are brought in they are still quarantined for 30 d. However, they receive the fenbendazole gel packs in lieu of water bottles and they are given small balls of cotton with a commercial permethrin as their nesting material for the entire 30 d. We still use our quality assurance sentinel program and collect serum and lymph nodes; however, we also do parasite testing on the colony animals as well as the sentinel animals in the form of fecal floats, tape test, colon cecum test, and fur/pelt tests. Since the initiation of these procedures we have been parasite free and hope to maintain that status. In conclusion, before we initiated these procedures we had an outbreak of mites and several outbreaks of pinworms. Since we initiated these procedures our 15-room animal facility has been mite and pinworm free. We feel that these measures have helped us tremendously, considering we bring in approximately 150 to 200 noncommercial animals a year, and believe that they may

help others as well.

P189 Effective Outreach: Choosing a "Multitool" Rather Than a "Tool Box" Approach to Designing and Developing Outreach Presentations

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Outreach is an essential component of laboratory animal science and medicine. It allows experts to address the technical, scientific, and moral complexities of animal-based biomedical research. It is a direct yet respectful response to campaigns and protests of animal rights activists. It puts a human face on a field that is so valuable to our society, yet so poorly understood. Laboratory animal practitioners are often asked to perform outreach to students of varying educational levels or to other specific groups. It is important for the presenter to realize that a single preprepared presentation cannot effectively appeal to all of these audiences because of differences in interest, background, and educational level. For instance, a presentation designed to encourage veterinary students to consider a career in laboratory animal medicine would likely not engage a group of high school students. On the other hand, it can be time-consuming and, to some degree, redundant to create an original presentation for each audience. It is possible, however, to construct effective, targeted presentations without devoting an inordinate amount of time and resources. This presentation aims to help current and potential presenters create effective, audience-specific presentations while minimizing development time. We provide an overview of different educational audiences, the differences and similarities between them, and how to use these differences and similarities to create effective presentations in a productive fashion.

P190 Auditing Laboratory Animal Biosecurity Programs

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Periodic evaluation of an institution's vivarium and policies/procedures, that constitutes a rodent biosecurity program, will help assure that best practices are in place to prevent a microbial pathogen outbreak. Such an evaluation occurred recently at all of our United States rodent-production facilities as part of a continuous improvement program. We created a site-visit audit form that will be reviewed during this presentation. This form covered 1) maintenance of building integrity and perimeter control, 2) control of the introduction and spread of pathogens, and 3) surveillance for pathogens and considerations should an outbreak occur. Included in the form were questions on routine matters such as mechanical cage washing efficacy and the comprehensiveness of the sentinel animal program along with less commonly addressed questions on whether routine rounds are done in adjacent support areas (including long-term storage and HVAC space) for the presence of feral rodent nesting activity. Also covered were questions to ask management and staff during the visit on the availability of, and ease of understanding, biosecurity policies and procedures along with who is authorized to make major or minor decisions on changes in these procedures on a day-to-day basis. Potential biologic security issues for each site were stratified as high, medium, or low and acted upon appropriately. Pharmaceutical, academic, and other biomedical research institutions will benefit from our "lessons learned" on how to conduct these types of audits, which differ from IACUC, AAALAC, and other reviews carried out in a vivarium. Attendees might also find our audit form to be a useful template for use in their own biosecurity programs.

P191 Study on Energy Consumption Monitoring and Energy-Saving of Laboratory Animal Facility

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High level of energy consumption remains a common problem in laboratory animal facilitiy. An energy monitoring system was used in our laboratory animal facility for energy consumption analysis and assessing of energy-saving method. We used a standard measuring method to unify the total energy consumption of electricity, steam, heat/cold, and others. We can calculate the square meter energy consumption and compare with other buildings. The energy consumption of 2 different steam supply ways pipeline and electric steam boiler were measured by the energy monitoring system, so as to assess the better energy-saving way. The total electric consumption of the animal facility is about 2,778,841.48 kwh, and the total steam consumption is about 12,244.9 GJ. The total energy consumption converted into standard coal and equivalent of coal were 1475.40 tons and 770.10 tons, respectively. We calculated the value of square meter energy consumption to be 103.08 kgCE. Compared with 4 different buildings monitored, the energy consumption of the animal facility is 8.52 to 21.88 times more. The steam consumption of 2 different suppling ways is 9925.00 GJ and 2651.00 GJ, respectively. The energy monitoring system can reflect energy consumption of laboratory animal facilitiesin real time, and it can be used for energy consumption statistics and energy-saving administration. Because of its high level of energy consumption, it is very important to find new ways to save energy. Steam supplied with electric steam boiler is a better way compared with pipeline. So we suggest using electric steam boiler when building new laboratory animal facilities.

P192 Experience in Management of a Mouse Facility Equipped with Hypochlorous Acid Water System

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It is important to control environmental microbes in the SPF animal facility. Numerous disinfectants have been used for this purpose. Hypochlorous acid (HOCl), a major inorganic bactericidal compound produced by neutrophils to kill the pathogen, is an effective and safe disinfectant. Our SPF mouse facility is equipped with HOCl water (HOClW) generator, which produces 50 ppm HOCl (pH 5.5 to 6.0) by mixing HCl and NaOCl solution. The HOClW supplies to each animal room and supporting areas through pipeline and is operated as the tap water. The pipeline also links to HOClW foggers in each room. The HOClW fine fog is spread automatically for 2 min every 7 min during working hours. All the autoclaved materials (mouse cages with bedding, diet, bottles, rubber stoppers, and others) are kept open in the SPF animal room. There are no bacteria detected on the surface of those autoclaved materials kept open in the SPF animal room for 1 wk. We monitored the bacteria number in the air and in the drinking water by blood-agar culture every month for the past 5 y. The results showed that the HOClW fog reduces the air bacteria to almost zero. Our mouse drinking water was treated with reverse osmosis, UV, and ozone, without autoclave. There were a few bacteria in the fresh drinking water. However, in the presence of 1 ppm HOCl, no bacteria were detected. The mice drinking HOClW for up to 6 mo appeared to be normal in terms of growth rate and reproduction performance. Our results indicated that HOClW effectively reduces the airborne and waterborne microbes in the animal room and is safe for the mice. Therefore, it can be applied to the management of the laboratory mouse facility.

P193 The European Mouse Mutant Archive (EMMA)

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The European Mouse Mutant Archive (EMMA) offers the

worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. A full description of these services can be viewed on the EMMA website at http://www.emmanet.org. The EMMA network is comprised of 14 partners who operate as the primary mouse repository in Europe and is funded by the participating institutes and the European Commission FP7 Capacities Specific Program. EMMA's primary objectives are to establish and manage a unified repository for maintaining mouse mutations and to make them available to the scientific community. In addition to these core services, the consortium can generate germfree (axenic) mice for its customers and also hosts courses in cryopreservation. All applications for archiving and requests for mutant mouse strains are submitted through the EMMA website. Mouse strains submitted for archiving are evaluated by EMMA's external scientific committee. Once approval has been granted depositors are asked to send mice of breeding age to one of the EMMA partners for embryo or spermatozoa cryopreservation. Strains held under the EMMA umbrella can be provided as frozen materials or rederived and shipped as live mice depending on the customer's needs. However, certain strains that are in high demand are maintained as breeding colonies to facilitate their rapid delivery. All animals supplied by EMMA are classified as SPF in accordance with the FELASA recommendations. EMMA is a founding member of FIMRe (International Federation of Mouse Resources) and actively cooperates with other leading repositories.

P194 High Mobility Group Box 1 as a Potential Regulator of Inflammation and Determinant of Scar Formation in Fetal Wounds

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Previous studies have shown that a different type of healing occurs in embryonic skin during the first 2 trimesters of development. In mice before embryonic day 15 (E15), wounds exhibit a unique pattern of healing leading to regeneration. In contrast, fetal wounds generated at late stages of development heal with inflammation and scar formation. Currently, reasons for differences in wound-induced inflammation in early and late embryonic skin are not entirely understood. We hypothesize that differences in expression or signaling of high mobility group box 1 (HMGB1), a nonhistone DNA binding protein that acts as a damage-associated molecular pattern (DAMP), control the inflammatory response in fetal wounds. Cellular injury results in the release of HMGB1 from the cell, where it stimulates inflammation by binding to receptor of advanced glycation end products (RAGE) or toll-like receptors present on inflammatory cells. To characterize HMGB1 in fetal repair, immunohistochemical staining of uninjured and wounded skin from the scarless (E15) and scar-forming (E18) time periods was analyzed. Unwounded E18 skin showed prominent nuclear staining for HMGB1 in basal keratinocytes. HMGB1 was also present in the nucleus of basal keratinocytes in E15 skin but the staining was much less intense, suggesting that there is lower epidermal expression of HMGB1 in E15 skin. In E18 wounds, there was a striking reduction in keratinocyte HMGB1 staining the wound margins, indicating that HMGB1 is released by keratinocytes in response to injury. RAGE protein was detected in cultured dermal fibroblasts suggesting that HMGB1 may directly affect fibroblasts after injury. Studies are being conducted to examine the effects of HMGB1 on collagen production and alpha-smooth muscle actin expression in fibroblasts. Overall, these data suggest HMGB1 may be responsible for the observed differences in inflammation in early and late fetal wounds. Future studies will determine the effects of HMGB1 on the production of scar tissue in vivo.

P195 Strain-Specific Effects of Ketamine-Xylazine-Acepromazine, Ketamine-Xylazine, and Isoflurane Anesthesia in BALB/c and C57BL/6 Strains of Mice

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The mouse is the most frequently used animal model for biomedical research. To meet research needs, a surgical plane of anesthesia is commonly induced, yet information is limited regarding strain-specific variability in physiologic responses. Anesthetic protocol selection is often based on availability of equipment, invasiveness and duration of surgical manipulation, and comfort level of the investigator. Rarely is an anesthetic protocol selected based on the strain of the mouse used, yet published reports indicate strain-specific differences such as alveolar anesthetic concentration, blood pressure, and heart rate. The purpose of this study was to identify strain-specific responses of mice subjected to differing anesthetic protocols with the aim of providing guidance in selecting the most appropriate anesthetic protocol and reduce the overall number of animals by decreasing mortality due to anesthetic complications. C57BL/6 and BALB/c mice (n = 10 for each anesthetic protocol) were induced to a surgical plane of anesthesia using 3 protocols: inhalant isoflurane (ISO), injectable ketamine-xylazine (KX), and injectable ketamine-xylazine-acepromazine (KXA). Depth of anesthetic plane and respiratory rate were monitored at 10-min intervals. An electrocardiogram was obtained on a subset of animals. CBC and chemistry panels were analyzed at baseline and after anesthesia. Strain-specific differences in physiologic parameters and spinal reflexes were not observed between C57BL/6 and BALB/C strains. Inhalant isoflurane provided an easily controlled surgical plane for both strains and a statistically significant shorter induction and recovery rate when compared with KXA and KX. If an injectable protocol is desired, a dose of 150/20/3 mg/kg KXA effectively produced a surgical plane of anesthesia in both strains. We were unable to determine a dose of KX that consistently provided 20 min of surgical anesthesia without significant mortality. While significant differences existed between anesthetic protocols, more sensitive monitoring equipment may be required to reveal differences amongst strains.

P196 Development of a Magnetic Resonance Imaging Service Core for Mouse Models

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Establishing a multidisciplinary service for MRI of mouse models introduces technical and logistical challenges, but results in diverse offerings of improved research approaches with higher resolution and noninvasive imaging. An MRI service core was established with a 7-T MRI high-field scanner and a hyperpolarizer. General anesthesia of mice during image acquisitions is maintained using isoflurane from a remote vaporizer with active scavenging. A small animal monitoring system tracks mouse body temperature, respiration, and heart rate which can also be used in conjunction with an external trigger for gated imaging. Mice are placed on a cradle that slides into the coil. Body temperature is maintained by a fiber optic thermometer-controller feedback loop that provides regulated warmed air to the mouse inside the bore. Both jugular and tail vein catheters are used depending on the protocol. MRI modalities offered include anatomic MRI, diffusion MRI, spectroscopy, dynamic contrast enhanced (DCE) MRI, and dynamic nuclear polarization, each of which have unique equipment and animal preparation requirements. Protocols may require multiple catheter placements, injections prior to or during imaging, continuous infusions of multiple agents, and specialized restrainers and coils. As examples, spectroscopy requires a larger coil and a surface coil, and DCE requires a segmented, preloaded tail vein catheter. Jugular vein catheters are often preferred when multiple catheter placements are required. A 3-way connector with one-way valves is used with a double barrel infusion pump for continuous infusions of multiple agents and a saline push. Tumor development, progression, metastasis, and responses to therapy in mouse models of cancer are evaluated. Sequential images are acquired, reducing numbers of animals used. Establishing an MRI service core diminishes animal use and provides unique research approaches with clinical relevance that lead to discoveries of new diagnostic and therapeutic approaches.

P197 Establishment of Lewis Rat Model of Arthritis for Investigating the Role of Kallikrein-Kinin System Activation in the Synovial Homing of Endothelial Progenitor Cells

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Circulating endothelial progenitor cells (EPC) home to ischemic and inflamed tissues and participates in vasculogenesis. However, the homing mechanism of EPC remains poorly understood. Plasma kallikrein-kinin system (KKS) consists of plasma prekallikrein, factor XII, and high molecular weight kininogen (HK). Upon activation of the KKS, prekallikrein is converted into kallikrein, which cleaves HK

to release bradykinin. Bradykinin increases vascular permeability and inflammatory responses. In this study, we found the KKS activation regulates the mobilization of EPC in vitro and modulates the homing property of EPC in vivo. EPC, which were isolated from bone marrow of disease-free Lewis rats, expressed similar mRNA levels of endothelial cell lineage marker such as VE-cadherin, CD31, vWF, uPAR, hematopoietic progenitor cell markers Sca-1, CD34, and bradykinin type 2 receptor (B2R). They also expressed mRNA of multiple homing receptors including chemokine (C-X-C motif) receptor 4, CD49d, and CD62-E. Bradykinin, which stimulates B2R, enhanced EPC migration over endothelial cell layer at 100 and 1000 nM ($P < 0.01$ and $P < 0.001$, respectively), suggesting that B2R stimulates transendothelial migration of EPC. Tumor necrosis factor alpha, the major mediator in arthritis, enhanced transendothelial migration of EPC by bradykinin (168.56 ± 9.56 compared with 96.02 ± 10.23, $P < 0.001$), this priming effect of TNFα resulted from its induction of B2R expression. Arthritis in Lewis rats is associated with activation of KKS. In Lewis rat model of arthritis, fluorescently-labeled EPC were selectively recruited to the inflamed synovium and formed new blood vessels. In conclusion, bradykinin and cytokines such as TNFα, may synergize to regulate the mobilization of EPC, and that the KKS regulates the homing property of EPC by bradykinin. Thus, activation of the KKS constitutes an additional mechanism for synovial vasculogenesis in arthritis. This study provides a new insight into understanding the role of the KKS in vascular pathology and inflammation in arthritis.

P198 Comparison of Direct and Indirect Blood Pressure Measurements in Anesthetized Rhesus Macaques (*Macaca mulatta*)

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Hypotension is a common anesthetic complication, making accurate blood pressure measurement a critical component of anesthetic monitoring. Indirect oscillometric pressure estimation is frequently used due to the invasiveness of arterial catheterization. Previous studies have suggested that numerous factors can affect the accuracy of indirect pressure estimation, including cuff fit and location, patient size and musculature, and observer bias. To test these assertions, indirect blood pressure measurements were compared with direct arterial readings in 7 rhesus macaques prior to and during isoflurane anesthesia. Mean arterial pressure (MAP) was evaluated throughout a range of hypotensive and normotensive values (40 to 115 mm Hg). Direct and indirect measurements evaluated by paired *t* test were significantly different in all subjects ($P < 0.007$). Data were further analyzed using a modified Bland–Altman method, which revealed negative bias in indirect pressure measurements (mean bias = −7.18 mm Hg), indicating a tendency toward underestimation of true arterial pressure. Bias was reduced at lower MAP, suggesting that indirect measurements were most accurate at clinically critical pressures. Regression analysis revealed strong linear relationships between the 2 methods in 6 of the 7 subjects ($R^2 = 0.87$ to 0.99), indicating consistency in bias effects on MAP measurement. A weaker linear relationship ($R^2 = 0.52$) and greater bias (−11.29 mm Hg) were noted in one case where cuff size deviated substantially from 40% of limb circumference. Subsequently, same-sized cuffs were placed on the brachium and opposite antebrachium of the same animal. Increased bias was noted in readings from the looser-fitting cuff (−14.21 mm Hg compared with −7.00 mm Hg, $P < 0.001$), though strong linear relationships were evident in both cases ($R^2 = 0.99$). These results confirm the importance of appropriate cuff fit in accurate indirect pressure measurement. While indirect pressure measurements tended to underestimate MAP, this bias was consistent and also reduced at hypotensive values, indicating a high degree of clinical utility.

P199 The Effects of Irradiated Compared with Nonirradiated Feed on Fecal Shedding of Mouse Parvovirus

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Mouse parvovirus (MPV) can cause unwanted physiologic changes that can confound ongoing research. It is commonplace to screen rodent colonies for this agent using sentinel monitoring programs. However, sentinel detection of MPV is often inconsistent from one monitoring period to the next, resulting in a great deal of frustration at many institutions. Recently, it has been suggested that use of irradiated feed may prevent entry of MPV. Presumably this results from destruction of low levels of MPV in food. However, it has also been suggested that irradiated food may alter intestinal microbiota, which may decrease fecal MPV to levels not efficiently transmitted to sentinels. The objective of this study was to determine whether the use of irradiated feed decreased fecal levels of MPV. Weanling Swiss Webster mice were singly housed in ventilated racks and fed either irradiated diet ($n = 10$) or nonirradiated diet ($n = 10$). After 3 wk of diet acclimation, all mice were given one inoculation of a tissue homogenate of MPV1e. Fecal shedding was assessed by quantitative PCR twice a week for 4 wk postinoculation. In 2 separate study replicates, there were no differences in onset or peak of MPV shedding. There was a trend towards lower levels of MPV in feces of mice fed irradiated diet; however, no statistically significant differences were found. These findings suggest that the use of irradiated feed should not impact transmission of this agent to sentinels.

P200 *Helicobacter ganmani* Does Not Induce Typhlocolitis in A/J and C57BL/6 Mice

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Helicobacter ganmani is one of the most prevalent *Helicobacter* contaminants of laboratory mice, second only to *Helicobacter hepaticus*. While abundant data exists describing the diseases associated with *H. hepaticus* infection, little is known about the pathogenicity of *H. ganmani*. The objective of this study was to evaluate the host response to *H. ganmani* colonization in *H. hepaticus* induced disease-resistant C57BL/6 and disease-susceptible A/J mice. To this end, groups of 10 A/J and C57BL/6 mice were inoculated with *H. ganmani*, *H. hepaticus*, or *Brucella* broth (sham). Cecal lesion scores, cecal gene expression, and *Helicobacter* load were measured at day 4 and 90 postinoculation. Cecal lesion scores were significantly higher in *H. hepaticus*-infected A/J mice at day 90 postinoculation; however, cecal lesion scores were not significantly different in *H. ganmani* colonized A/J or C57BL/6 mice compared with the sham inoculated cohorts at either time point. Preliminary data from cecal gene expression analysis suggests no difference in gene expression between *H. ganmani* and sham-inoculated mice. Based on these results, we conclude that *Helicobacter ganmani* fails to induce the typhlocolitis that typifies *H. hepaticus* infection in either susceptible or resistant mouse strains.

P201 Effects of Environmental Enrichment on the Production of C57BL/6 Mice Kept in Different Housing Systems

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The goal of this study was to evaluate the effects of environmental enrichment on the performance of C57BL/6 mice kept in conventional animal facilities (open cage; OC) and individually ventilated caging system (IVC). We analyzed the interactions between treatments (enrichment compared with no enrichment) and housing systems (OC compared with IVC) for 5 mo evaluating: fertility rate, parturition interval, prolificity, number and percentage of weaned pups, percentage of preweaning mortality, and weight gain of weaned pups. We used 32 breeding pairs, divided into 4 groups (group A: nonenriched open cage; group B: enriched open cage; group C: nonenriched IVC; group D: enriched IVC) with 8 breeding pairs per group. We obtained the following significant results: 1) significant difference among groups A and B (A, $n = 170$; B, $n = 189$, $P < 0.05$), and groups A and D (A, $n = 170$; B, $n = 168$, $P < 0.05$), where there was higher mortality in group A; 2) interaction among a significant average of weaned × treatment (A, m = 6.57; B, m = 6.69; C, m = 5.89; D, m = 6.26; $F = 6.83$; $P = 0.014$); 3) interaction among a significant percentage of preweaning mortality × treatment (A = 58%; B = 21%; C = 31%; D = 17%; $F = 6.83$; $P = 0.014$), where enrichment had a positive effect. In terms of average weight gain, males and females kept in enriched open cage showed significantly higher weight gain. Therefore, in the multifactorial analysis, there was a wisespread relationship between the percentage of pups weaned and the treatment, with the enrichment treatment providing the best results. The prevailing interactions on weight gain were between the open cage housing system × treatment with enrichment.

P202 Neurokinin-1 Receptor Antagonist (Aprepitant) Has No Effect on Viral Load against Feline Immunodeficiency Virus

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Despite the success of antiretroviral therapies in controlling HIV infection in humans, very little is done to treat FIV in domestic cats. One important difference between HIV and FIV is the use of CD4 and CCR5 by most HIV strains found in humans and the use of CD134 and CXCR4 by FIV for viral entry into host cells. The use of these receptor/coreceptor pairs for cell entry by different viruses affects not only drug resistance, but also cellular tropism. Previous work has shown that neurokinin1 receptor antagonists inhibit HIV1 replication in lymphocytes and macrophages infected in vitro. While some studies suggest the effect may be related to downregulation of CCR5 receptors in target cells, other results dispute this conclusion. Therefore, the exact mechanism of action against HIV remains to be determined. To determine if this drug has similar antiviral effects on FIV and to further probe the mechanism of action of these drugs against viral replication, we conducted a safety trial in purpose-bred SPF cats and a double-blinded, placebo-controlled clinical trial in asymptomatic domestic cats naturally infected with FIV. Twenty client-owned cats were assigned randomly to receive either the treatment drug (125 mg of aprepitant per day), or a placebo for 30 d. Viral loads in plasma, CD4:CD8 lymphocyte ratios, and plasma levels of drug and substance P were measured at days 0, 14, and 28 in each cat. CBC and serum chemistry profiles were also analyzed at each time-point to determine off-target effects. Although the drug was administered to healthy and FIV-positive cats with no adverse effects, there was no effect on viral loads during the course of the study. The results of this study suggest downregulation of CCR5 may be a critical mechanism of action for the inhibition of viral replication by neurokinin1 receptor antagonists.

P203 Enhancement of Urine Samples from Mice

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Historically, all animals have been fasted prior to blood and urine collections for toxicology studies. Because of the mouse's propensity to not drink if not fed, this often resulted in varying degrees of dehydration and low blood and urine volumes. Thus, fasting was abandoned for mice with resultant dramatic improvements in obtainable blood volumes with no detrimental interpretive effect on other hematology or clinical chemistry parameters. However, the usable volume and quality of urine samples remained a quality issue. Urine samples in the past and until recently were collected via dripping into an open container positioned below the cage allowing for considerable evaporation. In spite of mesh filters over the outflow collection tube, urine samples tended to be flocculent with varying amounts of diet debris contamination. We have developed a method by which a gelatinous form of food is offered to mice during urine collection to eliminate the contamination issues and the physical collection device has been modified to mitigate the potential for evaporation. Two internal studies were conducted using the newly created protocol for urine collection in mice. These studies demonstrated clear advantages of this procedure over the previous protocol for collecting urine from mice. The number of samples that met the minimum volume requirements for analysis using an automated urine chemistry analyzer were increased by 56%. In addition, the quality of the urine was also enhanced as evidenced by the analysis of the data produced by the analyzer and the drastic reduction of foreign sediments in the microscopic evaluation. There were no physiologic or toxicologically meaningful differences relative to the different diets that would affect interpretation of hematology or clinical chemistry results. We have adopted this as our standard protocol for urine collection in mice.

P204 Structural and Functional Comparison of Generic and Branded Enoxaparin

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Low molecular weight heparins (LMWH) have historically been used in the long-term management of thromboembolism in human and veterinary patients. Enoxaparin (E) has been used to treat venous and arterial thrombosis in cats. In human medicine there is often the introduction of generic brands of E with some showing a difference in anticoagulant and antiprotease behavior in comparison to the branded E. It was hypothesized that branded enoxaparin (BE) and a generic version of enoxaparin (GE) may exhibit different behavior in the anticoagulant and antiprotease assays when supplemented in feline plasma. A colony of female random source cats (n = 7) were divided into 2 groups with group 1 receiving BE (n = 4) 1 mg/mL SC, and group 2 receiving GE (n = 3) 1 mg/mL SC. Blood was drawn (3 mL) at baseline, 3, 5, 7, and 24 h. Blood was immediately transferred to a 3-mL sodium citrate tube (3.2%: 1 part sodium citrate to 9 parts blood). Assay run consisted of activated partial thromboplastin time (aPTT), common pathway test, and thrombin time (TT 5 U/mL). After tests were run on the whole blood, blood was spun to obtain plasma and tests were repeated. In every test both drugs peaked at the 3-h time point and were back to baseline levels by the 24-h time point. Both drugs demonstrated anticoagulant affects on the samples; however, in the common pathway test and TT the strength of the drug directly related to the individual cat. In the aPTT test there were stronger results in the whole blood than was found in the plasma; however, neither drug outperformed the other. This leads us to conclude that both BE and GE demonstrate equal anticoagulant properties.

P205 Effect of Pain Management on Immunization Efficacy in Mice

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Recognition and management of pain following research procedures is important for animal welfare, but is particularly challenging for rodents due to their status as a prey species. Although immunization with complete Freund adjuvant is commonly accepted to cause pain, rodents are not typically provided analgesics due to concerns that this may affect the desired immune responses. This study tested the hypothesis that immunization-related pain in mice can be effectively relieved without compromising the effectiveness of the immune response. Three commonly used analgesics (acetaminophen 300 mg/kg/d in drinking water, meloxicam 2 mg/kg SC once daily, buprenorphine 0.1 mg/kg SC twice daily) were given for 3 d postimmunization. Immunization pain was assessed by behavioral responses, including unrestricted horizontal locomotion in an open field, forced walking on an automated treadmill, and voluntary running in home-cage running wheels over several days. Effects of analgesics on antibody responses were assessed using immunoassays. Open field activity and the distance traveled during forced gait analysis and voluntary running were each decreased following immunization in the leg. Each of these responses returned near baseline when immunized mice were treated with acetaminophen or meloxicam, but voluntary running remained decreased for mice treated with buprenorphine. Analgesic treatment did not significantly affect the number of vaccine responders or the mean or maximum antibody titer following repeated immunization with recombinant protective antigen from *Bacillus anthracis*. We conclude that open field activity and distance traveled during forced gait analysis or voluntary running are sensitive indicators of pain following immunization in rodents. Furthermore, use of common analgesics does not affect immune responses when multiple vaccine doses are provided. Since studies requiring immunization are common in laboratory animal research, results of this study should enhance humane animal use through appropriate assessment and management of immunization-associated pain.

P206 *Escherichia coli* O157:H7 Infection in Dutch Belted and New Zealand White Rabbits

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Enterohemorrhagic *Escherichia coli* (EHEC) produce one or more types of Shiga toxins and are foodborne causes of bloody diarrhea. The prototype EHEC strain, *E. coli* O157:H7, is responsible for both sporadic cases and serious outbreaks worldwide. Infection with *E. coli* that produce Shiga toxins may lead to diarrhea, hemorrhagic colitis, or (less frequently) hemolytic uremic syndrome, which can cause acute kidney failure. The exact mechanism by which EHEC evokes intestinal and renal disease has not yet been determined. The development of a readily reproducible animal oral-infection model with which to evaluate the full pathogenic potential of *E. coli* O157:H7 and assess the efficacy of therapeutics and vaccines remains a research priority. Dutch belted (DB) rabbits are reported to be susceptible to both natural and experimental EHEC-induced disease, and New Zealand white (NZW) rabbits are a model for the intestinal manifestations of EHEC infection. In the current study, we compared the pathology caused by *E. coli* O157:H7 infection in DB and NZW rabbits. Twelve rabbits (6 animals per breed) were infected with 10^9 colony forming units of the *E. coli* O157:H7 bacteria via the orogastric route. Four rabbits (2 per group per breed) infected orally with phosphate buffered saline served as the control animals. Both breeds of rabbits developed clinical signs of disease and intestinal lesions after experimental infection. Both infected groups of rabbits exhibited diarrhea and weight loss, shed bacteria in their feces, and developed enteritis. In addition, one of the infected DB rabbits developed renal lesions. Diarrhea occurred in 67% of the infected NZW rabbits and in 60% of the infected DB rabbits on one or more days after infection. Of the infected NZW rabbits, 83.33% exhibited weight loss, whereas 80% infected DB rabbits lost weight after infection. Furthermore, 100% of the infected NZW rabbits and 80% infected DB rabbits developed enteritis. Control animals did not display any histopathology or clinical signs of disease. Our findings provide evidence that both breeds are susceptible to *E. coli* O157:H7 infection and that both may be useful models for investigating EHEC infections of humans.

P207 Mouse Small Intestinal Loop Model to Probe the Role of Kca3.1 in Host: Microbe Interactions

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Paneth cells contain defensin-rich secretory granules that are released into the intestinal lumen in response to microbial stimulation. The secreted antimicrobials contribute to host defense and to homeostasis with the colonizing microbiota. The intermediate-conductance Ca^{2+} activated K^+ channel, KCa3.1, is involved in regulating defensin granule release from Paneth cells. We hypothesized that pharmacological activation of this channel with the KCa3.1 activator, SKA31, would increase lumen killing of defensin-sensitive pathogens, while its inhibition with the blocker, TRAM34, would conversely decrease this effect. To test our hypothesis, a mouse intestinal bowel loop model was used to investigate host-microbial interactions in vivo. Three groups of animals were studied: control (n = 6), TRAM34 treated (n = 5), and SKA31 treated (n = 6). After surgical isolation of the distal small intestine, the bowel loop from mice in each group was directly inoculated with 1×10^6 colony forming units (CFU) of virulent *Salmonella enterica serovar typhimurium*. Two hours after inoculation of bacteria, the bowel loop, mesenteric lymph nodes and spleen were collected for analysis of bacterial CFU. As hypothesized, activation of the channel with SKA31 resulted in a 2-fold enhancement of microbial killing, while TRAM34 mediated inhibition of the channel resulted in a 3-fold decrease in microbial killing. Unexpectedly, we did not detect changes in the 2 major phyla of mouse intestinal microbes, Firmicutes and Bacteroidetes, between control animals and those treated with either the activator or inhibitor. This study demonstrates the successful application of the intestinal loop surgical model to study potassium channel regulation of Paneth cell function in host defense and pathogen interactions.

P208 Effect of Honey and Salt on Laboratory Sprague–Dawley Rat (*Rattus norvegicus*)

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Honey has been used as home remedy for centuries for numerous ailments. Conversely, salt is known to increase blood pressure and other health problems. However, there is no or very less scientific evidence of these claims. Hence, this scientific investigation was carried out to see the effect of honey and salt alone and together on health of rats. Earlier similar studies were carried out on Japanese quail. In this experiment we used 16 rats in each experiment and divided into 4 groups (groups 1, 2, 3, and 4). The rats were kept in commercially made boxes (18 × 10 × 9 in.) in a room temperature at 72 ± 2 °F. All animals were provided routine rat feed and tap water to drink ad libitum during the 8-wk period of experiment. group 1 (control) rats received tap water for drink (no honey or salt); group 2 received 10% honey solution (clover honey dissolved in tap water); group 3 received 0.5% salt solution (iodized salt dissolved in tap water) and Group 4 received a mix of honey and salt (10% honey and 5% salt dissolved in tap water). Every day enough fresh feed (total 200 g of feed) and drink (total 450 mL of drink solution) were provided to all groups. Digital balance was used to record the daily feed consumption by subtracting the leftover feed from the total feed provided (200 g), and individual body weight was also measured and recorded. At the end of experiment the average feed consumption was found as: group1, 38 ± 4.38 g/d; group 2, 21 ± 4.36 g/d; group 3, 32 ± 3.21 g/d; and group 4, 24 ± 4.35 g/d. The lowest body weight gain (30 g) was found in group 2 (who consumed the honey solution) followed by group 4 (honey and salt), then group 3 (salt), and the highest body weight gain (70 g) was in group 1 (the control group). This experiment shows that the consumption of honey can curtail feed intake and weight gain significantly ($P < 0.05$) even when fed with salt. This experiment shows that the consumption of honey has a positive effect on health and helps eliminate some negative effects from other substances such as salt.

P209 Obtaining High Titer Polyclonal Antibodies in Rabbits Using a Modified Double Primary Inoculation

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Traditionally, the use of more than one injection containing complete Freund adjuvant (CFA) has been highly discouraged. According a published article, high titer antibodies could be obtained using a double primary inoculation with injections spaced 3 d apart. Based on this, and with IACUC approval, a pilot study was conducted to see if modifications could be done to maintain the increased immune response, while lowering the total volume to avoid the adverse effects seen with repeated CFA usage. A pilot study was conducted using 5 rabbits started, along with 25 animals started on a standard schedule. The pilot animals were administered an antigen emulsion with a total volume of 0.5 mL on day 0 and again on day 3 with the same dosage and volume. The control rabbits received a single injection on day 0, total volume of 1.0 mL. Both groups received identical booster immunizations on day 28 and bleeds were taken 14 d later. Observations were performed 3 times a week for a total of 10 wk to track nodule and lesion formation. Results showed identical lesion and nodule formation rates for both groups. To determine titer, bleeds were screened by ELISA, the pilot group showed titers averaging 6 times higher than those of the standard group and subsequent bleed titers continued to improve. This technique has now been repeated using various antigens with surprising titer results along with no increase in lesion formation. This method has proven to be a refinement in the polyclonal antibody process by allowing fewer animals to be started on projects along with reducing the overall number of immunizations needed to obtain high tier, functional antibodies.

P210 Modulation of Innate Immunity by a Bacterial Phospholipase

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Listeria monocytogenes is an intracellular bacterial pathogen that has the ability to escape from vacuoles to multiply in the cytosol of host cells. Escape from vacuoles is mediated in part by a bacterial phospholipase C (PC-PLC). PC-PLC is made as a proenzyme whose maturation occurs specifically in acidifying vacuoles. A mutant strain of *L. monocytogenes* that constitutively secretes active PC-PLC in the cytosol of infected cells does not affect host cell viability or the ability of bacteria to multiply intracellularly. However, the mutant is significantly attenuated in vivo suggesting that it is compromised in its ability to effectively colonize the host. To determine if the PC-PLC mutant stimulates a stronger immune response to infection, we assessed whether it could protect mice against infection with

wildtype strain. Groups of 4 to 5 6-wk-old female BALB/c mice were infected via gavage or intravenously with wildtype, mutant, or both strains. Bacterial counts from the spleen at 72 h postinfection show a statistically significant decrease ($P < 0.05$) in the numbers of wildtype bacteria retrieved from coinfected mice when compared with mice infected with wildtype alone demonstrating a partial protective response. Whole genome microarray analysis was then performed to assess differences in transcriptional host response to infection with wildtype or the mutant strain, using mouse bone marrow-derived macrophages as the host. We have identified significant differences ($P < 0.05$) between the wildtype and mutant strains at 2 separate time points, including total number of up- or downregulated genes and pathways when compared with uninfected controls and condition variations in principal component analysis. We plan on further elucidating the mutant's effects through translational analysis and variations during in vivo infection. These data provide evidence that constitutive activation of PC-PLC by *L. monocytogenes* attenuates the bacteria and provides protection against wildtype infection. Further, this study begins to elucidate genome wide-differences induced in infected cells from both strains.

P211 Dermal Application of Emerging Threat Agents on Unanesthetized Nonhuman Primates

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The skin is the most probable route of exposure to emerging threat agents, posing a threat to the warfighter as well as the civilian population. Understanding the penetration rates, persistence on the skin, and the dermal hazards of these emerging threat agents will allow for development of more effective treatments and decontamination measures. The unanesthetized nonhuman primate (NHP) model allows for an effective comparison to possible exposure in humans. An unanesthetized animal model is essential to studies evaluating the dermal effects of emerging threat agents as anesthetics and analgesics are known to have profound physiologic effects that can change the rate of absorption into and penetration through the skin. An unoccluded dose site offers additional challenges because of safety concerns for individuals handling these animals after challenge as well as for individuals handling contaminated caging and equipment. To facilitate in-hood handling of animals, specialized equipment was needed. Fabricated in-hood caging provided improved access to the animal along with a metabolic pan for collecting urine and feces. A removable plexiglass shield prevented urine and feces from exiting the hood and provided protection for a telemetry receiver. A holding device was fabricated to safely restrain the unanesthetized animal for dosing with the emerging threat agent and for the collection of blood samples. A primate jacket was modified with a large port between the shoulder blades that allowed for access to the skin during dosing and provided unoccluded covering of the skin while in the cage and restraint device. All animals were acclimated to the cage, restraint device, and modified jacket prior to the start of the study. Animals were also acclimated to the movement between the fabricated in-hood cage and the restraint device. These procedures and equipment allow for a safe and standardized way to percutaneously dose unanesthetized NHP using an unoccluded dose site.

P212 The Effects of Submandibular Vein Blood Collection on the Health of Female NCr nu/nu, CD1, and CB.17 SCID Mice

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Blood collection in the mouse is a vital tool in research studies. Submandibular vein blood collection has been reported in scientific research as an effective method for obtaining blood with minimal stress to the animal and without using anesthesia. However, detailed documentation of the impact of the procedure on animal health is limited in published literature and generally refers to mice with fur. Nude mice are used extensively in oncology preclinical studies. To evaluate the impact of submandibular vein bleeding in nude mice, a comparison was performed in furred mice as well as in mice with different levels of immunity. Groups of 15 female CR CD1, NCr nu/nu, and CR CB.17 SCID mice were subjected to a single submandibular vein bleed with a 4-mm lancet. Clotting time at the site of the puncture was noted if it exceeded 5 s. Blood was processed for CBC, glucose, insulin, and cytokine levels to establish a baseline for future studies. The animals were monitored daily for up to 5 d following the initial blood collection. Observations included food and water consumption, behavior, and body weight. Gross examinations of the puncture site and surrounding tissue were also made so as to give clinical scores to indicate the level of trauma observed. Histologic samples of the skin, muscle, mandibular lymph node, and salivary glands at the puncture site were taken to correspond with clinical observations. Food and water consumption, behavior, and body weights were found to be unremarkable between groups. Analysis of clinical scores showed statistically significant differences between the hairless NCr nu/nu, the furred CD1, and CB.17 SCID mice, implying that NCr nu/nu mice are susceptible to increased hematoma and tissue damage when compared with CD1 and CB.17 SCID mice. Clinical scores in CD1 and CB.17 SCID mice were not distinguishable, suggesting that immune status does not acutely influence the level of tissue damage. In conclusion, submandibular vein blood collection is an effective method for acquiring blood samples in NCr nu/nu, CR CD1, and CR CB.17 SCID mice. Nude mice require special consideration to minimize tissue damage.

P213 Comparison of Scrotal and Abdominal Approaches to Vasectomy in the Mouse

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Vasectomized males are used to generate pseudopregnant embryo recipients and thus are a crucial component of any transgenic mouse program. Males can be vasectomized via either an abdominal or a scrotal approach, but there is no consensus on which procedure is more favorable in terms of reducing postoperative discomfort. We sought to compare these techniques using fecal corticosterone metabolite (FCM) concentrations and assessment of nocturnal activity following surgery, hypothesizing that the abdominal approach would cause more postoperative pain, and thus have higher FCM concentrations and decreased nocturnal activity. Twenty-one 5- to 6-wk-old Crl:CD1(ICR) males were used for these experiments. All were acclimated to frequent placement into an empty shoebox cage for feces collection. Fecal samples were collected at 0800, 1200, 1500, 1800, and 1700 on a baseline day. The following day, animals were anesthetized, given meloxicam (0.05 mg SC), and assigned one of 3 surgical treatments ($n = 7$ animals per group) between 0900 and 1000: scrotal, abdominal, or control (anesthesia/analgesia only). Feces were collected on the day of surgery according to the same schedule as baseline day. FCM were extracted from feces in 80% methanol and measured using a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay. Mice were video-recorded from 1900 to 0700 (dark phase of 12:12-h light:dark cycle in our facility) on the evening after surgery, and activity level was scored at 8 time points throughout the night. Based on a subset of animals, differences in FCM concentrations did not significantly differ between groups (one-way ANOVA), and nocturnal activity scores between groups also did not differ significantly, though additional data is currently being analyzed. At this time, preliminary results indicate that when analgesics are used perhaps neither surgical approach is preferable; ongoing experiments may show significance.

P214 Characterization of *Helicobacter* spp. Isolated from Eastern Chipmunks

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Helicobacter spp. have been identified in a wide variety of animals. Humanely trapped Eastern chipmunks, *Tamias striatus*, from south-central Pennsylvania, were evaluated to determine if the wild-caught animals typically harbor enterohepatic *Helicobacter* spp. Liver, feces, and cecal contents were cultured under microaerobic conditions. Curved to spiral motile bacteria morphologically consistent with *Helicobacter* spp. grew from the feces and cecal contents of all 4 chipmunks tested. No *Helicobacter*-like bacteria grew from the livers. The initial cultures contained a mixture of motile bacteria of different morphologies by transmission electron microscopy. Population 1 was approximately 0.3 µm wide and 3.7 to 5.5 µm long with bipolar sheathed flagella. Population 2 was 0.4 µm wide and up to 4 µm long with unsheathed bipolar flagella. Neither population had detectable

urease activity. From a strain purified from population 1, 1572 bases of the 16S rRNA gene were sequenced and found to have the greatest sequence similarity with *H. typhlonius*. It had a 215 bp intervening sequence (IVS) which, however, was more similar to the IVS of *H. muricola* than the IVS of *H. typhlonius*. There was no IVS in the 23S rRNA gene, which had 96% sequence similarity with *H. aurati*. Procedures are underway to purify a strain from population 2. It has been reported previously that sequence analysis of the 16S and 23S rRNA genes may yield different phylogenetic trees for species within the *Helicobacter* genus. Analysis of the 23S rRNA gene is reported to be more reliable for determining evolutionary relatedness, so the strain we sequenced may be more closely related to *H. aurati*, isolated from hamsters, than to *H. typhlonius*, which is found in mice. These results suggest that chipmunks may harbor at least one novel species of *Helicobacter*. Studies are underway to further characterize strains isolated from both morphologic populations.

P215 Expression of Capsid Protein of a Newly Identified Rat Minute Virus and Application in Serodiagnosis and Surveillance of RMV Infection

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Rat parvoviruses are among the most prevalent infectious agents found in contemporary laboratory rat colonies. Despite the limited clinical diseases and histopathology lesions, rat parvoviruses have significant deleterious effects on research due to the immunomodulatory effects both in vivo and in vitro and the interference with oncologic research. Recently identified rat minute virus type 1d (RMV-1d) was the first molecularly characterized RMV variant identified in Asia. In this study, the goal is to develop a highly accurate, high-throughput serologic assay to diagnose the novel parvovirus infection in rats. First, the major capsid viral protein (VP2) gene of RMV-1d was cloned and the recombinant VP2 (rVP2) was expressed using a baculovirus expression system. The rVP2 proteins were applied as antigens in ELISA to detect antiRMV antibody in sera from naturally infected rats. The newly developed RMV-1d rVP2 ELISA offers a rapid, inexpensive, and accurate method for the screening of laboratory rat colonies for RMV infections. A surveillance of RMV infection in laboratory rats in Taiwan will also be performed on serum samples collected between 2007 and 2011 using the newly developed RMV-1d rVP2 ELISA.

P216 Experimentally Induced Effects on Serum Markers of Skeletal Muscle Injury

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In vivo procedures such as fasting, dosing, and blood collection have the potential to unintentionally affect experimental results, complicating data interpretation. While not always possible to eliminate experimentally induced effects, understanding those effects facilitates accurate data interpretation. In this study, multiple dosing routes and blood collection procedures as well as fasting were evaluated in the rat for effects on traditional and novel serum markers of skeletal muscle injury (AST, ALT, Myl3, TIMP, FABP3, sTnI, cTnT, cTnI, parvalbumin, CK). In experiment 1, groups of animals (n = 5 per group) were left untreated (fed or fasted), dosed with saline via multiple routes (intravenously, intraperitoneally, orally, and intramuscularly), or subcutaneously dosed with the skeletal muscle toxicant TMPD, and then fasted. Blood was collected 24 h after dosing for analysis. In experiment 2, 4 groups of animals (n = 5 per group) were administered saline via oral gavage for 6 d and the effects of blood collection routes evaluated. In this experiment, 2 groups of nonanesthetized animals were serially bled from either the tail vein or jugular vein on day 1 and novel markers of skeletal muscle injury were measured; 2 additional groups were added as controls. On day 7, terminal blood samples were collected for analysis. Hematology, standard clinical chemistry and novel markers of skeletal muscle injury were measured in all terminal blood samples collected in both experiments. Histopathology on several muscles, liver and kidney was also evaluated. The results from experiment 1 showed that fed animals had higher levels of certain muscle injury markers (CK protein, FABP3, parvalbumin) as compared with overnight fasted animals. Dosing with normal saline by the intravenous route had no effect on markers of skeletal muscle injury, while a few of the animals dosed orally, intraperitoneally, or intramuscularly were outliers for one or more serum markers; however, none of these animals had relevant histologic changes. Six analytes (FABP3, Myl3, sTnI, AST, ALT, and TIMP) discriminated between TMPD-dosed animals and saline-dosed controls although the fold-increases over control were not similar for all discriminating markers. In experiment 2, serum markers of skeletal muscle injury were generally higher in the serial samples collected from the tail vein as compared with the jugular vein with peak serum levels observed between the 2- and 8-h timepoints. However, the magnitude of this effect was minimal as compared with the response elicited by TMPD treatment in the previous experiment and any effects of serial blood collection site were not persistent. We have shown that experimental procedures can result in unexpected effects on markers of skeletal muscle injury.

P217 Hand-Held Jugular Phlebotomy Technique for Nonanesthetized Mice

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The acquisition of multiple blood samples from individual mice has been increasingly present in our study designs, either to decrease the number of animals necessary for use on study or to increase the number of samples that can be acquired from a small amount of test article. Current bleeding methods for mice have proved to be dissatisfactory because the methods either result in an insufficient amount of obtainable blood volume or require anesthetics which may alter an animal's metabolism. After investigating the published phlebotomy techniques, it was deemed necessary to develop our own technique to meet our study goals. A method development project for a nonanesthetized jugular vein phlebotomy technique was conducted over 3 time-periods to see if it was possible to obtain a target blood collection volume of 0.25 mLwithout compromising the collection site or the overall health of the animal. Ten mice were bled once at 3 different time points. Each mouse was bled at the first time point, then 16 h after the first time point, and then 24 h after the second time point. A clinical veterinarian was brought in before the final blood collection to evaluate the overall health of the animals. No negative findings were recorded. After a better method of restraint was developed, the same project was conducted 2 more times with continuous improvement in both ease of collection and acquisition of target volume. It was concluded that an experienced technician with sufficient training could use this phlebotomy technique to reproducibly obtain a maximum blood volume of 0.25 mLwithout anesthetic or the need for euthanasia following blood collection. Since the initial development of this phlebotomy technique, we have used this collection method on multiple studies and have not observed any apparent impact on animal health.

P218 Evaluation of Automated Blood Sampling for Low Volume Serial Sampling and Dried Blood Spot Pharmacokinetic Applications in Mouse and Rat

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Preclinical pharmacokinetic (PK) studies provide critical information regarding the in vivo disposition of potential new therapeutic agents, aiding in the selection of lead candidates with the best chances of clinical success. Advances in automation technology offer alternatives to manual sampling; however, any gains in efficiency that can be realized by automation must not be offset by losses in accuracy and data quality. In the present study, automated blood sampling (ABS) was evaluated as a means to conduct either routine plasma PK studies with low volume sampling, or blood PK studies with dried blood spot (DBS) sampling. To evaluate low volume serial sampling for plasma PK, mice were administered an intravenous dose (compound A), and sampled by carotid artery cannula. Plasma clearance (CLp), steady-state volume of distribution (VSS), and half-life ($t_{1/2}$) were determined as primary endpoints. Results indicated reasonable PK parameter agreement (approximately 30% difference) compared with those obtained by conventional manual methods with terminal sampling. Notably, the automated methodology required 80% fewer animals, with the added advantage of serial PK from individual animals. Automated DBS sampling was evaluated using both mice and rats with intravenous administration of either compound A or compound B. Results for compound A in mice suggested good agreement (<10%

difference) between PK data obtained via plasma analysis from serial sampling and blood analysis via DBS, when blood-to-plasma ratio was taken into consideration. Variability (%CV) in CL was higher in the DBS study than the plasma study. In a second experiment, rats were administered an intravenous dose (compound B), and sampled by carotid artery cannula for DBS analysis. PK parameters obtained were compared with those from a previous study where manual DBS sampling was employed, and found to be statistically indistinguishable ($P > 0.05$) with similar variability. These data indicate ABS technologies are capable of reliable low volume sampling for plasma PK or blood PK via DBS in rodents, offering increased flexibility over manual sampling practices with reduced animal utilization.

P219 Surgical Technique for Simultaneous Measurement of Blood Pressure, Electrocardiogram, and Temperature in a Single Mouse for Chronic Cardiovascular Applications

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Until recently, researchers collecting chronic cardiovascular (CV) data from mice have had to choose between blood pressure (BP) and electrocardiogram (ECG) transmitters; prioritizing the signal of most interest or choosing to implant parallel animal groups to capture both signals. A new device enabling simultaneous collection of BP, ECG, temperature (temp), and locomotor activity in mice eliminates the need for separate groups and allows for a more complete CV assessment in mice. Six male mice (C57BL6/J) were implanted with the new device. The catheter was placed in the left carotid artery. Small skin incisions were made over the right pectoral muscle and the left caudolateral thorax, and ECG leads were routed to these incisions to create a lead II configuration. The transmitter body was then implanted subcutaneously along the flank. Chronic data collection over a 3-wk period demonstrated that the new device provides a more refined approach to mouse CV studies by eliminating variability associated with collecting from 2 separate groups of mice. Furthermore, it reduces the number of animals, transmitters, and supporting materials used and provides a more complete CV assessment for applications such as heart failure or myocardial infarction.

P220 High-Resolution Linkage Mapping of the Rat Hooded Locus

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The hooded phenotype showing nonpigmented hairs in the abdominal skin is one of the coat color phenotypes seen peculiarly in the laboratory rat. The hooded locus showing autosomal recessive inheritence has been mapped on chromosome (Chr) 14 in previous investigations. Through genetic fine mapping using feral rat-derived inbred strain IS and hooded phenotype strain, LEA, we narrowed critical region of the hooded locus and revealed that only *Kit* gene, known as a marker of melanocytes and one of the coat color genes, existed in this region, suggesting strongly that the *Kit* is a gene responsible for the hooded phenotype. Nucleotide sequence analysis revealed a G to C transversion in exon 2 of *Kit* gene; however, it was synonymous mutation. Further, the expression of *Kit* mRNA were not different in fetal neural tubes and neonatal and adult skins between IS and LEA rats. We then examined *Kit*-positive cells and melanin granules were observed in the nonpigmented hair follicles, although *Kit*-positive cells, possibly melanocytes, were observed in the same nonpigmented hair follicles in the LEA rat abdominal skin. These results suggest that the synthesis of melanin is impaired possibly due to the malfunction of *Kit* expressed in melanocytes residing in the nonpigmented hair follicles of hooded phenotype rats. Molecular mechanisms of malfunction of the *Kit* and substantial mutation of *Kit* gene remain unknown, but should be resolved in future investigation.

P221 Murine Norovirus: Isolation and Detection in Mouse Colonies from Brazilian Animal Facilities

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Murine norovirus (MNV) an enteric murine pathogen wich belongs to Caliciviridae family is a nonenveloped virus with positive-sense single-stranded RNA genome. It is widely distributed and one of the most prevalent infectious agents in laboratory mice. However, there is no data about the prevalence of MNV infection in Brazilian animal facilities. Our goal was to focus on isolating MNV from naturally infected mice and determine the prevalence of MNV infection in these colonies. An RT-PCR to detect MNV in feces of naturally and experimentally infected animals was also established. Fecal samples and mesenteric lymphonodes were collected from different mice strains and a fecal suspension 20% was done. Virus isolation was performed through serial blinded passages of fecal suspensions using a murine macrophage cell line RAW264.7. SPF BALB/c and SWISS mice (4 wk old) were used to produce policlonal antibodies prior to performing a serological assay. Inoculated RAW264.7 cells displayed a suggestive cytophatic effect characterized by vesiculated cells and detachment in the third day post inoculation, after the seventh passage. An RT-PCR was performed using RNA polymerase gene primers designed to detect MNV 1, 2, 3 and 4 as reported previously. All the fecal samples and supernatant of infected cells were sucessfully amplified. The nucleotide sequencing and electronic microscopy is in process to confirm the virus identity. To estimate the prevalence of MNV infection in mice colonies, a total of 11 Brazilian facilities were investigated, including 88 animals. MNV was detected in 23.86% of fecal samples analyzed. Furthermore, PCR products of positive samples were examined and the nucleotide sequence homology of the RNA polymerase gene in comparasion with characterized MNV strains showed an identity ranging from 96% to 98%. To the authors' knowledge, this is the first report of MNV isolation and detection in mouse colonies in Brazil.

P222 Optimal Duration of Jacket Habituation Based on Cardiovascular Endpoints in Beagle Dogs and Cynomolgus Monkeys

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The invasive telemetry system and snapshot recording of ECG are commonly used to monitor cardiovascular (CV) endpoints in safety pharmacology and toxicity studies. Due to the recent development of noninvasive jacket telemetry systems, CV endpoints can be monitored for longer periods on toxicity studies in unrestrained dogs and monkeys. It is essential to habituate animals to the jacket prior to the data collection period to obtain good quality data. The purpose of this study was to determine the optimal habituation period based on heart rate response. Heart rate (HR) was collected from 4 jacketed monkeys following 3 consecutive occasions of jacket habituation. Heart rate was also collected from 2 sets of jacketed dogs following 3 (set A: 18 dogs) and 7 (set B: 4 dogs) consecutive occasions of jacket habituation. Heart rate data collected following 3- and 7-d jacket habituation was compared with the testing facility's historical control data collected from nonjacketed, invasive telemetry models in both species. The historical control data used for this study consisted of an average HR collected from 107 monkeys and 145 dogs. Heart rate collected from jacketed monkeys (following 3-d habituation) and dogs (following 3- and 7-d habituation) are comparable to data from nonjacketed animals. Therefore, we concluded that a 3-d habituation is optimal to prevent undesirable effects in the CV data that may result from insufficient jacket habituation.

P223 Effects of Lipopolysaccharide on Physiologic and Pharmacological Parameters Following Ketamine-Xylazine Administration at an Anesthetic Dose in Rat

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Lipopolysaccharide (LPS) is a constituent of the bacterial cell wall that is known to induce fever in animals. Fever, occurring during infections and inflammatory processes, causes a diminution of liver metabolism, blood pressure, and renal filtration responsible for an increase of drug plasmatic concentrations. The main objective of this study was to evaluate the physiologic (rectal temperature, cardiac

and respiratory frequency, oxygen saturation, and the withdrawal reflex) and pharmacological changes in rats following a ketamine-xylazine administration following the administration of different LPS concentrations. Sprague–Dawley rats were used: control group saline administration ($n = 5$) and 3 groups ($n = 6$ per group) with mild, moderate, and high fever (LPS 1, 10, 100 µg/kg, respectively). LPS was injected 2 h before ketamine-xylazine administration. Blood samples were taken prior to and following intraperitoneal administrations at 5, 15, 30 min and 1, 2, 6, and 24 h for complete blood chemistry and haematological profiles as well as analysis of plasmatic drug concentrations by tandem liquid chromatography-mass spectrometry. All animals were anesthetized with an intramuscular ketamine (80 mg/kg) xylazine (5 mg/kg) combination. Rectal temperature was significantly lower only for the low LPS dose ($P < 0.005$). Cardiac frequency was lower in the low LPS group ($P < 0.001$) and higher in the high LPS group at 1 and 2 h ($P < 0.001$ and 0.05, respectively). There were no differences in respiratory frequency and blood parameters. Oxygen saturation was significantly lower at 5, 15, and 30 min in the low and moderate LPS group ($P < 0.001$). High and moderate LPS groups had longer recovery time (1 to 2 h) compared with controls. Ketamine pharmacokinetics were not affected by LPS; however, for xylazine the AUC and the Cmax of the moderate and high LPS groups were increased ($P < 0.001$). In conclusion, LPS has a profound effect on physiologic and pharmacological parameters of the ketamine-xylazine combination and dose adjustments could be made to modify anesthesia if required.

P224 In Vivo Imaging of the Mouse Eye by High Frequency Ultrasound

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High frequency ultrasound (US) is a useful technique for examining the rodent eye. It provides an unprecedented opportunity to visualize the anatomy and pathology of the eye associated with clinical and experimental research. US is an inexpensive, safe, and noninvasive method compared with MRI or other imaging modes. When other techniques are not possible, that is, when an opacity of the lens is present, critical information about ocular health and disease is rapidly provided by US examination, revealing anatomic information in real time. For this study, adult mouse eyes were imaged using several high-frequency probes with center frequencies of 30, 40, and 55 mHz. All images were obtained while the mouse was under appropriate anesthesia to facilitate image stability. Skintape held the eyelids open and a sterile, water-soluble coupling gel was applied to the eye for sound transmission. Sagittal, dorsal, and transverse images were first obtained to capture any differences in the anatomy of the eye. When a lesion was found an oblique plane was used to better delineate the abnormality. Ocular tissue including the cornea, lens, retina, and vitreous body were easily identified in these imaging planes. During the study several pathologies were detected in otherwise healthy mice. Lens abnormalities such as cataract formation and rupture of the lens capsule were noted. Some of the more discrete findings were retinal detachment and changes in the globe shape and size (microphthalmia). US image resolution and field of view (FOV) is dependant upon the transducer frequency. The 30- and 40-mHz transducers provide a deeper and wider FOV (12.7 mm and 6 mm, respectively) at the expense of resolution. The 55-mHz probe has the most shallow FOV, 4.5 mm, but gives the highest resolution, down to 75 µm. Accordingly, the anterior portions of the eye were best observed with the 55-mHz probe, while the 30 and 40 mHz were best for the posterior and periorbital areas. Using US to characterize the mouse eye provides a valuable means of noninvasively analyzing genetic variants of development and disease models in the laboratory mouse.

P225 α-Tocopherol Concentrations in Serum of Healthy Mice Following Supplementation with Synthetic or Natural Vitamin E in Food or Water

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Vitamin E is an antioxidant with therapeutic indications in mice, including the treatment of mouse ulcerative dermatitis syndrome. Natural sources have been shown to be better used by animals than synthetic supplemental sources. Therefore, diet and source of supplementation can affect serum concentration of vitamin E. Logistically, provision of fortified food is difficult when mice are enrolled in studies requiring special test diets. An alternative is to provide supplements in water. We evaluated serum vitamin E levels in mice to determine if liquid vitamin E can substitute vitamin E food. Group housed female Crl:CD1 mice were maintained either on a 99 IU/kg vitamin E (dl-α-tocopheryl acetate; controls) pelleted diet, a 3000 IU/kg vitamin E pelleted diet (food group), 3.0 or 1.5 IU/mL micellized liquid vitamin E (d-α-tocopherol; high or low liquid group) in pH 2.5 to 3.0 water. Mice were monitored once daily. Water and food were not changed for 14 d. Five mice per group were euthanized on days 7 and 14 and blood was collected by cardiocentesis. Sera were separated, protected from light and frozen at −80 °C. Pooled (by treatment group per time point) sera were analyzed for α-tocopherol by high performance liquid chromatography with fluorescence detection. Mice offered vitamin E food or water had consistently higher serum vitamin E levels compared with controls: 2.0- and 3.0-fold increase in food group, 2.4- and 2.5-fold increase in high liquid group, 1.4- and 2.2-fold increase in low liquid group after 7 and 14 d, respectively. Vitamin E in water, at half the concentration, raised mean serum levels by nearly half as much, which highlights the linear dose-response relationship between dietary intake and serum levels. In conclusion, micellized vitamin E in water increases serum vitamin E levels comparable to that of fortified food. Further, the results demonstrate that micellized vitamin E remains stable in acified water for at least 14 d. Micellized vitamin E may be administered in acidified drinking water when administration of fortified food is not possible.

P226 Differences in Activity in Male and Female B6C3F1 Mice Using the Open Field and Voluntary Running Wheel

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While conducting behavior phenotyping tests on genetically engineered mice, we observed greater locomotion in female mice compared with male mice in the open field chamber. The purpose of this study was to verify and quantify activity in male and female B6C3F1 mice using the open field and the voluntary running wheel. Mice were individually placed in the open field ($n = 38$) for 30 min. Locomotion and location within the chamber were documented. Mice were then singly housed in cages ($n = 19$) with free access to a running wheel. Running wheel revolutions were electronically recorded every hour for 36 d. The hour with the highest number of revolutions each 24-h period was identified and used to calculate the maximum amount of activity and the rate activity increased and decreased was also calculated using the hourly revolutions. Female mice had significantly higher levels of locomotion and spent significantly more time in the margins of the open field than males. Females demonstrated significantly higher number of revolutions on running wheels than males during both light and dark cycles. It was also observed that males reached their maximum activity on the running wheels significantly earlier and increased their rate of activity faster than female mice. We also observed a significant variation among females compared with males with respect to the maximum amount of activity on the running wheels. These findings suggest that sex differences should be carefully considered when conducting studies that use the open field or voluntary running wheel in the B6C3F1 mouse and possibly other mouse strains.

P227 Automated Pharmacokinetic Sampling in the Rat by Dried Blood Spot and Liquid Blood Methods

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There is an increased trend to predict the pharmacokinetic (PK) characteristics of small molecule compounds in humans in early drug discovery through rat single species PK scaling. To enable efficient rat PK screening of discovery compounds, automated sampling of blood time points are employed. Typically, 150 to 200 µL of whole blood is collected at each of the 9 to 11 time points over a 24-h period. Recently, with the development of dried blood spot (DBS) technology for drug level quantitation, an opportunity exists for the automated collection of time points by DBS. This work reports the modification of a Dilab accusampler that is used to collect both liquid blood (180 µL) and dried

blood spots (20 µL) simultaneously. Dual cannulated (CAC and JVC) Male Wistar-Han rats were separately dosed with imatinib at 2 mg/kg IV (n = 3 rats) and 10 mg/kg PO (n = 3 rats). The accusampler was programmed to sample 200 µL of whole blood at different time points then dispense 180 µL of liquid blood into tubes and also spot the 20 µL of liquid blood onto a DBS card. The accusampler was modified through a combination of software script updates, replacement of hardware and utilization of a secondary collection platform. Overall, the PK results from Imatinib by automated blood spot collection were comparable to manual blood spot collection. Similar in vivo plasma and blood PK results were obtained for imatinib. By splitting a time point sample between liquid whole blood and DBS collection, enables ex vivo determination of blood to plasma ratios and reporting of plasma concentrations when DBS samples are primarily collected. Automated DBS collection in the rat allows for reduced animal numbers to assess PK, biomarker, and pharmacodynamic responses which is in concordance with 3Rs principles.

P228 Heat-Shock Protein 70 and Nuclear Factor Kappa B: Implications in Necrotizing Enterocolitis in Sprague–Dawley Pups

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Necrotizing enterocolitis (NEC) causes mortality in infants which have term and preterm birth. In the premature population, 10% of infants will develop NEC and 40% of those infants will die. Multiple stressors contribute to a cascade of events. Due to functionally immature gut, intestinal epithelial injury results in sepsis and can lead to multiple organ failure and death. Systemic increase of proinflammatory cytokines, chemokines, and various proteins have been cited as contributors to inflammation and gut permeability. Gut permeability may also be protected from environmental insult with activation of Heat Shock Protein (Hsp) 70. The family of Hsp are activated with stressors such as thermal challenge, trauma and injury and HSP 70, in particular, is known to reduce the effects toxic insult and intestinal mucosal damage. Our model of NEC using Sprague–Dawley rat pups demonstrates that Hsp70 is among endogenous factors found in mammalian colostrum and milk. When activated, Hsp70 can attenuate the inflammatory cycle and inhibit nuclear factor kappa B (NFκB) which is thought to play a key role in inflammation and tissue damage. Briefly, rat pups were caesarian-delivered prematurely, as a stressor, at gestational day 20 and housed in a 37 °C incubator or allowed to deliver and nurse normally with the dam. Premature pups received regular formula every 3 h at a volume of 100 to 150 µL via oral gavage. Pups were euthanized at postnatal day 4, and intestinal tissue was harvested and processed for further biochemical and immunohistochemical analysis. Results showed significant differences between the formula-fed and mother-fed pups. Flux studies with FITC labeled Dextran demonstrate more gut "leakiness" in the formula-fed pups. Our conclusion is that HSP 70 confers a protective effect against gut permeability, or leakiness, that may help to inhibit necrosis in the intestine.

P229 Efficacy Studies of Gel-Delivered Analgesics in Rodent Models of Pain

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Our purpose was to determine if commonly used analgesics could be effectively delivered to rodents using a dietary gel. Efficacy was assessed by measurement of a reduction in pain behaviors following injection of an inflammatory agent or an incisional surgical procedure. Carprofen was dissolved into a hydration gel (98% water) for mouse studies while buprenorphine was evenly mixed into a hazelnut gel for rat studies. Control or drug-containing gels were made available to animals ad libitum 12 h prior to the start of formal procedures and were changed out once per day throughout the assessments. At t = 0 h, mice received an injection of complete Freund adjuvant (CFA) while rats had the plantaris muscle on their left hind paw incised (plantar incision surgery). All groups had similar gains in bodyweight over the course of the studies and appeared to maintain good overall health as assessed by daily observations. Testing of thermal (Hargreaves) and tactile (von Frey) thresholds and paw volumes indicated significant differences between groups with respect to the level of pain behaviors that developed in response to the tissue injury. Animals receiving gel-delivered analgesics displayed reduced pain behaviors compared with controls. The gel-delivered drug groups had similar reductions in pain behaviors compared with animals treated with conventional injections of the analgesics. These studies provide encouraging preliminary data on the feasibility of delivering typical opioid and nonopioid analgesics to rodents. The gel-based technology helped improve postoperative pain control and recovery from surgery while being a convenient and cost effective approach for the laboratory.

P230 Analysis of Specific Immune Response in Mice Induced by Oral Immunization with *Trichoplusia ni* Larvae Producing Virus Derived Antigens

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In vaccine development, common strategies of antigen delivery include infection with live or attenuated viruses, or injection with purified recombinant proteins. In our previous study, we found that specific immune response was induced in mice by feeding with *Trichoplusia ni* larvae producing the mouse minute virus VP2 (MMV-VP2) protein expressed in a recombinant baculovirus. The ability of the MMV-VP2 protein to self assemble into virus-like particles (VLP) when expressed in the baculovirus system might be critical for oral immunization with infected *T. ni* larvae. To further analyze this hypothesis, we investigated 3 groups of baculovirus expressed proteins: 1) the VP2 capsid proteins of mouse parvovirus type 1 and type 2 that are capable of forming VLP; 2) DsRed protein with the ability to form aggregates; and 3) mouse norovirus capsid protein VP1, in which the formation of VLP is controversial. Baculoviruses expressing the target proteins were injected into *T. ni* larvae. Protein expression was confirmed by Western immunoblotting, and the experimental animals were fed with larvae producing the antigenic proteins. Blood samples collected from mice at various time points were analyzed by ELISA to monitor the development of specific immune response. The highest number of seroconverted animals was determined for MPV1-VP2 and MPV2-VP2 as 40% and 100% in C3H mice, respectively. Fewer animals (24%) were seroconverted with MNV-VP1, and only one mouse developed the DsRed specific antibody. These results suggested the ability of protein to self-assemble into VLP is critical for the induction of specific immune response through oral immunization with larvae producing the antigen. Further analysis demonstrated that titers of the antibodies produced in mice by feeding with larvae were comparable to those obtained through the natural route of infection. Furthermore, a neutralizing effect of these antibodies was demonstrated with 3 logs decrease of the virus titer. Thus, our results indicate a possibility of using larvae expressing target protein in the form of VLP as an oral vaccine to generate protective immune response.

P231 Pupillary Light Response in a Guinea Pig Model Exposed to Organophosphate Agents

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Here we report a study to more precisely define and quantify the relationship between organophosphate agent exposure, including pesticides and nerve agents, with cholinesterase inhibition and the ocular biomarkers that are induced. A thorough investigation of these relationships was conducted to subsequently refine pupillary algorithms for the automated detection of organophosphate exposures with greater specificity regarding level of exposure, the extent of cholinesterase inhibition, and the temporal presentation and persistence of the ocular biomarkers. A guinea pig model was exposed to varying concentrations of parathion (pesticide), soman, and VX (nerve agents) to methodically detail both the temporal and quantitative occurrence of pupillary deficits (anticholinesterase biomarkers) to determine the most sensitive, accurate diagnostic algorithms in these animal models. Dose-response curves and temporal-response curves for both pupillary deficits and generalized symptoms were developed for each agent used. Based upon previous studies dose ranges were conducted between the LD50 down to 3000-fold below the LD50 to incorporate lethal and sublethal exposures without decreasing the potential sensitivity of the ocular biomarkers. In addition, cholinesterase assays were performed at various time

points postexposure. These cholinesterase inhibition assays in the guinea pig model will allow improved correlation to human data that already exist and map a direct relationship between exposure, enzyme inhibition, and ocular deficits that will be vital for future development of both diagnostic and treatment protocols. All animals that showed signs of organophosphate intoxication had a reduced pupillary response. The reduction of pupillary response was proportional to the level of intoxication with fatally exposed animals losing all pupillary response 30 min prior to death. These findings support the use pupillary response in determining if an animal has been cutaneously exposed to organophosphates.

P232 Development, Validation, and Implementation of ELISA Specific for 19 Rodent Pathogens

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Immunologic methods are commonly used to diagnose infection by various pathogens. These methods are versatile, sensitive, relatively simple, and cost efficient. The development of a reliable serodiagnostic assay requires 3 steps: development and evaluation of the specific antigen and/or antibodies, development and validation of the corresponding serological assay, and implementation of the assay in a diagnostic process. Here, we report the results of the development and implementation of indirect ELISA specific for 24 various rodent viruses, bacteria, and parasites. We developed the methods of amplification and purification for mouse and rat viruses (MMV, MPV type 1 and type 2, MHV-A59 and MHV-JHM, EDIM with NCDV as surrogate, Sendai virus, PVM, TMEV (GDVII), MAV-Florida, MAV-K87, MCMV, REOIII, MNV, LCMV, hantavirus, ectromelia virus based on vaccinia virus, SDAV, RPV and RMV), bacteria and mycoplasma (CAR *Bacillus*, *M. pulmonis*, and *M. arthritidis*), and parasite (*E. cuniculi*). Antigens specific for different rodent pathogens were generated with 2 major approaches: 1) prepared as a whole pathogen amplified, purified, and inactivated and 2) produced in a form of recombinant protein using baculovirus expression system. Each prepared antigen was implemented in ELISA and evaluated for analytical sensitivity and analytical specificity. To complete validation of the ELISA, 19 feasibility studies were performed with more than 5900 tested rats and mice. To obtain 95% confidence of the assay with 95% ± 5% of the expected diagnostic sensitivity, serum samples from 720 mice and 230 rats infected with different pathogens were analyzed. We showed that analytical sensitivities of institutional ELISA were very similar or higher compared with those of the commercially available reagents. Institutional ELISA plates of different specificities produced in scale-up process demonstrated high consistency and precision of the plates with coefficient of variation less than 10% compared with the commonly excepted industrial standards of 20% for serological assays. Continuous monitoring of the implemented assays showed 95% to 98% of diagnostic sensitivity and specificity. Thus, these developed and validated antigens and corresponding ELISA can be used in testing of rodent pathogens.

P233 Jacketed External Telemetry Electrocardiograph Collection Procedures in Nonhuman Primates and Canines

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External telemetry systems are nonimplantable and noninvasive allowing for the collection and assessment of electrocardiograph (ECG) data from conscious, nonrestrained animals including nonhuman primates and canines. Traditional collection of ECG with animals not implanted with telemetric devices requires the use of chemical or physical restraint, which can stress the animal and be detrimental to data interpretation. Collection of data in this traditional fashion also limits the length of the collection to just a few minutes. The external system allows for continuous collection of ECG data on up to 36 animals simultaneously for periods up to 24 h and has been shown to provide data of comparable quality to that of implanted telemetric systems. The external system is also adequately sensitive to detecting changes in electrocardiograph morphology. The external system allows animals to be free in their homecage and minimally restricted by a jacket that protects the ECG leads and also houses the transmitting device. To use the system it is beneficial for animals to be appropriately acclimated to wearing the jackets (and collars for canines) required for collection. Personnel are appropriately trained to use the computer software and input study specific protocols and set up the equipment for individual animals. This training also includes learning to prepare the animals for the external lead placement and proper jacket application.

P234 Coagulation Parameter Differences between *Macaca mulatta* and *Macaca fascicularis*

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Macaca mulatta (rhesus) is used extensively for anticoagulant therapy development. Availability, cost, and size has led to an increased use of *M. fascicularis* (cynomolgus) for testing. We observed genetic diversity among macaques from different sources and wanted to compare coagulation responses to examine potential interspecific disparities. Blood was drawn, transferred to tubes with 3.2% sodium citrate, centrifuged to obtain plasma, and stored at −70 °C. Clotting tests performed included prothrombin time (PT), activated partial thromboplastin time (aPTT), heptest and thrombin time (TT) assays using fibrometers. Commercially available chromogenic substrate assays for antiXa and antiIIa were also performed in the presence of heparin (H) and enoxaparin (E). Pooled samples were also serially diluted and percentage of antithrombin determined. The PT and TT activities were higher for cynomolgus compared with rhesus whereas aPTT and heptest were lower. Similar interspecies responses for aPTT were observed with increasing concentrations of H; with E, differences were observed at higher concentrations. The rhesus demonstrated a stronger inhibition of antiIIa activity when supplemented with H; however, with E, the differences in inhibition were reduced between species. Similar responses were observed for antiXa for both species and drugs. ATIII activity was higher in cynomolgus at greater than 50% dilution. The data show striking differences for coagulation parameters and responses to heparins between the 2 species of macaques. A clear understanding of the differences in these species is essential when interpreting past data and assessing new anticoagulants for efficacy, safety, and pharmacokinetics.

P235 Identification of *Campylobacter lanienae* from Laboratory Chinchillas (*Chinchilla laniger*)

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Chinchillas are commonly used as a model of otitis media. Gastric ulcerations have been previously reported on postmortem examinations of pet and laboratory chinchillas, but no etiologic agent has been described. Recent postmortem evaluations of chinchillas in our research colony revealed mild to moderate lymphoplasmacytic gastritis, duodenitis, and typhlocolitis. The aim of this study was to survey chinchillas for bacterial agents in the order Campylobacterales that are known to cause inflammatory gastrointestinal diseases in other species (*Helicobacter* spp. and *Campylobacter* spp.). Fecal and gastrointestinal tissue samples were collected at necropsy from 27 juvenile male animals that were euthanized for use in an unrelated study. Fecal DNA was extracted using a commercial extraction kit, and samples were evaluated by PCR using 16S rRNA genus-specific primers for *Helicobacter* spp. (C97/C05, 1200-base-pair amplicon) and *Campylobacter* spp. (C99/C98, 300-base-pair amplicon). None of the animals were positive for *Helicobacter* spp., but 12 animals (44.4%) were positive for *Campylobacter* spp. Purified products from the *Campylobacter* spp. PCR were sequenced and found to have >99.5% 16S rRNA homology (215/216 identities) with *Campylobacter lanienae*, a gram-negative, microaerophilic curved rod-shaped bacterium originally isolated from the feces of slaughterhouse employees. This organism has also been isolated from the feces of cattle, swine, and sheep. PCR using 16S rRNA species-specific primers for *C. lanienae* (CLAN76F/CLAN521021R, 920-base-pair amplicon) confirmed the identity of the bacterium. This represents the initial characterization of a bacterium in the order Campylobacterales in chinchillas and the first time *C. lanienae* has been identified in animals with gastrointestinal lesions. Currently, we are isolating the bacterium from fecal and gastrointestinal samples to ascertain the colonization dynamics of *C. lanienae* and characterize whether the organism is associated with gastrointestinal pathology. The implications of *C. lanienae* as a possible gastrointestinal pathogen in chinchillas and as a potential zoonotic agent emphasize the importance of our findings.

P236 Comparison of Methods Used for Sustained Controlled Hypothermia in Rats

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Experimental therapeutic hypothermia is attempted in a wide array of conditions in humans including stroke, traumatic brain injury, cardiac arrest, and burn injury. Different methods have been used to achieve hypothermia in laboratory animals in order to study its effects on these and other conditions. However, the procedural details of hypothermia-inducing methods and the reliability of different methods in producing sustained hypothermia are not well described in published reports. The goal of this study is to test the reliability and ease of use of the 3 most commonly used hypothermia-inducing methods. Sixteen male Sprague–Dawley rats were randomly assigned to control or one of 3 hypothermia methods; ice bath, alcohol pads, and chill pad. Rats were anesthetized and their abdominal hair was removed. The ice bath method involved placing the rat on a plastic sheet floating in ice water. In the second method, rats were placed on gauze pads soaked in 70% isopropyl alcohol, while the tail, feet, and ears were rubbed with isopropyl alcohol pads. Rats were placed on a commercial water circulating cooling pad for the chill pad method. Core body temperature, heart rate, and blood oxygen saturation were measured every 15 min for 2 h. Body temperature and heart rate were not significantly altered in the control rats during the 2-h period. All 3 methods of hypothermia significantly decreased body temperature and heart rate within 30 min. However, the chill pad tended to produce more sustained hypothermia in the intended target range of 28 to 30 °C. Contrary to our expectations, oxygen saturation appeared to increase with time in both hypothermia and control rats. Based on these observations, we conclude that the chill pad method is a safe and convenient method to produce sustained controlled hypothermia in rats.

P237 4-Stage Model of CAR *Bacillus* Infection In Vitro

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The cilia-associated respiratory *Bacillus* (CARB), an unclassified, extracellular, gram-negative filamentous bacterium, colonizes the ciliated respiratory epithelium of rodents and causes chronic respiratory disease (CRD). Experimental infection of sensitive BALB/c mice with CARB induces production of cytokines (TNFα, IFNγ, and IL4) and specific antibodies (IgM, IgG, and IgA) but these responses do not suppress onset of pneumonia. In order to reveal the mechanism of CARB pathogenesis, we tried to establish in vitro model and found that 1-mo continuing infection in mammary cell culture with 4 stages using Vero E6 cells. BALB/c-nu/+ mice were intranasally inoculated with SMR strain of CARB. Lung homogenates of pneumonia were added to the cell cultures (Vero E6, DBT, 3T6, and BALB/3T3) and morphologic changes were monitored using phase contrast microscopy. Grown CARB was confirmed by immunofluorescence assay and PCR. Three and 8 d after inoculation, cells infected with CARB were analyzed by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). CARB was reported to grow in mammalian cell culture. Based on this system, bacillary growths were observed in all cell cultures 2 wk postinfection but remarkable and ongoing proliferation over 1 mo was noted in Vero E6 culture. Hereafter, we used CARB obtained from Vero E6 culture and 4 stages of 1-wk interval each were observed in this model. We speculate that these stages are: 1) CARB maturation process (getting bold shape), 2) CARB anchoring on cells to start division, 3) dividing continued, and 4) death of CARB and cells. Especially in the second stage, robust proliferation of CARB on the cells showed mossy and gregarious colonies by SEM and TEM observation resembling the in vivo histopathologic finding of CARB's CRD. Since signaling pathway of Vero E6 cells in virus infection was well studied, this Vero E6-CARB infection model can be applied to clarify cell-based CARB infection and immunity.

P238 Central Pain Following a Hematoma Located in Deep Brain Nuclei in Sprague–Dawley Rats Can Be Reversed with Gabapentin

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The objective of this study was to evaluate pain sensitization in rats following the induction of an intracerebral haemorrhage located in the basal ganglia and/or thalamus using the Rosenberg model. Thirty male Sprague–Dawley rats weighing between 175 to 300 g were used. In the first experiment, 3 groups of 6 animals were used to evaluate pain threshold using Hargreaves test (thermal sensitivity). Following 3 d of behavioral testing (baseline values), animals in each group were injected intracerebrally either with 0.5, 1, or 2 µL of a collagenase solution (0.5 U/2 µL type VII collagenase) inducing a hematoma in the right caudoputamen nucleus and/or thalamus. They were then tested for the next 9 consecutive days. No pain-related behavioral changes were observed following injections with 0.5 and 1 µL of collagenase. However, with 2 µL, reaction times were significantly faster on days 3, 4, 5, 6 ($P < 0.0001$), and 7 ($P < 0.006$) in the right and left hind paws compared with baseline values. The lesion was localized only in the caudoputamen nucleus for animals receiving 0.5 and 1 µL of collagenase whereas lesions extended in the ipsilateral thalamic nuclei (lateral-dorsal and lateral-posterior nuclei) for animals receiving 2 µL of collagenase. In a second experiment, gabapentin reversed mechanical allodynia, evaluated with von Frey filaments, and hyperalgesia, evaluated with Hargreaves test, in rats ($n = 6$) following a collagenase-induced (3 µL) hematoma. In conclusion, these preliminary results suggest that central pain was induced in rats with a collagenase-induced intracerebral haemorrhage localized in the caudoputamen nuclei most probably associated with lesions to the thalamus, and concurrent allodynia and hyperalgesia were reduced with gabapentin treatment.

P239 A Panel of 96 Single Nucleotide Polymorphisms for Genetic Monitoring of Rats

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Genetic monitoring is necessary to assure the genetic integrity of laboratory animals when bred, especially when there is a potential genetic risk such as similar coat colors of different strains. While microsatellite markers or biochemical tests can be used for genetic monitoring, single nucleotide polymorphisms (SNP) are more easily available in current databases, and are more amenable to high-throughput analytic technologies. We, therefore, set out to design such a more efficient genetic monitoring panel and used data obtained from 720 SNP to design a 96-marker panel that can be used for genetic quality control of all rat inbred strains and outbred stocks. The panel contains SNP that are heterozygous (polymorphic) in outbred stocks, making it possible to assess both strain/stock identity and the population genetic characteristics of outbred stocks. Markers were detected on an analyzer using a bead array and an allele-specific chemistry. The panel was shown to reliably differentiate all major rat strains and stocks at our institution. For example, Goto-Kakizaki (GK/MolTac) and Fischer (F344/NTac) rat strains are differentiated by 34 markers, and Wistar Hannover GALASTM (HanTac:WH) outbred rats are differentiated by at least 2 fixed homozygous markers from any other outbred stock, and heterozygosity of that stock can be assessed by 59 markers. The 96-SNP genetic monitoring panel will allow for genetic quality control of laboratory rats at a higher level than previously possible.

P240 Rabbit Supraspinatus Motor Endplates are Unaffected by a Rotator Cuff Tear

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Fatty infiltration is a significant complication of rotator cuff tears. Pathogenesis of the fatty infiltration has not been reported but it is speculated that nerve damage due to impingement may play a role. We hypothesize that changes in innervation at the motor-end-plate contributes to fatty infiltration. Four 4-mo-old New Zealand white rabbits had a unilateral supraspinatus tendon transection at the greater tuberosity insertion under isoflurane anesthesia. All attachments of the tendon to the surrounding tissues, including the infraspinatus were released, allowing the tendon to retract. Buprenorphine (0.1 mg/kg SC) was given at the time of surgery and a fentanyl patch was placed (delivery of 0.25 µg/h) on the dorsal cervical area for postoperative analgesia. At 3 mo, the rabbits were euthanized. The supraspinatus

muscle and suprascapular nerve were completely dissected from the supraspinatus fossa. The supraspinatus was sectioned into multiple slices for confocal microscopic motor end plate functional analysis and histologic analysis of osmium tetroxide stained sections for fatty degeneration. Paired student *t* test was used to analyze the data with $\alpha = 0.05$. There was an increase in fatty infiltration in the surgically resected supraspinatus muscles compared with the control supraspinatus muscle (19% and 3%, respectively). The degree of denervation ($P = 0.10$) and partial denervation ($P = 0.84$) was not significantly different between control and experimental muscle. We conclude that denervation of the motor endplate is not required for the induction of fatty degeneration in rotator cuff tears. Understanding the cause of fatty infiltration is critical to orthopedic surgery because its presence limits repair ability.

P241 Health Effects of Intravenous Tail Vein Injection Volume in CD1 Mice

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Investigators doing research often need to administer intravenous bolus injections of substances into the lateral tail veins of mice. The maximal dose volume for these injections has been recommended at 5 mL/kg or about 150 to 200 µL for a 25- to 30-g mouse. The scientific literature lacks rigorous documentation of the health effects of bolus mouse tail vein injections in excess of that maximal upper limit with the exception of some studies relating to hydrodynamic delivery of DNA and tumor cells into mice. Our hypothesis is that a 1-mL IV injection of 0.9% normal saline in the lateral tail vein will not result in significant hematological, biochemical, or tissue abnormalities in CD1 mice. Ten female CD1 mice weighing between 30 to 35 g were injected with 1 mL sterile, preservative-free 0.9% saline in their lateral tail veins. The injections were administered with a 28-gauge needle attached to a 3-cc syringe over a time period ranging from 20 to 25 s. Baseline hematological and biochemical blood values obtained 1 wk following shipment to our facility were compared with those taken 24 h following the 1-mL bolus tail vein injections. Preliminary results suggest some hematological and biochemical trends (increased WBC, blood glucose, and serum potassium levels) indicative of a mild stress response and other transitory phenomena. Results of microscopic analysis of selected organs (heart, lungs, liver, spleen, brain, kidneys, and pancreas) are still pending. In summary, preliminary data suggest that a 1-mL IV bolus injection of normal saline may result in mild, temporary changes in the hemogram and blood chemistry profiles but no significant clinical abnormalities in CD1 mice.

P242 Rag1 Knockout via Zink-Finger Nuclease Technology in Fragile LEW Zygotes

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Embryonic stem cells as tools for the genetic manipulation are only available from a few strains of mice and rats. Transfer of genetic modifications onto other backgrounds is time consuming and expensive. Zinc-finger nuclease (ZFN) technology represents a strain-independent approach for genetic manipulation in rodents. Most gene targeting experiments in the rat via ZFN were done using outbred Sprague–Dawley rats because of high fecundity and good response to gonadotropins. Here we report the first disruption of a Rag1 allele in the LEW/Ztm inbred strain, serving as the genetic background for a large family of congenic strains. mRNA encoding for a pair of Rag1 specific ZFN were injected into the pronuclei of zygotes from LEW/Ztm rats. Two cell embryos were transplanted the following day to foster mothers. Surveyor mutation detection assays were performed to identify Rag1 mutations and to exclude off-target mutations in the surviving offspring. Mutations were confirmed by sequencing. Germ-line transmission was validated by birth of Rag1 mutated offspring derived by mating of the Rag1 mutated founder to a wildtype sibling. PCR amplification followed by restriction digest was used for genotyping. After pronuclear injection of ZFN mRNA into 623 zygotes 314 were transferred to foster dams. Of 52 offspring born 42 pups were weaned. Of these, one female was identified as being heterozygous for the Rag1 mutation. Sequencing revealed a 5 bp mutation (the insertion of a cytosine and a deletion of 4 bp) leading to a frame shift mutation. The mutation provokes a premature stop codon, resulting in a putative protein of only 198 amino acids as compared with the wildtype protein with 1040 amino acids. No additional off-target mutation could be identified in the 10 loci that show the highest sequence homology to the Rag1 target site. Germ-line transmission was confirmed providing a normal Mendelian ratio with 6 heterozygous and 5 wildtype offspring in the first litter. The data emphasize the high potential of ZFN to introduce genetic manipulation independent of the genetic background of the rats. Thus, the ZFN technology may serve as a time saving and cost-effective approach to generate new rodent models, and help to reduce the number of animals by avoiding animal consuming backcrossing.

P243 Differentiation of Substrains of C57BL/6 Using Single Nucleotide Polymorphism Markers and Allele Specific Oligonucleotide Markers

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Substrains of inbred strains arise when breeders are deviated from their original colony. The resulting descendants can separate into sublines due to genetic drift either caused by residual heterozygosity in the original population, recent spontaneous mutations that become fixed, or in the worst case by genetic contamination. Established substrains of inbred strains of mice are widely distributed. Unfortunately, their genealogy is not always properly reported, which then will create problems as to which substrain to be selected as control or further backcross partner. A huge problem is the intensive usage of different substrains, especially of C57BL/6, as controls and genetic background for congenics. These strains/stocks quite often harbor one or more genetically modified genes as well as a mixed genetic background (of different (sub)strains). Despite appropriate breeding techniques applied by the vendors, accompanied by genetic monitoring programs, quite often researchers derive their animals from various sources without paying attention to genetic uniformity of the strains and use, but also deliver their "combined" models to others. We used a set of markers consisting of more than 30 SNP and some DNA length polymorphisms to differentiate B6 substrains from several European and American sources to define their respective substrain specific genotype. The markers used have in part been developed by us or published by others. This set of markers was then used to genotype the genetic background of B6 substrains actually carrying one or more genetically modified genes. Our findings revealed clear differences between B6 substrains and, moreover, heterozygous B6 colonies have been detected, even when defined as being homozygous. Mice with mixed B6 substrain background were not only detected amongst the experimental colonies at our vivarium, but also amongst those received from commercial providers.

P244 Comparison of 2 Methods for Cryopreservation of Mouse Spermatozoa

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Most of the genetically modified mouse strains are maintained on a C57BL/6 background. Besides the fact, that different substrains do exist, sperm from C57BL/6 mice is difficult to cryopreserve. Here we compare 2 methods (A and B) for the cryopreservation of 3 C57BL/6 substrains that are frequently used in Germany. The sperm of 5 males per strain and freezing-method was cryopreserved: C57BL/JRj, C57BL/6JCrl, C57BL/6NCrl, and B6C3F1/Crl (control). Sperm was prepared with standard procedures in cryoprotection medium (CPM). CPM was composed of 18% raffinose and 3% skim milk in H_2O. CPM was supplemented with 477 µM monothioglycerol (MTG) for method B. For method A holes (3 mm deep, ø 5 mm) were melted into a block of dry ice (−80°C) using a steel punch and 50µL of the sperm suspension was filled into each hole. Five minutes later the suspension had formed small "pellets", which were transferred to precooled (−196°C) 1-mL cryocontainers and placed into liquid nitrogen for storage. For method B, the sperm suspension was loaded into 0.25-mL French straws, which were then heat-sealed. The straws were incubated in nitrogen gas for 10 min, before they were plunged into liquid nitrogen. Prior to freezing and directly after thawing the sperm motility was calculated. B6C3F1/Crl oocytes were fertilized using standard procedures with the thawed sperm. Unfrozen sperm was also used for IVF as control. Both freezing methods reduced sperm motility significantly in all 3 strains. The fertilization ability depended on the freezing method and the substrain. However, the cryopreservation with method B resulted

in significantly higher fertilization rates than method A only for substrain C57BL/6NCrl. For the other substrains and for the control the differences were not significant. Therefore, both methods can be used for the cryopreservation of sperm from C57BL/6 substrains. The fertilization ability of frozen thawed sperm is more dependent on the substrain than on the method used.

P245 Tracking Tumor Production and Metastases by Fluorescence Imaging

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Orthotopic models play a significant role in the understanding of cancer formation and spread, and also provide a physiologic environment for evaluation of novel diagnostic and therapeutic agents. Since monitoring the growth of orthotopic tumor is often challenging, we have instituted a reporter gene approach to track primary and metastatic prostate cancer (PC3). Noninvasive imaging was used to monitor the success of implantation, tumor growth, and the formation of metastases with a noninvasive fluorescence, optical imaging system. Using nu/nu mice, PC3-dsRed-expressing cells (1×10^6 in 10 µL) were inoculated into one prostate lobe ($n = 140$). At week 4 postsurgery, mice were imaged to monitor the success of implantation and subsequently imaged at weeks 6, 8, 10, and 12 to monitor tumor growth and metastasis, if present ($n = 110$). A dual-labeled, near infrared fluorescent and radiotracer, antibody was used to target and confirm tumor and metastasis presence and growth. Prior to week 4, fluorescence in the region of the tumor implants was not significantly higher than background levels. At week 4, we detected fluorescent signal indicating the presence of tumor cells at the implantation site in several mice. Imaging studies performed at weeks 6 and 8 detected metastases to the lumbar and renal lymph nodes, indicating the high sensitivity of the imaging system. Using a customized, noninvasive fluorescence, optical imaging system, we were able to detect primary tumors at 4 wk and metastasis by 8 wk postsurgery. Fluorescence imaging agents could be used for tracking metastases in the clinical setting and guiding physicians during nodal dissection.

P246 Establishment of Total Limbal Stem Cell Deficiency Model in Rabbit

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Recently, many studies have suggested that transplantation of cultured autologous limbal stem cells has potential therapeutic effects for restoration of the corneal epithelium. A reliable animal model of limbal stem cell deficiency is considered. The purpose of this study was to establish a total limbal stem cell deficiency model in rabbit by comparison of 2 methods. Sixteen New Zealand white rabbits were divided into 2 groups: group 1 (surgical technique) and group 2 (surgical technique with alkali burning). Under general anesthesia (zoletil/xylazine), drops of 0.5% alcaine were instilled onto the cornea at the beginning of the procedure. In group 1, a limbal stem cell deficiency was created in each rabbit's right eye with surgical technique; the corneal limbal epithelial lamella was excised completely with a number 15 scalpel blade. Group 2 was applied the alkali burning in addition to surgical technique; the limbal region was swabbed gently with a sterile cotton stick using 1-N NaOH and then washed with 0.9% NaCl. The nonoperated left eye served as control. The corneal surface was also examined for smoothness, clarity, and vascularization by slit-lamp examination. Histologic evaluation with hematoxylin and eosin staining and impression cytology were performed to assess the phenotype of corneal epithelium. By examining corneal opacity, fluorescein stained areas and neovascularization, the scores of corneal surface in group 2 were higher than that of group 1. Eight weeks after the injury, all eyes in group 2 showed moderate to severe corneal vascularization with epithelial defects, whereas the same effect took 10 wk in group 1. In impression cytology, all treated eyes in group 2 were positive for PAS staining, indicating the presence of conjunctival goblet cells, whereas 4 eyes in group 1 was negative, indicating the partially recovery spontaneously. Histologic findings showed corneal defects covered the goblet cells, new vessels and connective tissues in group 2 compared with group 1. These results suggested method of surgical technique with alkali burning is more reliable than that of surgical technique as animal model of total limbal stem cell deficiency. Our animal model might be useful in preclinical study of evaluating the effects of the cell therapy for limbal stem cell deficiency.

P247 The Incidence of Mouse Hepatitis Virus and *Helicobacter* Species in Wild Rodents and Wildlife Species around an Animal Research Facility

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Wild rodents and other wildlife species can introduce pathogenic agents that may infect research animals and impact study outcome. For over 30 y, our Quality Assurance Laboratory has been collecting specimens from wild rodents and other wildlife species to determine their microbial profile in an effort to reduce the risk of transmission of pathogenic agents to research animals. Our goal here is to summarize our most important findings and to report the incidence of pathogenic agents, including mouse hepatitis virus (MHV) and *Helicobacter* species in wild rodents. Wild rodents were humanely captured in traps and blood, tissues, and feces were collected for serological and PCR assays. Sera was tested for antibodies to MHV using ELISA and confirmed positive by indirect fluorescent antibody. Fifteen of 51 (29.4%) wild mice tested positive for antibodies to MHV. A region of the MHV sequence for the *S* gene was analyzed from wild and resident mice infected with MHV. The gene sequence of the MHV in all of the wild mice was 100% homologous to the *S* gene sequence of the MHV found in sentinel and resident mice from a previous MHV outbreak in our colony. The MHV sequence in the wild mice was 95% homologous to RCV-SDAV. Fecal samples were also collected from wildlife around our research facility and assayed via PCR for *Helicobacter* species. A total of 38 of 51 (74.5%) wild mice, 4 of 4 flying squirrels, 2 of 2 moles, 1 of 1 opossum, 1 of 1 Norway rat, 0 of 4 voles, 0 of 2 snakes, 0 of 3 Canada geese, and 0 of 3 box turtles were PCR positive for *Helicobacter* species. We concluded that MHV and *Helicobacter* species were the most prevalent pathogenic agents detected in wild mice. These findings confirm that wild rodents present a significant health risk to research animals and emphasize the importance of a successful pest control program.

P248 Clinical and Subclinical Elephant Endotheliotropic Herpesvirus Infection in Asian Elephants (*Elephas maximus*)

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Elephant Endotheliotropic Herpesvirus (EEHV) hemorrhagic disease accounts for approximately half of all Asian elephant deaths that occur in captivity. Survival from EEHV-associated disease depends on rapid diagnosis and treatment of affected elephants. We have recently described a novel real-time quantitative PCR (qPCR) assay that detects EEHV1A and B, which are most commonly associated with mortality in captive Asian elephants in North America. Using this assay we determined that 3 of 5 healthy Asian elephants within a single screened herd have been previously infected with EEHV1 and they frequently shed virus in trunk secretions. Currently, the prevalence and molecular epidemiology of EEHV1 infection in a large population of captive Asian elephants in North America is unknown. In addition, management of clinically ill animals would benefit with the availability of data describing viral blood loads and shedding in fluid secretions during the course of clinical disease. To address these outstanding questions we monitored the kinetics of EEHV1 viremia and shedding in body fluids of both subclinical and clinically ill Asian elephants. We found that viral loads peaked in blood samples of clinically ill elephants between 4 to 21 d following appearance of clinical signs. Virus was detectable in nasal secretions with slightly delayed kinetics relative to viremia and was still detectable for several weeks following recovery. We found that EEHV1 was detectable in trunk washes from all 24 elephants included in the survey. Each elephant displayed a different frequency and magnitude of viral shedding in trunk secretions. In addition, viral gene subtyping analysis identified 6 unique EEHV1 species amongst this cohort of elephants. These data suggest that EEHV1 is a ubiquitous virus and is capable of producing

both subclinical and lethal infections in Asian elephants. This work is of significance for all veterinarians and elephant managers involved in the care of Asian elephants.

P249 The Development of an Inducible Cough Model in the Conscious Dog

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Cough is a common and possibly debilitating symptom of chronic airway diseases such as COPD, asthma, idiopathic pulmonary fibrosis, and cancer. In order to test treatment options for cough a reproducible animal model is needed. Guinea pigs have been the most commonly used animal model of cough; however, we wished to develop a model of inducible cough in a species that could also be used for pharmacokinetics and dose estimation for human trials. A cough model in a sedated dog is described in the literature, but in our experience we found it to be inconsistent and difficult to use due to inability to consistently regulate depth of sedation which appeared to suppress the cough reflex. Instead we developed a model of inducible cough in the conscious dog using a percutaneous transtracheal catheter to introduce a chemical tussive agent into the airway. Under light anesthesia, a temporary catheter was percutaneously placed in the dog's trachea under bronchoscopic guidance. The dog was then allowed to fully recover and cough was induced with several different stimulants introduced via the catheter. Resulting subsequent coughs were counted visually and recorded with a respiratory pneumograph belt. In the anesthetized model we were able to induce cough in 3 of 8 dogs (all less than 10 coughs) with citric acid, while in the conscious model 8 of 8 dogs had a cough response ranging from 30 to 400 coughs. With this model we were able to obtain consistent cough response within dogs using citric acid as a tussive agent. Dogs recovered quickly with little evidence of tracheal injury and were able to have the procedure repeated within 2 wk time, thus allowing each dog to serve as its own control and to be reused multiple times. This model could prove to be useful in further development of antitussive agents.

P250 Age and Associated Growth Correlated with Complications of the Femoral Catheter Component of a Vascular Access Port System in Juvenile Cynomolgus Monkeys

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Body weight for juvenile cynomolgus monkeys (*Macaca fascicularis*) was measured for 97 animals. Individual body weights were collected from birth up to 80 wk of age. Body weights were measured weekly throughout the study to assess growth and body weight gain in infant/juvenile cynomolgus monkeys. Beginning at animal receipt, all animals received twice daily feed rations. New world primate diet was initially provided with a gradual change to old world primate diet over the course of 6 wk. The basal diet was also supplemented with fresh fruit and vegetables 4 to 5 times weekly and certified enrichment 3 to 4 times weekly. Ninety-two of the animals were surgically implanted with a vascular access port attached to a polyurethane central venous catheter introduced into the external iliac vein to facilitate infusion administration. As the animals grew, complications with the catheter system increased (that is, increased back-pressure, inguinal edema, and others). Fluoroscopy images were collected to evaluate and assess the possible nature of the complications. The onset of edema and other complications were observed at an average age of approximately 50 wk. The average body weight for males and females at this age was 1.56 and 1.38 kg, respectively. As the animals grew older and increased in weight, the incidence of catheter displacement or malfunction increased, mainly due to animal growth.

P251 Intravenous Infusion and Restraint in Juvenile Beagle Dogs

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Juvenile toxicology studies are mandated by both European and US regulatory agencies prior to conducting the initiation of pediatric clinical trials. An agency guideline, *Guidance for Industry Nonclinical Safety Evaluation of Pediatric Drug Products*, was finalized in 2006 and provides general information on the conduct of nonclinical safety evaluation of drugs intended for the pediatric population. Nonrodent juvenile studies may be conducted in various species, but are most commonly performed in dogs or nonhuman primates. A common justification for the use of the dog is the ability to evaluate toxicology parameters during the critical developmental period that is the human-equivalent of neonatal/infant age ranges. However, juvenile toxicology studies present several challenges, including the technical aspects of dose administration to small, young animals. Many drugs are dosed by intravenous infusion, making the development of an effective and efficient method in the juvenile beagle dog crucial. During this development, consideration for restraint, location, and duration of intravenous infusion, age of the test subject, dose frequency, maximizing animal comfort, and minimizing animal stress were addressed. As the dog aged from PND 14, with limited mobility and small size, through PND 91, the age at which adult dog procedures may be used, methods and procedures were evaluated and adapted. The present evaluation concluded that intravenous infusion of juvenile beagle dogs as young as PND 14 was feasible, thus enabling dosing over the full juvenile toxicology study range from neonate to adolescent.

P252 A Novel Method for Detecting *Staphylococcus aureus* in Haired Rats

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Staphylococcus aureus is a ubiquitous opportunistic pathogen present in the nasal passages of 25% to 30% of humans. The incidence of *S. aureus* in rodent vendor rooms may range from less than 5% to 90%. The presence of *S. aureus* in immunocompromised rodents can be a serious problem. Culture procedures for detecting *S. aureus* include oropharyngeal or skin swabs or feces. This report compares 3 sampling methods for detecting *S. aureus* in haired rats: 1) contact agar plates applied to the inside(s) of animal cages, 2) oropharyngeal swabs, and 3) pooled fecal samples. Commercially available Baird-Parker and tryptic soy broth with 5% sheep blood contact plates were applied to the inside of 5 cages containing haired rats (3 per cage) positive for *S. aureus*. Cages were sampled 2, 3, 24, and 48 h after cage change. Plates were incubated at 35 °C for 24 to 48 h and presence of *S. aureus* confirmed using coagulase test. Oropharyngeal swabs and feces were collected, placed in thioglycolate broth and subcultured at 24 h to blood agar and phenyl ethyl alcohol agar plates. *S. aureus* was detected in 20 of 25 (80%) samples from *S. aureus*-positive cages by cage-side Baird-Parker plate application. In contrast, only 12 of 20 (60%) oropharyngeal swabs and 10 of 20 (50%) fecal samples were culture positive for *S. aureus* indicating that the Baird-Parker contact plates are a simple method for the detection of *S. aureus*. In addition the Baird-Parker contact plates, applied 2 to 3 h sfter cage change is more accurate than applying plates 24 or 48 h after cage change due to potential bacterial overgrowth. It was concluded that Baird-Parker contact plates are a rapid, effective, stress free method for detecting *S. aureus* in nude or haired rodents. Results should be interpreted by a trained microbiologist.

P253 Protection Effect of a Combination of Antioxidants on Oxidative DNA Damage of Mice

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Our goal with this study was to evaluate the protective effect of a combination of *Fructus rhodomyrti*, *Ginkgo biloba*, *Ganoderma lucidum*, and *Fructus cannabis* (CSCT) on oxidative DNA damage in vitro and in vivo. Spleen lymphocytes were treated with different concentrations of CSCT, and then treated with H_2O_2. After that, the comet length and percentage of cells with migrated DNA were measured and compared with negative control groups, positive control groups, and CSCT protective groups. The mice oxidative stress group and CSCT protection group were established and compared according to determination of serum total antioxidant capacity (TAP) and other indicators as well as the serum level of 8-OHdG. The CSCT combination can reduce H_2O_2-induced DNA damage to varying degrees. The TAP and other indicators as well as the serum 8-OHdG

level were significantly different between the CSCT protection group and the oxidative stress group. These results suggest that the CSCT combination might have antioxidative effects, which means it could significantly reduce free radicals-induced oxidative DNA damage in a certain concentration range in vivo and in vitro.

P254 Mouse Malignant Mesothelioma Results Found To Be Murine Polyoma Virus PCR Positive: Virus Integration without Expression

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In the fall of 2010, the Department of Comparative Medicine (DCM) sent AB12 tumor cells from an investigator's cell line to a laboratory's Diagnostics for Infectious Disease real time (rt) PCR testing. Days after obtaining a positive polyoma virus rtPCR result, the investigators informed DCM that the tumor cell line had previously been injected into 2 groups of mice (8 wk and 1 wk prior). DCM immediately recreated a timeline of events, which included mouse locations and movements. Specific areas were quarantined, injected animals were moved to a biosecure location (BSL2), and a testing/diagnostic plan was formulated. Only the mice from the second injections remained and all animals in the facility were housed in standard static microisolation caging. Upon further inquiry, the investigator's laboratory reported the cell line had been tested elsewhere in 2004 and was serologically negative for common murine pathogens (specifics unknown). Additional testing by DCM included: rtPCR polyoma retest of the cell line, rtPCR polyoma testing of tissues from the injected mice, serology, and rtPCR on tissues from other mice housed in the same BSL2 facility. Running a MAP test was considered, but due to time and cost, we chose to continue serologic and rtPCR testing from animals that were considered 'high risk'. After approximately 1 mo of follow-up testing, no animals had seroconverted or were rtPCR positive, except for tissues (skin and tumor) from animals directly injected with the AB12 cell line. Later, IFA was run on the cell line, and no detectable polyoma virus antigen was found. These findings support the hypothesis that there was virus integration into the tumor cell genome, but not expression (infection). These novel results should be considered when deciding how and when to test tumor cell lines at other institutions.

P255 Packed Cell Volume, Plasma Proteins, and Blood Sugar Level as Predictors of Approaching Sexual Maturity in Japanese Quail

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The Japanese quail (*Coturnix japonica*) is a very popular animal for use in scientific research. Its contribution to studies on pre- and postnatal growth, reproduction, endocrinology, nutrition, and toxicology are noteworthy. Continued characterization is, therefore, essential for obtaining precise and dependable results. The research as reported deals with postnatal growth of Japanese quail females and simultaneous physiologic changes in packed cell volume (PCV), blood glucose level (BGL), and plasma protein level (PPL) from advancing age to sexual maturity (laying of first egg) at approximately day 50. On day 21, 15 hatch-mate female chicks of uniform body weight were selected for this project. The birds were housed in an air-conditioned room (73 to 75 °F) in suspended changes with 3 to 4 birds per cage (15 × 12 × 12 in.) with free access to feed and water under a photoperiod of 14:10-h light:dark cycle. The birds were weighed and blood sampled (0.25 mL) from the brachial vein at day 4 intervals. The blood was processed for PCV using hematocrit tubes (14,000 rpm for 5 min), PPL using the refractometer, and BSL using glucometer. Following day 36, the PCV level began to rise gradually above the preceding levels of 38.9% ± 1.6% to 41.4% ± 2.5% on day 40, to 43.2% ± 0.8% on day 44, and to 45.8% ± 3.2% on day 48. PPL also increased gradually from the preceding level of 3.32 ± 0.8 to 5.64 ± 1.2 g/dL on day 48. However, BGL decreased gradually from the preceding level of 266.5 ± 21.5 mg/dL to 228.6 ± 20.1 mg/dL on day 48 ($P < 0.05$). The data reflects that PCV, PPL, and BGL can be used to assess approaching sexual maturity in Japanese quail.

P256 Testing the Ability of Hemostatic Products to Protect against the Chemical Warfare Agent VX

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Hemostatic agents are used to prevent soldiers from bleeding to death in the field. This study addresses their effects on a bleeding wound contaminated with the highly toxic organophosphate chemical warfare agent VX. In vitro studies showed that a granular hemostatic agent was the most promising candidate for blocking the absorption of VX, and it was selected for in vivo testing. We first determined that the median lethal dose (MLD) of VX when placed on the ear of an anesthetized pig was 60 µg/kg. Animals received between 2% and 4% isoflurane to keep them below the plane of anesthesia. Next, 300 µg/kg of VX was applied to the ear with a glass chamber surrounding the application site, which was left untreated as a control or treated with the granular product. All of the control pigs developed signs of severe agent intoxication with a mortality rate of 83%. In contrast, the granular-treated pigs all survived with 83% exhibiting mild to no intoxication. We then created a nonbleeding wound in the axillary area of an anesthetized pig, applied neat VX into the wound and determined the 6-h MLD to be 28.6 µg/kg. We expanded on this work by applying 5× MLD (143 µg/kg) of VX into the wound, cutting the vein/artery bundle and when the pocket was half-filled with blood, pouring the granular product into the pocket. Of the animals tested in this manner, 3 out of 6 died prior to the 6-h endpoint. Because the granular product causes clots to form distant from the wound, it is no longer used in the field and has been replaced by a hemostatic impregnated gauze. In the same 5× MLD bleeding wound model 5 out of 6 animals treated with the hemostatic gauze died prior to the 6-h endpoint. Thus, although both hemostatic agents provide benefit, both failed to significantly protect against a 5× MLD challenge of VX in a bleeding wound.

P257 Immuno-Antimicrobial Therapy for Treatment of Chronic Staphylococcal Infection with Pre- and Postexposure Vaccination

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Staphylococcus aureus is a common biofilm producing pathogen that is frequently involved in the development of nosocomial and community acquired infections. The prevalence and severity of staphylococcal infections is increasing, and the emergence of new, highly resistant strains continues to impede efforts to reduce morbidity and mortality rates associated with the bacteria. The increased incidence of severe staphylococcal infections, in addition to the prevalence of antimicrobial resistance, requires that new therapeutic measures be developed against this pathogen. We hypothesize that the combination therapeutic regimen of antibiotics and vaccination will reduce the incidence and severity of staph-associated infections and implant failure. Mice were implanted with a subcutaneous catheter inoculated with luciferase expressing *S. aureus*. Five mice from each treatment group received subtherapeutic antibiotics, *S. aureus* biofilm vaccine, or combination therapy postexposure. Another 5 mice from each group received preexposure vaccine developed from *S. aureus* biofilm antigens 10 d prior to subcutaneous catheter implant. Biofilm antigens were collected at 2, 5, and 10 d post culture. In both the pre- and postexposure experiments, all mice achieved initial luciferase expression of approximately 106 photons/s. The preexposure vaccinated animals' luciferase expression decreased to below the level of detection by 13 d postinfection, while those receiving postexposure biofilm vaccination and/or antibiotic therapy maintained luciferase expression of approximately 106 photons/s until at least day 21 postexposure. At this time, the combination and antibiotic only-treated groups decreased to below detectable levels while the untreated and vaccine only groups maintained luciferase expression at approximately 106 photons/s until day 32 postinfection. These results indicated that preexposure vaccination with biofilm antigens did not reduce bacterial numbers compared with sham vaccinated mice. However, combined vaccination and antibiotic therapy commencing postexposure did improve bacterial resolution compared with nontreated controls, demonstrating the potential for vaccination with biofilm antigens combined with antibiotic therapy to improve recovery from chronic *S. aureus* infections.

P258 Determination of the Prostate Gland Size in Beagle Studs Using Transabdominal Ultrasonography

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Prostate cancer is the most prevalent cancer of men in the western hemisphere. Very few animal models exist that develop spontaneous prostatic disease. The dog is one of the few animal models that does develop spontaneous prostatic disease to include prostatic carcinoma and benign prostate hypertrophy. Moreover, the dog has a single lobed prostate similar to man unlike the mouse and rat. As in man, prostatic disease is seen in older dogs, especially those over 5 y of age. Though there are some inducible models of prostatic disease in dogs the need for spontaneous models exists. The purpose of this study was to evaluate the size and volume of the prostate gland in beagle studs. Thirteen beagle studs ranging in age from 3 to 7 y of age (mean, 4.6 y) were evaluated. Measurements of the greatest craniocaudal (L), transverse (W), and dorsoventral (D) were made using a 4- to 7-MHz curved linear array transducer. Volumes were calculated using the measured prostatic volume (Vm) formula of [1/2.6 (L × W × D)] + 1.8 (cm^3). Body weights (kg) were also recorded. Mean prostatic volume was 11.25 ± 1.7 cm^3. Mean body weight was 11.8 ±1.3 kg. The mean transverse measurement (width) was 3.46 ± 0.3. These values are representative of the prostate dimensions of a working stud in a commercial breeding facility.

P259 Consistency in Tumor Growth Measurements: The Importance of Intertechnician Variability

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Tumor volume in mouse and rat xenograft models is a primary endpoint for many oncology studies. Longitudinal quantification of tumor volume provides a growth index, used for evaluating response to treatment, a vital data set for our research. This data is also important for randomization of mice prior to starting an experimental treatment. Calipers are commonly used to measure tumor volume while the animal is restrained by a technician. Consistency in taking measurements and animal handling is critical to prevent the introduction of confounding variance into the data. Realizing that there will always be some individual variation in collecting data of this type, technicians in our department are trained to measure tumors in a consistent manner and typically record volume data 2 to 3 times a week per study. As a metric for our training program we evaluated the intertechnician variability for tumor volume measurement. During the course of 2 longitudinal studies, tumor volume was recorded by the trainer and the trainees simultaneously during each measurement session. Our comparison revealed significant difference between individual technicians. While each technician's data showed consistent trends in growth, over time there was large variation in the absolute value of the measurements between technicians. Our data suggest that only one individual should perform tumor volume measurements for a given study and that using multiple individuals for data collection will compromise study integrity. Further, the importance of consistent handling techniques will also be discussed. Studies hinging on tumor volume measurement require a dedicated study technician and careful planning to avoid data compromise.

P260 Infectivity of *Helicobacter pullorum* in Brown Norway and Sprague–Dawley Rats

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Helicobacter pullorum is an enterohepatic *Helicobacter* spp. recently reported as a natural infection in mice and rats. We previously reported persistent infection of singly housed brown Norway (BN) rats over a period of 6 mo following oral gavage with approximately 1 × 10^9 CFU every other day for 3 doses. Lower bowel tissue collected at necropsy confirmed that the cecum and colon were sites of *H. pullorum* colonization. All fecal and tissue PCR products were amplified using species-specific primers for *H. pullorum* cytolethal distending toxin B (cdtB). A concurrent 22-wk dirty bedding transfer study, during which 4 cages of pair-housed Sprague–Dawley (SD) rats were exposed to bedding from the *H. pullorum*-positive BN rats at a 3:2 ratio of dirty to clean bedding, did not result in colonization of the cecum or colon. To determine if the failure of *H. pullorum* colonization in SD rats was due to the method of exposure or host resistance, 10 additional SD rats were infected with *H. pullorum* by oral gavage with approximately 1 × 10^9 CFU of *H. pullorum* every other day for 3 doses. Feces were collected and analyzed at 2 wk postinfection (PI). In contrast to the BN study where 6 of 6 rats were positive for *H. pullorum* by fecal PCR at 2 wk PI, only 1 of 10 orally dosed SD rats was positive for *H. pullorum* at 2 wk PI. Although experimental *H. pullorum* infection by oral gavage was successful, conventional bedding transfer to SD rats was unsuccessful, implying that a critical infectious dose of *H. pullorum* was not achieved. These data suggest that SD rats are not suitable colony sentinels for detection of *H. pullorum*. Experimental dosing data, to date, may indicate a relative host resistance to *H. pullorum* in SD compared with BN rats.

P261 *Pneumocystis carinii* Infection in Mice and Rat Colonies from Brazilian Animal Houses

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Pneumocystis carinii is classified as a fungal pathogen and it is an opportunistic organism that can cause lethal pneumonia in immunocompromised mammals. *P. carinii* and *P. wakefieldiae* sp. nov., are species found in laboratory rats while laboratory mice can be infected with *Pneumocystis murina* sp. nov., formerly known as *P. carinii* f. sp. *muris*. Immunocompetent mice become infected with *P. murina*, but clear the infection without developing lesions. This organism can be detectd by PCR as well as by histopathology. The aim of this work consists in establishing a PCR assay in our routine of laboratory animal health to perform an initial trial to detect *P. carinii* in mice and rat strains from Brazilian animal facilities. SPF and nonbarrier mouse and rat colonies from 9 animal facilities were screened. We tested 59 animals representing 29 rats (8 strains) and 30 mice (20 strains) from both sexes and ages. Both animals exhibiting and not exhibiting typical signs of chronic pneumonia were examined and lung tissue was collected for DNA extraction and histologic preparations. Specific stains were used to verify the presence of *P. carinii* cysts. DNA was extracted using a commercial kit and the PCR reaction was performed using a specific primer sequence directed to the rRNA gene. *P. carinii* was detected in immunocompetent, immunodeficient and genetic modified animals. The infection was confirmed by PCR in 9 rats and 12 mice, representing 31% of positive rats and 40% of positive mice. Histopathology demonstrated the presence of *P. carinii* organisms in some of the tissues analyzed confirming the clinical disease.The present study confirmed that PCR is able to detect the infection at early age as well as in the absence of clinical signs. Therefore, PCR technique can be a useful tool to determine the prevalence of *P. carinii* in colonies of mice and rat from Brazilian animal facilities.

P262 Optimized Sperm Cryopreservation Media for Mice

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Sperm cryopreservation has become an acceptable and cost-effective procedure for archiving the genomes of genetically modified animals. During the sperm freezing process, ice formation and reactive oxygen species formation have been proven to be closely associated with post thawed sperm capability to fertilize oocytes. We examined supplementation of sperm cyroprotective agent (CPA) with free-radical scavengers to potentially improve the thawed sperm motility, viability, and subsequent fertilizing capacity. Various concentrations of antioxidant supplement (AOS) and polyvinyl alcohol (PVA) and the effects they had on post thaw motility and in vitro fertilization (IVF) efficiency were tested. Sperm were harvested from male mice and 10 µL sperm per CPA suspension was loaded into each 0.25-mL cryopreservation straw. Straws were sealed on both ends and placed into a freezing canister which was floated on liquid nitrogen for 10 min, and then submerged. Straws were thawed in a 37 °C water bath for 2 to 3 min. After thawing, sperm was diluted and assessed using a computer assisted sperm analysis system for motility and used in an IVF procedure. Embryos generated from IVF were cultured overnight and 2-cell stage embryo development was assessed. The results indicated that supplementation of CPA with 10 µL/mL AOS provided a significantly greater protection of sperm motility, and

significantly higher IVF rates were achieved with C57BL/6NCrl mouse sperm frozen in CPA + 10 µL/mL AOS + 100 µg/mL PVA. Based on these results, the modified sperm cryopreservation media were used to freeze sperm from 5 inbred strains of mice and perform IVF. The IVF rates were 55.7%, 26.4%, 87.3%, 87.8%, and 26.2% in C57BL/6NCrl, 129S2/SvPasCrl, FVB/NCrl, DBA/2NCrl, and BALB/cAnNCrl, respectively. These results are consistent with recent reports. Over a 1.5-y period, 49 distinct mouse lines were archived by sperm cryopreservation and later recovered by IVF. The recipient pregnancy and live-birth rates were 93.2% and 39.9%, respectively. This new sperm cryopreservation media will increase success in archiving and recovery of genetically modified mouse models.

P263 Mouse Parvovirus-1 Induced Cytokine Expression in Amniotic Fluid and Adult Female Mice Sera

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Mouse parvovirus-1 (MPV1) is one of the most prevalent infectious agents in modern laboratory mouse colonies. Though MPV1 infection results in subclinical signs and no pathology, it can seriously impact biomedical research because infection elicits significant immunomodulatory effects both in vitro and in vivo. Reported alterations include inhibition of normal and cancerous T cells and the potentiation of tumor allograph rejection. The mechanisms responsible for these effects are poorly defined. Recent neurodevelopmental studies have demonstrated that the proper spatiotemporal cytokine expression in the brain plays an essential part of normal brain development. Thus, an alteration of this expression by MPV1 could alter the normal development of the brain. The aim of this study was to characterize the cytokine profiles in MPV1 infected and noninfected mice to study the impact of MPV1 infection on prenatal brain development. Mice were inoculated orally with MPV1 (100 ID50). Once infection was confirmed by qPCR and serology, mice were time-mated. Mice were euthanized and blood collected for cytokine analysis using cytokine-protein arrays. The results indicated that amniotic fluid from MPV infected embryos had elevated IL6 and decreased IL2, IL3, and IL5. Adult female sera had elevated IL12, VGEF, and SFC and decreased IL2, IL3, IL5, IL9, IL10, IL17, IFNγ, GCSF, and TNFα. These cyotkine alterations may impact fetal neurogenesis and development.

P264 Combined Immunotherapy and Antimicrobial Therapy for Treatment of Chronic Staphylococcal Osteomyelitis

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The high prevalence of *Staphylocccus aureus* nosocomial infections, combined with rapidly developing resistance to traditional antibiotic regimens, suggest a need for novel therapies against this common pathogen. We found that combined immune-antimicrobial therapy with IFNγ and penicillins generated strong synergistic killing of intracellular *Staphylococcus*. Therefore, we investigated the effectiveness of combined therapy in a mouse model of chronic osteomyelitis. Osteomyelitis was induced in CD1 mice by placing a 1-cm steel pin precoated with a luciferase expressing *S. aureus* biofilm in the tibia. Mice (n = 4 to 5 per group) were treated with subtherapeutic doses of piperacillin, combined with a once weekly heat-killed *S. aureus* vaccine. Treatment was initiated immediately or 10 d after implantation. Group 1 received piperacillin (50 mg/kg IP) every 12 h; group 2 received 3 *S. aureus* vaccines; group 3 received both piperacillin and vaccine; group 4 were untreated controls. Bone infection was quantitated every 3 d using an IVIS imaging system and bacterial load present upon the pins were represented by luciferase expression as photons per second. Mice receiving immediate treatment in groups 1, 3, and 4 developed a steady increase in bacterial growth as evident by an increase in luciferase expression peaking at 10 d after implantation at 8 × 105 photon/s. Luciferase expression was 104 photons/s in groups 3 and 4 and persisted at 5 × 105 photons/s in groups 1 and 2 at 30 d after implantation. Unexpectedly, we noted an increase in luciferase expression in vaccinated mice compared with untreated mice. Mice receiving delayed treatment in all groups developed a steady increase in luciferase expression again peaking at day 10 after implantation at 7 × 105 photons/s. Luciferase expression waned in all groups to 104 photons/s at 27 d after implantation. Neutropenia was induced with cyclophosphamide to minimize cellular influx and protection of the biofilm prior to implantation and were treated 10 d after implantation as previously stated. Neutropenic mice receiving delayed treatment developed a steady increase in in luciferase expression in all groups which peaked at 7 d after implantation at 2 × 106 photons/s and waned in all groups by day 20 after implantation to 4 × 104 photons/s. These results indicated that combined vaccination and antibiotic therapy did not improve resolution of osteomyelitis, regardless of timing of therapy or presence of neutropenia. Our studies indicate that vaccination may not improve the efficacy of antimicrobial therapy for treatment of chronic osteomyelitis.

P265 Influence of Mouse Parvovirus on Cytokine Production in Mice

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Mouse parvovirus (MPV) is a continual concern in mouse colonies. The presence of the virus can negatively impact research results through its immunosuppressive effects on T cells, but its serologic detection remains inconsistent. In order to determine a more reliable way to detect MPV in mouse colonies, a better understanding of the host immune response during the course of infection is necessary. The aim of this project is to evaluate the T-cell responses in MPV infected mice to determine if regulatory T cells influence the host immune response. Toward this aim, T-cell responses in BALB/c, C57BL/6, and Swiss Webster mice inoculated with MPV1e were compared with sham-inoculated mice. Infection was confirmed by fecal PCR. Ten mice from each strain were euthanized after 5 d, representing an acute infection, and 10 mice from each strain were euthanized after 6 wk, representing chronic infection. Mesenteric lymph nodes and spleen samples were taken from all of the mice and their cellular contents analyzed by flow cytometry to evaluate Th1, Th2, Th17, and regulatory T cell responses. All strains had an increase in CD4+ and CD8+ T cells, and decrease in Foxp3+CD4+ T cells, IL17+ T cells, and IL10 production. There were variable strain dependent changes in CD11c, CD11b, and NK1.1. cells, and TNFα and IFNγ production. These results demonstrate the complexities of the host immune response to MPV and how it may potentially impact the antibody response and subsequent serologic detection of MPV. With a better understanding of the host immune response and the role of regulatory T cells, more reliable diagnostic assays could be developed to enhance the detection and minimize the research effects of MPV infection in mice.

P266 Selection in NOD-*scid IL2Rγ*^{null} Mice of Gene-Modified Human T Cells Expressing Engineered Human Enzymes that Confer Resistance to Lymphotoxic Drugs

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The efficacy of adoptive T cell immunotherapy depends on the persistence of transferred gene-modified T cells in vivo. In this study we examined a strategy to select for adoptively transferred gene-modified T cells over endogenous lymphocyte pools with lymphotoxic drugs in NSG mice during lymphopenia driven homeostatic expansion. The lymphopenic environment in NSG mice favors human T cell homeostatic proliferation and survival due to decreased competition for cytokines, increased space availability in lymphoid compartments, and the absence of $CD4^+CD25^+$ regulatory T cells. Here we assess 2 mutated human enzyme transgenes, dihydrofolate reductase ($DHFR^{FS}$) and inosine monophosphate dehydrogenase II ($IMPDH2^{IY}$), for conferring resistance of engineered T cells to clinically relevant immunosuppressive drugs methotrexate (MTX) and mycophenolate mofetil (MMF), respectively. We demonstrated that human T cells engineered with a self-inactivating lentiviral vector to coexpress $DHFR^{FS}$ and $IMPDH2^{IY}$, are resistant to as much as 0.1 µM MTX and 2.5 µM MPA in vitro. The lentiviral vector was determined replication incompetent via p24 ELISA assays prior to administration of these lenti-transduced T cells into mice. Physiologically relevant and nontoxic MTX and MMF doses were also established in NSG mice prior to performance of the in vivo selection experiments. Lenti-transduced T cells that were only 29% positive for $DHFR^{FS}$/ $IMPDH2^{IY}$, expression were then engrafted (10^7 IV) into 6- to 10-wk-old NSG mice receiving 2 × 10^7 irradiated NS0 cells engineered to secrete human IL15 3 times a week to provide a systemic supply of human IL15 in vivo. Upon

administration of MTX and/or MMF, the preferential survival of $DHFR^{FS}$/$IMPDH2^{IY}$, -positive human T cells was detected by flow-cytometric analysis of retroorbital eye bleeds. Our results suggest that expression of $DHFR^{FS}$ and in vivo administration of MTX allows for potent selection of T cells that is more robust than $IMPDH2^{IY}$/MMF and comparable to dual selection with MTX and MMF together. This work in an NSG mouse model sets the stage for the clinical utilization of MTX mediated selection of gene-modified cells following adoptive transfer.

P267 Prostaglandin-Based Treatment of Dystocia in the Laboratory Mouse

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Murine dystocia is a significant clinical condition in laboratory animal medicine (LAM), but current treatment protocols rarely save the dam or produce a viable litter. As transgenic mice become more valuable, the impact of unsuccessful treatment of dystocia is more significant. Several drugs are used to treat murine dystocia, including oxytocin, Ca gluconate, ketoprofen, saline, dextrose, and antibiotics, but doses and results are inconsistent. An improved strategy for treating dystocia in the laboratory mouse would represent an important advance in LAM. Oxytocin (OT) and prostaglandin (PGF2α) stimulate smooth muscle contraction, but their roles in murine parturition are unclear. PGF2α is likely important in this process, since COX-1 k/o mice have delayed luteolysis and decreased expression of OT receptors. Uterine contractions are absent in mice lacking PGF2α receptors or parts of the PGF2α synthetic pathway, and OT fails to rescue this effect. Thus, we hypothesized that prostaglandins may provide a more effective therapeutic approach than oxytocin to counteract dystocia in the laboratory mouse. Mice of various strains at 2 to 15 mo old were used in this study if in good clinical condition, when reported for dystocia, by unbiased veterinary staff. Outcomes of murine dystocia cases were recorded after subcutaneous or intraperitoneal treatment with 1.0 IU OT + 2.5 µg PGF2α (n = 9) or 2.5 µg PGF2α (n = 22), and compared with previous cases treated with 1.0 IU OT (n = 20). Production of live pups was recorded by unbiased observers and compared by Fisher exact test. Live pups were produced in 2 of 9 (22%; P = 0.22) mice treated with 1.0 IU OT + 2.5 µg PGF2α and 2 of 22 (9%; P = 0.54) mice treated with 2.5 µg PGF2α, compared with 1 of 20 (5%) mice treated with 1.0 IU OT. There was no significant difference in outcomes of these treatment groups. These results suggest that PGF2α provides no advantage over OT as therapy for murine dystocia. Early detection and effective treatment of murine dystocia are difficult because mice give birth at night, and dystocia may be ongoing for several hours before treatment starts. More information about murine dystocia may indicate strain predilections or clinical signs that will allow successful treatment or prevention of this condition in the future in laboratory mice.

P268 An Evaluation of Learning and Memory Based on Hand Dominance in Infant Nonhuman Primates Using the Wisconsin General Testing Apparatus

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Learning and memory testing is an important component in segment III reproductive toxicology studies in nonhuman primates. The correlation between learning, memory, and handedness was examined using the Wisconsin General Testing Apparatus. Data were collected on 8 infant cynomolgous macaques (*Macaca fascicularis*), 5 males and 3 females, approximately 200 d of age. Possible differences between genders were not investigated in this study. Animals went through 17 sessions of an adaptation phase prior to being weaned, and through 5 sessions after weaning. Following adaptation, the animals were examined for handedness over 10 sessions. An animal was considered to be right-handed or left-handed based on the observation of the animal using either the right or the left hand during more than 70% of the trials. Three of the subjects were determined to be right-handed animals and 2 were left-handed; the remaining animals were classified as ambidextrous. Following the handedness evaluations the animals were progressed to a 30-session learning and memory phase. This began with a testing phase (20 trials per session) that evaluated how many days it took an animal to associate an object with a reward. The memory phase examined the number of days to associate a second object with the reward. In general, weanlings that exhibited right hand dominance completed the learning phase in the least amount of days and completed the most number of reversals. The results suggest that the rate of learning might correspond to handedness.

P269 In Vivo Characterization of Ki-1/57 Protein

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Ki-1/57 protein was first discovered in Hodgkin cell lymphoma and has been shown to be involved with protein expression control by mRNA presplicing events. Although many molecular and biochemical studies have been carried out in vitro, the function of the Ki-1/57 protein continues to be unclear. Therefore, we carried out the characterization of the Ki-1/57 protein in vivo using C57BL/6 mice and analyzing the localization of this protein in different tissues. Seven to 8-wk-old, SPF C57BL/6 mice were used. The protocols were evaluated and approved by our Animal Ethics Committee, and international guidelines for the use and care of research animals were followed. We extracted the kidney, lung, brain, colon, thin intestine, thymus, spleen, skeletal muscle, liver, and heart for morphologic analysis. They were fixed and processed for cuts in paraffin and submitted to immunohistochemistry. Ki-1/57 appeared in specific locations in some tissues. Moreover, for isoform studies of the Ki-1/57 protein in the different organs, Western blot was performed by eletroforese in SDS-PAGE, which appeared in nitrocellulose membranes by immunoblotting with Ki-1/57-specific antibody (A26). It was possible for us to see that the Ki-1/57 presence, although widely distributed in tissues in a generalized manner, had a specific location in different cellular types found in many organs, such as the kidney, thin intestine, colon, spleen, and brain. Furthermore, 2 different isoforms had been verified in these organs, and their presence determined to be independent of each other. This cell-specific location and the presence of different isoforms of this protein suggested an organ-specific function for Ki-1/57. The determination of the Ki-1/57 function is related directly with the function exerted by the cellular type in its organs of origin, whose analyses need further studies. This also shows the importance of an in vivo analysis when collecting data to determine the function of a protein that is still unknown, even after in vitro molecular and biochemical analyses indicates its function in a generalized way.

P270 Long-Term Maintenance of Chronically Catheterized Rats Used in Standard Self-Administration Study Designs

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Intravenous self-administration is a regulatory required operant conditioning assay used to predict the reinforcing properties of new chemical entities prior to drug approval by the United States Food and Drug Administration. Animals are trained to lever press to receive infusions of a standard drug of abuse. Vehicle and various doses of a new compound are usually tested against baseline patterns of responding for the known drug of abuse. The duration of these studies usually approximate a minimum of 3 mo with animals self-administering drug 5 to 7 times a week. Catheter patency issues have always been the chief experimental confound in these study designs. Over the last 4 y, numerous changes were instituted to all aspects involved in the catheterization and maintenance of catheterized animals including 1) the surgical procedure, 2) design of and material used in the catheters, 3) concentrations of lock solutions, 4) catheter maintenance schedules, and 5) the incision/exteriorization site care. The current standard of care provides for greater than 80% (108 of 126) of animals patent for more than 90 d, 52% (26 of 50) patent greater than 180 d, and 28% (14 of 50) patent greater than 260 d. Out of 126 instrumented animals summarized for this report, the average patency life of the catheters was 114 d. In compliance with the 3Rs for the use of animals in research, the sample size needed to complete a standard self-administration study has been significantly decreased by the current standard of care. Such operational and study design changes result in a total reduction in cost and, more importantly, results in a reduction in total number of animals used and increases the general health of experimental subjects while on study.

P271 *Helicobacter bilis* Monoassociation in Gnotobiotic Swiss

Mice Stimulates B Cell Hyperplasia and Cross-Reactive Immune Responses to Altered Schaedler Flora

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We reported that *Helicobacter bilis* infection of 42 germfree (GF) Swiss Webster mice for 3 to 12 mo caused concurrent mild, multifocal typhlocolitis that correlated with peak *H. bilis* colonization levels at the cecal-colic junction. Compared with 39 germfree control mice, *H. bilis* infection induced robust IgG and IgA responses to *H. bilis* accompanied by dramatic hypertrophy of gut-associated lymphoid tissues (GALT). To characterize the immune response to *H. bilis* in the absence of other microbiota, cecal-colic tissue obtained from 3 GF and 3 *H. bilis*-infected mice at 9 mo postinfection were stained immunohistochemically for CD3 (pan T cell), B220 (pan B cell), F4/80 (macrophage), and Ki67 (cell proliferation). Positively stained cells were counted for each marker in 5 fields of well-defined lymphoid follicles and 10 intestinal crypts. Sera from 3 GF controls, 6 *H. bilis*-infected mice, and 5 barrier-maintained SPF mice were screened by ELISA for IgG reactivity to *H. bilis*, 4 members of ASF and as a control, *H. trogontum*, a *Helicobacter* isolated from rats and mice. Compared with GF, *H. bilis*-infected mice had more prominent B cell staining in GALT that was also higher in Ki67 staining, and a lower level of T cell staining, thus resulting in a higher B:T cell ratio. GF and *H. bilis*-infected mice had minimal macrophages in lymphoid follicles but Ki67 staining was higher in GALT of *H. bilis* infected mice. Similarly, Ki67 staining of intestinal glandular epithelial cells was higher in *H. bilis*-infected mice compared with GF. *H. bilis*-seropositive mice had IgG cross-reactivity with *H. trogontum* and ASF antigens, consistent with literature demonstrating *H. bilis* may cause seroconversion to other microbiota and contribute to inflammation. This GF Swiss mouse model will allow for further investigation of the mechanism responsible for cross-reactive immune responses initiated by *H. bilis* and may support *H. bilis* infection as a confounder of colitis models in research mice.

P272 Development and Validation of a Chronic Cisterna Magna Catheterization Model in Canine

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Analysis of cerebrospinal fluid (CSF) supports biomedical research to determine the ability of agents to cross the blood-brain barrier. There are a number of published methods for serial CSF collection, but the fact remains that model robustness is unpredictable due to variable catheter patency rates. We have developed and refined this model over a 12-mo period in the anesthesia and analgesia regimen with a fentanyl constant-rate intravenous infusion and in patient positioning without need for a stereotaxic device. The surgery consisted of a routine approach to the cisterna magna and small opening created through the occipital membrane. A custom-made catheter was inserted, directed rostrally, and secured with preplaced mattress sutures. Eleven dogs have been instrumented within in a 7-mo period and postoperative clinical signs were limited to minor neck stiffness in one dog, and superficial incisional complications in a minority of animals. CSF was collected weekly for 4 wk in the postoperative period to analyze cells and protein levels. Protein levels in week 1 averaged 35.1 mg/dL and in week 2, 39.4 mg/dL. Animals were transferred for scientific use at week 2 to 3 when protein levels normalized (<25 mg/dL). In 11 animals, 66% were successfully transferred. At week 4 to 5, 73% remained patent. At week 8, 12, and 26, 27% were patent. Animals were sent to necropsy and histopathology for evaluation and to determine cause of patency loss. Pathologic findings for 2 of the nonfunctional animals showed the catheter adhered to the meninges. In conclusion, our surgical technique and refinements have enabled us to successfully create and validate a chronic canine CSF access model. In addition, this model is time limited and should be used for experimental purposes as soon as possible due to the unpredictability and variability of patency rates and catheter tip occlusion.

P273 Variability in Time Course of Diabetes Development in Zucker Diabetic Fatty Rats

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Zucker diabetic fatty (ZDF) rats display progressive hyperglycemia, pancreatic failure, and are fully diabetic by 12 wk of age on average. However, there is variability in the time course of development of diabetes in the ZDF rat. Our aim was to investigate the average age at which ZDF rat becomes diabetic and to determine how the average values are influenced by variability in the development of diabetes. Male ZDF rats (n = 169) were studied between 2001 through 2010. Rats were received at 5 wk of age and studied from 6 to 12 wk of age. Rats had ad libitum access to rat chow and water. Blood glucose and plasma insulin were measured weekly after lights-on in the nonfasted state; data are presented as mean ± SEM. On average, the onset of hyperglycemia was between 7 and 8 wk of age, when mean blood glucose changed by 113 mg/dL (from 157 ± 3.1 mg/dL to 270 ± 8.8 mg/dL). However, only 37% of the rats developed hyperglycemia at 7 wk, while 2%, 41%, 5%, and 4% developed hyperglycemia at 6, 8, 9, and 10 wk, respectively. Also, 10% never developed hyperglycemia. On average, plasma insulin concentration peaked at 8 wk of age and declined from 4456 ± 216 pM to 3617 ± 202 pM at 9 wk. However, only 44% of rats showed a peak in plasma insulin at 8 wk of age, while 4%, 24%, 11%, and 3% peaked at 7, 9, 10, and 11 wk of age, respectively. Additionally, 14% never showed a peak in plasma insulin. These average data are consistent with the published literature. Documented variability in development of hyperglycemia and hyperinsulinemia can be accounted for in future studies, allowing modification of experimental design and/or analysis to use this valuable model of type 2 diabetes more productively.

P274 Topical Fungal Treatment of Northern Fowl Mite (*Ornithonyssus sylviarum*) in Poultry (*Gallus gallus domesticus*)

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Treatment of northern fowl mite (*Ornithonyssus sylviarum*) infestation in poultry in research facilities can be challenging. The mite has an exceedingly fast reproductive cycle (egg to adult in 5 to 7 d), and synthetic chemical treatments can be toxic to birds, applicators, and the environment. Such treatment may also potentially interfere with experimental research designs. This study aimed to evaluate the efficacy of topical application of an entomopathogenic fungus, *Beauveria bassiana*, in the treatment of a naturally occurring infestation of northern fowl mites in pen-housed roosters (n = 14; age, 18 mo). Two groups of 7 roosters each were used in 2 experiments: *Beauveria* (30 mL, 2.9×10^{10} spores per bird) compared with water (30 mL, control), and *Beauveria* (30 mL, 2.9×10^{10} spores per bird) compared with another common topical treatment, an insecticide (30 mL, tetrachlorvinphos/dichlorvos). A third experiment piloted a higher dose of *Beauveria* (300 mL, 2.9×10^{10} spores per bird) in the 7 birds unexposed to the insecticide. In the first 2 experiments, the fungus significantly reduced mite levels relative to a control group ($P < 0.05$), but failed to outperform RAVAP when used at an equal volume and frequency. The third experiment demonstrated that increasing the volume and frequency of *Beauveria* application improved outcomes to the point of complete elimination of the mite on the bird. The results presented here suggest that this fungus is effective at reducing or even eliminating the northern fowl mite on poultry, and can constitute an important part of an integrated pest management program if applied in sufficient doses. Further research is needed to document the most effective dose and frequency of application of *Beauveria bassiana* to control the northern fowl mite in poultry.

P275 A Comparison of Metabolic Characteristics among C57BL/6NTac, C57BL/6J, and C57BL/6JBom Diet-Induced Obese Mice with Environmental Conditioning

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The C57BL/6 mice are extensively used in research on metabolism and obesity for several reasons, not least of which is the susceptibility of this strain to obesity, hyperglycemia, and insulin resistance when fed a high fat diet (HFD), the diet-induced obese (DIO) mouse model. Since there are multiple substrains of C57BL/6 mice that have

identified genetic differences, we undertook a phenotypic analysis of metabolic characteristics in 3 substrains of C57BL/6: C57BL/6NTac, C57BL/6J and C57BL/6JBom on a regular, low-fat diet and a HFD. Environment can have a significant influence on feeding and metabolic activity, so we further subdivided the strains fed a HFD into 2 conditioning groups, those fed a HFD beginning at 6 wk of age at one of 2 commercial facilities: facility A (FA) or facility B (FB) and those fed a HFD beginning at 6 wk of age at the same facility where the testing occurred, facility C (FC). In general, the DIO mice from the B6NTac substrain were heavier than the B6J substrain at matched ages and conditioning sites. Additionally, the DIO strains that were fed the HFD at FC were heavier than the strains that were fed a HFD at the respective commercial suppliers (FA or FB), though the relative differences in body weight between the B6NTac and B6J substrains were consistent, regardless of the HFD conditioning location. The B6JBom substrain, which is not commercially available as a DIO model, was similar in weight to the B6J when both were conditioned to the HFD at FC. There was no difference in body weights among the B6NTac, B6J, and B6JBom on the regular diet. Important differences in glucohomeostasis were noted among the different DIO substrains. The 2 DIO strains from FA and FB were similar in glucose levels during an OGTT assay at 18 wk of age, but insulin levels were much higher in the FA mice, consistent with increased insulin content in the pancreas. Insulin resistance was greatest in both B6NTac DIO groups (conditioned at FA and FC) when tested in an insulin tolerance test at 21 wk of age. In summary, it appeared that the B6NTac substrain develops obesity and insulin resistance to a greater extent than the diet- and age-matched B6J and B6JBom mice.

P276 An Evaluation of 2 Methods for Serial Collection of Cerebrospinal Fluid in the Conscious Cynomolgus Monkey

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Serial cerebral spinal fluid (CSF) collection is necessary for studies involving test materials that are active in the central nervous system (CNS). Single-point exposures can be confirmed by an intrathecal puncture but this does not provide a time profile of exposure and must be conducted in anesthetized animals. The purpose of this study was to evaluate 2 models for placing an indwelling catheter in the lumbar intrathecal space to collect serial CSF samples from unanesthetized cynomolgus monkeys. The 2 models evaluated (n = 6 per model) were: 1) a surgically implanted intrathecal catheter via a hemilaminectomy of the L5 vertebra and 2) a percutaneously implanted intrathecal catheter through the L5 to L6 vertebral space using a "through the needle" catheterization technique. Animals receiving the hemilaminectomy were maintained for 6 wk with 2 serial collection phases at weeks 2 and 5. The percutaneously catheterized animals were maintained for approximately 96 h before removal of the catheter in phase I. A second percutaneous implantation was performed after a 2-wk holiday for the phase II collections. Serial CSF collections were attempted at least 8 times per animal over the 3 d of each phase. No clinical signs or changes in body weight or body temperature were associated with either method. Fluorographic confirmation of catheter tip placement indicated no difference in patency of catheter tips placed between L1 and T11. Clinical and anatomic findings indicated more significant changes in the percutaneous model. The hemilaminectomy model demonstrated a 50% success for bidirectional patency at all collection timepoints. The percutaneous model demonstrated a 50% and 96% success for bidirectional patency on phase I and phases II, respectively. In conclusion, the 2 methods evaluated here for serial collection of CSF from conscious, restrained primates are feasible for the serial collection of CSF for the determination of CNS exposure.

P277 UVB Exposure and Topical Estrogen Effects on the Development of Skin Cancer in a Pre- and Postmenopausal Mouse Model

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Topical or systemic estrogen and its effects on the skin have been studied in postmenopausal women, but data is lacking concerning usage of topical estrogenic compounds by younger, pre-menopausal women. It is hypothesized that topical estrogen application to previously UVB exposed skin accelerates skin carcinogenesis. The specifics aims of this study are to determine the effects of topical estrogen on ultraviolet light-B (UVB) induced skin tumor development and progression using Skh-1 hairless mice. Seventy female mice were divided into 2 groups and one group was irradiated 3 times weekly with 2240 J/m^2 for 10 wk to model human UVB exposure from childhood through early adulthood. Mice then received either ovariectomy (postmenopausal) or sham surgery (premenopausal) and were treated topically with 10 nmol 17β-estradiol or vehicle control 3 times weekly for 15 wk with no further UVB irradiation. Tumor numbers and size were measured weekly during the 15-wk treatment period. Neither unirradiated mice receiving topical estrogen or vehicle control developed tumors during the course of the study. Topical treatment with estrogen following 10 wk of UVB exposure in intact mice induced an increased tumor burden compared with UVB exposed mice receiving vehicle control. Ovariectomized mice had increased tumor burden regardless of topical treatments when compared with UV exposed intact mice. Studies are ongoing to determine potential mechanisms behind these observations. These data indicate that the application of exogenous estrogen to previously UVB-exposed skin can potentially initiate an increase in skin tumor development in premenopausal women. These findings have negative implications for the use of lotions and creams containing estrogenic compounds on sun-exposed sites by young women.

P278 Experimental Infection of *Helicobacter pullorum* in B6.129P2-IL-10^{tm1Cgn} Mice

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Helicobacter pullorum, an enterohepatic *Helicobacter*, is associated with gastroenteritis and hepatobiliary disease in humans and chickens and infection in barrier-maintained BN/MolTac rats and C57BL/6NTac and C3H/HeNTac mice has been described. For this study, infection of B6.129P2-IL-10^{tm1Cgn} (IL10$^{-/-}$) mice with *H. pullorum* was evaluated as a model for inflammatory bowel disease (IBD) as reported for other *Helicobacter*s isolated from humans and rodents. Twenty *Helicobacter*-free IL10$^{-/-}$ mice were orogastrically gavaged with 2×10^8 CFU of *H. pullorum* in *Brucella* broth every other day for 3 doses, while 12 *Helicobacter*-free controls were sham dosed with *Brucella* broth. Infection status was monitored every 2 to 3 wk by fecal PCR using *H. pullorum* cytolethal distending toxin B-specific (*cdtB*) primers and a *H. pullorum*-specific ELISA for serum IgG over 12 wk. Pooled fecal samples by cage were *H. pullorum* PCR positive by 2 wk postinfection (WPI). At necropsy 4 to 6 WPI, 5 of 10 mice had lost body condition, 4 of 10 developed rectal prolapse, 7 of 10 had seroconverted, and all 10 mice were PCR positive for *H. pullorum* (10 of 10 feces, 9 of 10 cecal, and 7 of 10 colonic samples). By 12 WPI, only 1 of 10 mice necropsied had lost body condition yet persistent infection with *H. pullorum* remained evident by PCR (10 of 10 feces, 8 of 10 cecal, and 10 of 10 colonic samples) and by seroconversion in 8 of 10 *H. pullorum* infected mice. Compared with *Helicobacter*-free control IL10$^{-/-}$ mice, there was a trend for clinical signs and typhlocolitis lesion scores in *H. pullorum*-infected IL10$^{-/-}$ mice to be most severe at 6 WPI with attenuation by 12 WPI, particularly as spontaneous inflammation progressively developed in control IL-10$^{-/-}$ mice. Although overall lesions and morbidity were less severe with *H. pullorum*, the acute nature of infection is consistent with the previously reported model of *H. trogontum*-associated IBD in IL10$^{-/-}$ mice. Because *H. pullorum* infects humans, the IL10$^{-/-}$ mouse model offers promise in identifying which host determinants may impact acute or more chronic, attenuated inflammatory responses to this emerging pathogen.

P279 Automated Blood Sampling Machine as a Powerful Tool for Studying Mechanisms of Insulin Resistance in Conscious Mice

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In research, it is often desirable to obtain pharmacological profiles from small animals, such as the mouse, where the blood sample size is limited by the size of the animal. The very low waste volume of a new automated blood sampling machine (ABSM) enables multiple sampling in conscious mice, and makes it possible to perform an intravenous glucose tolerance test (IVGTT) from a single animal, reducing the number of animals used and improving the quality of the dataset. Our aim was to characterize the high-fat fed mouse as a model for studying insulin resistance and to further evaluate the ABSM. Twenty male C57BL/J6 mice were fed a high-fat (60% fat) or a normal diet (11% fat) for 4 to 6 wk before interventing the IVGTT. One week before the IVGTT mice were fitted with a permanent

catheter in the right jugular vein and given 1 wk of recovery before the experiment. On the test day, mice and catheter were connected to the ABSM and fasted 4 h before the IVGTT. Blood samples for determination of plasma blood glucose (PGB) and plasma insulin (PI) were collected from the jugular vein, at times −5, 1, 6, 14, 20, 30, and 50 min by automated sampling. At t = 0 mice were administered an intravenous bolus of glucose (1 g/kg) through the tail vein. PBG was determined using the glucose oxidase method and plasma insulin was determined using ELISA method. Data are expressed as mean ± SEM. We observed an increased acute insulin response in mice fed a high-fat diet with peak insulin = 455.0 ± 71.8 pM, as compared with mice fed a normal diet, peak insulin = 206.2 ± 30.3 pM at time point t = 1 min after an intravenous glucose bolus. There were no significant differences in the insulin response at later time points and the glucose profiles were similar in the 2 groups. These results show that it is possible to study the acute insulin response in conscious mice after an IVGTT. ABSM is a qualified method to study insulin resistance and generate pharmacological profiles in conscious mice. The ABSM makes it possible to obtain high quality samples because of less animal handling, multiple sampling in each individual, low waste volume, and a consistent sampling technique at all time points.

P280 Porcine Plasma as a Viable Option in Clinical Chemistry Analysis

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As serum is the most popular medium submitted for clinical chemistry analysis, the objective of the study is to determine whether pig plasma may be confidently used to test for analytes in blood and which tests may preclude using plasma over serum. The Phenotyping Core Lab at our institution frequently receives porcine serum with requests for complete chemistry panels. Seldom does the lab receive plasma, although convenience would lend itself for its frequency. More often than not when chemistry panels are requested, CBC/differentials are also requested. When chemistry analysis is desired, curiously, investigators prefer to work with serum rather plasma where they may not need to be so discriminating. In extracting blood and ejecting it into one tube rather than 2 tubes (serum separator and anticoagulating), primary investigators will not only reduce overall time, but they will find the process cost effective; limiting the use of unnecessary tubes. By just using one anticoagulating tube, both an accurate CBC/differential can be taken and a chemistry panel may be performed. More than 15 animals were used in our study. Hemolysis, as a variable, was avoided in both serum and plasma. For the following clinical chemistry tests we found great similarity (> 92%) and no significant difference ($P > 0.15$): cholesterol, triglyceride, CK, ALT, AST, amylase, LDH, ALP, TBili, glucose, TPO, BUN, creatinine, ALB, Na, Cl, HDL, and GGT. Potassium was eliminated as a test considering that some anticoagulating tubes contain K2 EDTA. Only calcium and ALP did we find low similarity (< 16.5%) and significant differences ($P < 0.05$). Therefore, both porcine plasma and serum may be confidently used to determine the analytes of blood. For consistency, it is recommended that investigators use either plasma or serum but not both.

P281 Assessment of QTc Reference Values in Cynomolgus Monkeys with Arrhythmia

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The *Guideline of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH) describes the importance of animal models including monkeys for nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) induced by human pharmaceuticals. As monkeys and humans show similar ionic mechanisms of repolarization, differing from those of laboratory mice, interest in using monkeys for pharmaceutical studies has recently increased. In a previous study, we clarified signs of arrhythmia such as long QT syndrome in a breeding colony of cynomolgus monkeys (*Macaca fascicularis*). The present study investigated electrocardiographic reference values of QTc as a marker of arrhythmia in cynomolgus monkeys. We measured electrocardiographic data from 353 monkeys (191 females, 162 males) including aged animals. The QT, RR intervals and formula corrected for cardiac rate were calculated from the electrocardiographic data. QTc values were calculated using the formula $[QTc] = [QT]/[RR]^n$, and the reference value of QTc was determined from the frequency distribution. The exponent of the QT interval corrected for heart rate was 0.576, similar to the value from Bazett formula. Mean ± SD QTc determined from $[QTc] = [QT]/[RR]^{0.576}$ was 373 ± 31, with no significant difference between males and females. The frequency distribution of QTc suggested that a reference QTc value could be established that could serve as a marker of QT interval prolongation in cynomolgus monkeys. The frequency distribution indicated QTc prolongation in 50 monkeys with underlying diseases such as long QT syndrome, heart failure and diabetes. In conclusion, reference value of QTc appears to be useful as a marker of arrhythmias in cynomolgus monkeys. Establishing models of arrhythmia in nonhuman primates might be particularly useful for understanding biologic aspects of cardiology.

P282 Further Characterization of CD1-pcy/pcy Mice: Evidence for Polyuria and Glucosuria

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The most clinically significant type of polycystic kidney disease (PKD) in people is inherited as an autosomal dominant trait (ADPKD) and is the most common inherited nephropathy. A form of polycystic kidney disease has been described in CD1-pcy/pcy mice comparable to human ADPKD in renal cyst localization and slow disease progression. In a preliminary study of the progression of PKD in CD1-pcy/pcy mice, urine was collected from 5 males and 5 females over 24 h in metabolic cages at 32 and 35 wk of age; in addition to urinalyses, necropsies were performed at 35 wk of age. At 32 wk no males had glucosuria compared with 3 of 5 females with quantitative urine glucose between 130 to 186 mg/dL (mean control urine glucose was 40 mg/dL). At 32 wk the average urinary output for males was 2.55 mL with specific gravity ranging from 1.021 to more than 1.035, compared with 4.7 mL in females with specific gravity ranging from 1.016 to 1.020. Again at 35 wk, no males had glucosuria compared with 3 of 4 females that had quantitative urine glucose measuring between 217 to 251 mg/dL. The serum glucose values of mice at 35 wk were within reference range, using normal values for Crl:CD1 (ICR)BR mice at 32 to 34 wk. Based on these preliminary findings, we hypothesize that pcy mice, though not hyperglycemic, develop polyuria and glucosuria. Histologic evaluation of kidneys at 35 wk of age showed cystic dilated distal tubules, with flattened epithelium, containing eosinophilic, proteinaceous material. Along with dilation of the renal pelvis, the glomeruli were shrunken with variably dilated and often cystic spaces within the Bowman capsule. To our knowledge this is the first report of glucosuria developing concurrently with progressive renal lesions in this mouse model of PKD.

P283 Intrapericardial Delivery of Visible Microcapsules Guided by X-Ray Fused Magnetic Resonance Imaging

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Stem cells delivered to the pericardial space may offer a minimally invasive approach with diminished concerns for conduction or embolic events associated with conventional delivery methods. X-ray fused with magnetic resonance imaging (XFM) may be useful for guiding intrapericardial access and providing improved soft tissue detail over fluoroscopy. Microencapsulation of stem cells may offer an additional method to avoid rejection and enhance cell survival. In this study, we assess the use of novel X-ray-visible microencapsulated stem cells for intrapericardial delivery using XFM. To assess feasibility of intrapericardial delivery, barium-alginate microcapsules (7 to 10 cc) were infused into the pericardial space in 4 female Yorkshire pigs (40 to 50 lb) with normal cardiac function and pericardial integrity. Further, 4 pigs were studied to determine short-term outcomes of an intrapericardial approach for delivery of XCaps; animals were

randomized to receive XCaps, naked human mesenchymal stem cells or saline. Echocardiograms and whole heart cardiac MRI were acquired prior to and after intrapericardial delivery. For XFM, a cardiac-gated c-arm CT was obtained and fused with MRI endocardial and epicardial segmentation and overlaid on live fluoroscopy to guide percutaneous access to the pericardial space by an experienced interventional cardiologist (PVJ). Echocardiograms and cine-MRI images were analyzed to assess cardiovascular function and pericardial integrity; C-arm CT was used to assess visibility of XCaps. Upon euthanasia, the heart and XCaps were harvested for histopathology. XCaps were easily visualized via c-arm CT immediately and at 7 d after delivery. Echocardiograms and MRI confirmed the lack of pericardial adhesions and effusion as well as preserved global function at 1 wk after delivery using XFM. Pericardial adhesions were prominent in one animal without XFM delivery. XCap integrity was preserved with consolidation into a tissue-like patch on the epicardial surface in all animals with no histologic foreign body response. XFM-guided intrapericardial delivery of XCaps offers a potentially new route for safe delivery of cellular therapeutics that can be tracked using clinical angiographic systems.

P284 Effective Decapsulation of Artemia Cyst

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Artemia (brine shrimp) has been found to be a suitable food for many diverse groups of organisms in the animal kingdom. *Artemia* cysts (eggs) are metabolically inactive and can remain in total stasis for 2 y while in dry, oxygen-free conditions, even at temperatures below freezing. Once decapsulated and placed in an appropriate hatching environment, the cysts hatch within a few hours. Decapsulation is the removal of the outer layer *Artemia* cyst shells. This procedure can be performed on the cysts using a short term exposure to hypochlorite solution. In many protocols the amount of bleach used for decapsulation is less than 100% effective, which negatively affects the hatching efficiency and hatching synchrony. At present, the amount of beach used for decapsulation is not optimized. As a result, the hatching efficiency and hatching synchrony are low. The objective of the study was to determine the most effective decapsulation procedure and increase hatching efficiency of brine cysts. In this experiment, 3 different decapsulation methods were tested on *Artemia* cysts from the lakes near San Francisco Bay. Method 1 used 1.5 L of house hold chlorine bleach (a solution of approximately 3% to 6% NaClO) and 45 g NaOH for about 20 min. Method 2 used 2 consecutive decapsulation steps; first using 1.5 L of NaClO and 45 g of NaOH for about 20 min, followed by 0.75 L bleach and 22.5 g NaOH until the cyst color changes to orange. Method 3 used 2.8 L bleach and 44 g NaOH for approximately 3 to 5 min. The hydration, dechlorinating, and hatching steps are the same for all 3 methods. One bag of cysts (450 g) was hydrated in 2 L of reverse osmosis (RO) water and then mixed using a stir plate for 1 h, prior to decapsulation with one of the 3 methods described above. The brine was drained into a large screen and rinsed with RO water for approximately 2 min. Then dechlorination was performed with 45 g of $Na_2S_2O_3$ in 2 L of RO water for about 10 min. The result of the experiment revealed that method 3 resulted in a significantly higher hatching efficiency than methods 1 and 2. The decapsulation time is also significantly reduced using method 3.

P285 Increase of Calreticulin in Hearts of Cardiomyopathic Mice with Sialyltransferase Transgenes

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It is very important to clarify the etiology of cardiomyopathy since cardiomyopathy is a serious heart disease with various causes. Calreticulin is a multifunctional protein involved in the Ca^{2+} storage and the endoplasmic/sarcoplasmic reticulum (ER/SR) quality control mechanism. We examined a role of calreticulin in cardiomyopathy-like symptoms in a transgenic mouse line (4C30 strain) with sialyltransferase overexpression, a possible mouse model of cardiomyopathy. In addition, a possible involvement of calreticulin was also examined with a Syrian hamster model (J2N strain) of cardiomyopathy with delta-sarcoglycan deficiency. Soluble fractions of heart proteins were extracted from hearts of 10-wk-old 4C30 mice and their normal control C57BL/6NCr (B6Cr), and from left ventricles of cardiomyopathic Syrian hamsters (J2N-k) and their normal control (J2N-n). Using quantitative Western blot, amounts of calreticulin in the soluble protein fractions were obtained as calreticulin/GAPDH ratios. Calreticulin/GAPDH ratios in 4C30 (mean ± SD: 0.248 ± 0.063) were significantly higher than in B6Cr (0.025 ± 0.007, *t* test, $P < 0.05$, $n = 3$). In contrast, no significant difference was found between J2N-n and J2N-k hamsters ($n = 3$) at the age of 4 wk (1.620 ± 0.277 compared with 1.073 ± 0.412), or 4 mo (0.768 ± 0.037 compared with 0.556 ± 0.285). Increase of calreticulin was found only in 4C30, suggesting that altered protein glycosylation by sialyltransferase overexpression might activate the ER/SR quality control mechanism. Increased calreticulin may adversely affect Ca^{2+} metabolism, which may lead to the malfunction of the heart contraction machinery and result in cardiomyopathy-like symptoms in 4C30. Our results also indicate that the change in calreticulin amounts is not always accompanied with cardiomyopathy.

P286 Effect of Corncob Compared with Aspen Chip Bedding on Rat EEG and Pain Models

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Nonexperimental variables can impact animal studies. We switched from aspen chip to corncob for its absorbency. Subsequently, rats in EEG studies were sleeping less during light cycle recordings. Sleep reduction can negatively impact the validity of EEG drug studies. We conducted a study to determine whether the corncob bedding was the cause of reduced sleep. Male SD rats were implanted with electrodes over the frontal and parietal cortices with a reference electrode implanted over the cerebellum. The rats were maintained on a normal 12:12-h light:dark cycle (lights on at 0600). Quantitative analysis of the EEG was performed using fast Fourier transform (FFT). Prior to the study, rats ($n = 15$) were on corncob bedding in both home cages and recording chambers for 5 wk, thus, were habituated to the bedding. Only the EEG chamber bedding was changed during the study. All rats were recorded on both bedding types on different days over the course of the experiment. Results showed that corncob bedding resulted in a significant decrease in the amount of time spent in slow-wave sleep during hours 0900 and 1000 as compared with aspen chip ($P < 0.001$ and $P < 0.01$, respectively; Bonferroni post hoc test). A 2-way ANOVA showed no effect of time ($P = 0.2173$), with a significant effect of treatment ($P < 0.0001$) and interaction ($P = 0.0275$). In addition, altered mechanical sensitivities were seen in rat models of inflammatory and neuropathic pain when rats were housed on corncob bedding compared with aspen chip. Additional overt pain behaviors were observed in corncob housed rats including protection (lifting) of the injured paw, pushing the bedding aside and a preference for areas free of bedding. These findings support other published literature suggesting that rats prefer wood chip to corncob bedding. The IACUC requested that all corncob bedding be removed from rodent cages based on this data.

P287 Reporting and Use of Laboratory Animal Anesthetics and Analgesics

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Use of anesthetics and analgesics for research animal surgeries is an animal welfare concern, as well as an important methodological variable. A literature review was performed to assess published use of analgesics and anesthetics in laboratory animals undergoing surgical procedures. Five models that require survival surgery were chosen for review: laminectomy in mice; orthopedic studies in dogs; craniotomy for cell-injection in mice; craniotomy for instrumentation for single-cell recording in macaques; and thoracotomy for myocardial infarction induction in swine. The PubMed database was used to identify 25 recent research reports in each of these models. Reported choice of anesthetic and analgesic were noted for each paper. Papers were scored for use of anesthetic regimens with analgesic components intraoperatively, as well as for use of multimodal analgesia postoperatively. Overall, 21% of the 125 papers made no mention of anesthesia. As none of these procedures could physically be performed without the restraint of general anesthesia, this finding clearly reflects publication practices, and cannot be interpreted to accurately reflect actual animal anesthetic practices. Of those 86 papers that gave detailed information on

anesthetic choice, 42% used an anesthetic regimen with intraoperative analgesic activity. Thirty-six papers (29%) stated that analgesics were used, and 28 of these detailed their analgesic choice, with 2 of those documenting multimodal postoperative analgesia. Sixty-four papers (51%) documented animal anesthesia without mention analgesia; whether this reflects a publication bias against full reporting of analgesic use, or truly represents analgesic nonuse in the laboratory could not be determined; both possibilities raise concern. Differences in anesthetic and analgesic use by model are presented. The authors argue that better science and better animal care could result from full reporting of laboratory animal anesthesia and pain management.

P288 Refinement of an Intravenous Dosing Method Using a Temporary Indwelling Cannula

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During standard intravenous infusion toxicology studies rats are either surgically prepared via cannulation of the femoral vein for repeat dosing, or they are habituated to being restrained in a holding tube for up to 4 h and a temporary indwelling cannula inserted into the tail vein for single doses. Each of these methods has disadvantages for the animal; either being singly housed postsurgery and permanently tethered, or being restrained during dosing. Since 2008 we have used an alternative method of intravenous infusion using an indwelling catheter. This method was developed in preference to surgical cannulation for single dose intravenous whole body plethysmography studies, and has been used on over 300 rats. The technique uses a temporary indwelling cannula inserted into a tail vein and secured with tape which is then protected by an outer sheath and connected to an intravenous line via a blunt needle. The welfare benefits of this temporary method are: no surgery is required in preparation of the study, no restraint is required, and the animals can be group housed except during dosing. It has since been used on several different single-dose safety pharmacology study types successfully, for example, gastrointestinal function and Irwin studies and has also been validated for repeated dosing, weekly or twice weekly for up to 4 wk. We have now applied this technique to a maximum tolerated dose and repeat dose cycle intravenous toxicity study in the rat, with a further refinement by using a swivel. This now prevents the risk of the rat becoming entangled while it is free to roam in the infusion cage. In summary, the method and dosing regimens trialed were generally well tolerated. There were no in-life tail observations and expected pathology associated with cannulation was observed.

P289 Laser-Assisted In Vitro Fertilization for the Efficient Recovery of Genetically Modified Mouse Lines on C57BL/6 Background with Low Fertility

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Genetically modified (GM) mouse lines on C57BL/6 (B6) background are commonly used in biomedical research; however, low fertility may affect successful propagation of GM lines with the potential to negatively impact their use. Laser-assisted in vitro fertilization (LAIVF) employs the use of a laser to perforate the zona pellucida allowing easier penetration of the sperm into the oocytes thereby increasing the fertilization rate. A retrospective analysis of conventional IVF and LAIVF procedures was performed using data collected during a period of 3 y at our Division of Comparative Medicine transgenic mouse core facility. Conventional IVF and LAIVF procedures were performed for 25 and 33 GM lines on B6 background, respectively. The LAIVF procedures were performed for rederivation and rescue (85%), for rapid expansion of the line (9%), and for retrieving mice from frozen sperm (6%). Fertilization efficiency for the 2 types of IVF techniques was evaluated by calculating 2-cell (2-cells/oocytes) and live offspring (live pups per transferred embryos) rates. The 2-cell rates for conventional IVF and LAIVF were 10% and 45%, respectively, whereas the live offspring rates were 28% and 9%, respectively. Although the offspring rate was higher for conventional IVF, LAIVF was useful for obtaining 2-cells from all 33 GM lines and live pups were obtained from 32 of 33 GM lines. In contrast, 2-cells and live offspring were obtained from only 3 of the 25 GM lines by conventional IVF. Of these 3 lines, the conventional IVF and LAIVF 2-cell rates were 28% and 59%, respectively; whereas the live offspring rates were 29% and 8%, respectively. Overall, our data suggests that LAIVF is useful for the successful recovery of GM lines on B6 background with low fertility. On the other hand, when fertility is not an issue, conventional IVF may result in live offspring obviating the need for LAIVF.

P290 Production of Germfree Chimeric Mice Using Nonsurgical Embryo Transfer from Embryos Shipped Overnight in a Portable Incubator System

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Production of genetically modified chimeric murine models routinely requires the use of a surgical embryo transfer procedure. The use of nonsurgical embryo transfer is steadily gaining acceptance as an alternative method for transferring late stage embryos. In our transgenic core facilities production of chimeric mice is conducted by transferring blastocysts into restricted/defined flora recipients. Furthermore, we have the capability of generating germfree offspring by surgically transferring embryos to germfree recipients and maintaining them in gnotobiotic isolators. The objective of the study was to determine if germfree chimeric mice could be produced via nonsurgical embryo transfer procedure when implanted into germfree recipients and maintained under gnotobiotic conditions. An initial pilot (n = 3 replicates and 9 recipients) was conducted to determine if overnight shipment of stage 3.5 blastocyst embryos in a portable incubator system, by commercial courier, could result in live offspring following surgical and nonsurgical embryo transfer. Our results indicated that overnight shipment of micromanipulated blastocysts and subsequent nonsurgical embryo transfer resulted in production of live offspring similar with live born rates (28.9% to 40.4%) to those of embryos transferred surgically the day of micromanipulation. A second experiment was conducted (n = 2 replicates) to determine if micromanipulated blastocyst embryos shipped overnight, could be transferred into germfree recipients (n = 7), maintain their germfree status, and subsequent offspring maintain a specific health status following nonsurgical embryo transfer. Our results indicated that overnight shipment of ES cell-injected blastocysts and subsequent nonsurgical embryo transfer into germfree recipients resulted in production of live germfree chimeric offspring. Health test monitoring further indicated that all germfree offspring were completely free of any microbial or viral pathogens. This study indicates that nonsurgical embryo transfer is an alternative means of generating germfree chimeric offspring when housed under gnotobiotic conditions.

P291 Development of a Measles Nucleoprotein for Serological Screening of Macaques to Assess the Efficacy of Measles Vaccination

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Measles, a paramyxovirus is highly contagious and can spread through respiration. Primates bred in captivity are prone to catching measles infection when they come in contact with their human caretakers and may die in an outbreak. Therefore, it is common to vaccinate the animals in primate colonies to protect them from measles infection. Following vaccination, animals are routinely tested for measles antibody titers to assess the efficacy of vaccination. A recombinant nucleoprotein (NP) was produced using a baculovirus expression vector system and insect cells for serological screening of vaccinated macaques. The expressed protein was purified by ultracentrifugation and density gradient techniques. Purified protein was analyzed by ELISA, SDS-PAGE and Western blot. Multiplexed system assays (MSA) using purified 45kd measles NP and commercial partially purified measles viral lysate (conventional antigen) were developed to detect measles antibodies. Specificity of the measles MSA was determined using 27 sera from a superclean macaque colony. All sera were found to be completely negative thus confirming the measles NP and conventional MSA to be 100% specific. In a measles vaccination study involving 40 macaques (20 rhesus and 20 cynomolgus), sera were collected weekly post vaccination until 4 wk. These were tested by measles NP MSA, conventional MSA and IFA. The NP MSA and IFA results showed approximately 50% of the vaccinated macaques had high antibody titers by the second wee,k with numbers (and titers too) increasing to approximately 75% in the third and fourth week. In comparison, the MSA using conventional antigen performed poorly, detecting only 3% to 6% of the positives

for the duration of the experiment. However, nearly 20% of the animals never seroconverted after the first vaccination, that is, found to be negative by MSA and IFA. These animals finally seroconverted between 2 to 3 wk post booster vaccine shot. Data from this vaccination study proves that measles NP MSA is highly sensitive and specific in detecting the measles antibodies in macaque sera and is a useful tool in testing the efficacy of measles vaccination of macaque colonies.

P292 Using Recombinant Prospect Hill Virus (PHV) Nucleoprotein as an Antigen for Serological Detection of PHV Antibodies in Mice

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Prospect Hill virus (PHV) is a New World hantavirus that is not known to cause any human disease. A recombinant nucleoprotein (NP) was produced using a baculovirus expression vector system in insect cells and used for the detection of PHV antibodies in mouse serum. Infected SF+ insect cells were lysed to release the expressed PHV N-protein aggregates which were then further purified by ultracentrifugation and cesium chloride gradient. Potency of the PHV N-protein was determined by ELISA and multiplexed system assays (MSA). Purity of the purified 55kd PHV N-protein was assessed by staining of SDS-PAGE gels and Western blot (WIB) analysis. Specificity of the recombinant PHV MSA was determined by screening 995 mouse sera from historically known negative colonies. Only 2 samples gave a positive reaction by MSA which were then found to be negative by subsequent IFA and WB confirmation, that is, the specificity of the MSA using PHV N-protein is greater than 99%. PHV serology using sequentially collected sera from mice experimentally inoculated with Hantaan virus demonstrated detectable seroconversion but much later compared with the Hantaan specific recombinant antigen, day 28 compared with day 7, respectively. The average titer of antibody positive sera by PHV MSA was also much lower compared with Hantaan MSA, thus confirming minimal cross reactivity between the 2 antigens. Field trials were performed to determine the prevalence of PHV in various academic and commercial mouse populations. Only 3 borderline positive samples resulted from screening of 430 sera which were subsequently confirmed negative by IFA, that is, no true positives were found in the tested samples. Later no real positives were found among more than 17,000 mouse sera samples from various institutes tested over a period of 6 mo. Data from the field studies suggests that serologic testing by MSA using PHV N-protein as antigen is highly sensitive and specific in detecting PHV antibodies in mice sera and that PHV is nonexistent in the laboratory mice. In addition, low cross reactivity between the 2 hantavirus MSA for PHV and Hantaan emphasizes the importance of using virus-specific antigens for screening of laboratory rodents.

P293 Development of a Recombinant *Pneumocystis carinii* Protein as an Antigen for Serological Screening of Laboratory Rats

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A new recombinant protein was developed for serological screening of *Pneumocystis carinii*, previously known as rat respiratory virus (RRV) in laboratory rats. The recombinant *P. carinii* protein was produced using a baculovirus expression vector system and insect cells. The expressed protein was purified using standard purification techniques including ultracentrifugation and a density gradient. Purified recombinant protein was analyzed by ELISA, gel electrophoresis (SDS-PAGE), Western immunoblot (WIB) and multiplexed system assays (MSA). Qualified 43kD *P. carinii* recombinant protein was used as MSA antigen for serologic detection of rat *P. carinii* antibodies. *P. carinii* immunofluorescence assay (IFA) was also developed using partially purified cysts from homogenized lung tissues from *P. carinii*-infected rats. In a small prevalence study (n = 687), serum screening of rats from various academic and commercial institutions was performed by IFA. Approximately 15% were positive for *P. carinii* antibodies. Analysis of over 7500 commercial and academic laboratory rat sera showed a similar prevalence of 17% when screened by MSA. The specificity of the MSA and IFA were determined by screening 396 sera samples from historically known negative rat colonies. All samples tested negative by IFA with 4 samples scoring as low positives by MSA. These discrepant sera samples were further analyzed by WIB. The samples were found to be false positive by *P. carinii* MSA, that is, specificity of *P. carinii* MSA assay was found to be greater than 99%. Additional serological screening was performed on rats of different age groups in a *P. carinii*-positive colony. Sera from 6 weanlings (3 wk old) was positive by MSA and/or IFA but *P. carinii* negative, indicating the presence of maternal *P. carinii* antibodies. Seven-week-old animals tested negative by both MSA and IFA. However, at 10 wk old, the detection rate increased to 3 of 6 and 4 of 6 positive by MSA and IFA, respectively. All 18 tested retired breeders were found to be positive by both MSA and IFA. Results from the above studies suggests that serologic testing by MSA using a recombinant protein as an antigen is highly sensitive and specific in detecting *P. carinii* antibodies in rat sera.

P294 Methods for Continuous Subcutaneous Infusion in Rats

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Continuous subcutaneous insulin infusion is increasingly used as a means of insulin delivery for those with type 1 diabetes, but can also be used for administration of medications for certain illnesses such as Parkinson disease or cancers. Research of these drugs is often done in rats. However, infusion of certain drugs at a single subcutaneous site poses significant risk of necrotic skin lesions, as well as problems such as leakage from the administration site when fluid is infused over a long period via standard indwelling catheters. This paper describes methods for subcutaneous infusion administration that reduces the probability of leakage and dermonecrosis by dispersing fluids under a large area of skin. In a study of 9 animals, 2 methods for subcutaneous infusion were tried. In method 1, 3 animals were catheterized with a straight catheter of polyurethane construction with side exit fenestrations. The catheter was inserted through an incision between the scapulas and routed subcutaneously to the dorsal flank. In method 2, a looped polyurethane catheter design was employed. This catheter was inserted in 6 animals through an incision between the scapulas. Prior to insertion, a blunt tool was used to clear a fan shaped pocket in the dorsal subcutaneous layer above the flanks for the catheter to lie in. The catheter was then grasped in the center of the loop with an alligator forceps and advanced through the incision to the animal's flanks. The forceps was used to help spread the loop so that fluid would be delivered to the entire dorsal lumbar area. Animals were attached to a tether and harness system and infused continuously with saline at a rate of 0.1 mL/kg/h for 14 d, then at 0.3 mL/kg/h for 14 additional days. During the last 24 h, with sterile 1% aniline blue dye was infused to confirm target delivery and diffusion. Neither catheter exhibited signs of leakage at the catheter/skin interface. Results showed that both catheter designs provided for adequate delivery over 4 wk. The looped catheter design appears to be superior in delivery when fluid must be uniformly dispersed over a large area, while the straight catheter has the advantage of targeting delivery to a particular site.

P295 Use of Body Surface Temperature Obtained with an Infrared Thermometer as Early Endpoint Criteria in Orthopox Infection Studies

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Many investigations have verified the usefulness of infrared thermometry in determining body surface temperature in laboratory mice. Body surface temperature, obtained with a laser-sited infrared thermometer, is one of the criteria used by our laboratory at the Integrated Research Facility to determine if mice have reached endpoint euthanasia parameters in studies of orthopoxvirus infections. In a series of 4 experiments of cowpox or vaccinia virus infection in BALB/c mice, body surface temperature euthanasia criteria were altered, and the numbers of mice found dead were measured. Initially, as many as 60% of the mice were found dead in the first experiment, with a lower endpoint body temperature criterion. After evaluating temperature data from this experiment, investigators of the next 3 experiments raised body temperature endpoint criterium to 28 °C. The percentage of mice found dead was reduced to 16%, 11%, and 15%, respectively. Raising the body surface temperature in these infectious models to meet an earlier endpoint criterion can reduce the number of animals required for a study and potential for distress in the end stages of orthopoxvirus disease.

P296 Confirming Pregnancy via Ultrasound Imaging Significantly Reduces the Number of Animals Required to Provide Time Pregnant Genetically Altered Mice for Studies

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In mice, studies that involve the harvest of embryonic tissue at specific developmental time points requires the generation of timed-pregnant females. The traditional way to generate time-pregnant females is to check for a copulatory plug in the morning as evidence that mating has occurred. Due to variability in male fertility, approximately 40% of the females observed to have a plug are found not to pregnant at the time of the experiment. This created a major impact to our collaborators' experimental timelines and wasted difficult to obtain reagents including the complex mutant mice that we provide for these studies. To address this problem, a high throughput procedure for imaging plugged females via ultrasound with E dates of 7.5 to 12.5 d was developed. The process involved anesthetizing the animals with isoflurane, chemical removal of abdominal fur, imaging the animals on a heated stage, and monitoring for recovery from anesthesia. We found that an experienced technician can accomplish an imaging session in 6 min with a high degree of accuracy. In addition, the implementation of ultrasound imaging to confirm pregnancy significantly reduced the number of animals used due to the transfer of nonpregnant females and provides a more reliable value added service.

P297 Efficacy of N-Acetyl-L-Cysteine for Treatment of Neurodegeneration in Neimann–Pick C1-Deficient Mice

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Niemann–Pick Type C (NPC1) disease is a neurodegenerative cholesterol storage disorder that is typically fatal by late adolescence. A mutation in the NPC1 gene accounts for 95% of all cases. NPC1-deficient mice faithfully recapitulate the human disease, in particular the cholesterol accumulation and oxidative stress phenotype, and the patterned loss of Purkinje neurons in the cerebellum. The goal of our study was to test the hypothesis that mitigation of oxidative stress will provide neuroprotection and delay the neurodegeneration in the NPC1 mouse model. For this study, mice were treated with N-acetyl-L-cysteine (NAC), an antioxidant that readily enters the central nervous system and in cells serves as a precursor for glutathione, an endogenous antioxidant. NPC1 and WT littermates were treated with either 125 or 250 mg/kg/d NAC provided ad libitum in their drinking water. Treatment was initiated either at week 4 or 6. The mice (n = 8 males per group) were monitored for weekly weights, neurologic function by assessing severity of tremor, and survival. NAC-treated NPC1 mice exhibited increased weight gain as compared with untreated NPC1 mice. The 250 mg/kg/d 4-wk group showed a 15% increase in weight at 10 wk ($P < 0.05$). Significant reductions in tremor amplitude were observed at 11 Hz in the group initiated on 250 mg/kg/d at 4 wk (59% at 9 wk, $P < 0.05$) and 125 mg/kg/d at 6 wk (70% at 9 wk and 53% at 10 wk, $P < 0.05$). Mean survival was significantly increased in the 250 mg/kg/d 4-wk group (8.4%, $P < 0.001$), the 125 mg/kg/d 4-wk group (6.5%, $P < 0.005$) and the 125 mg/kg/d 6-wk group (5.7%, $P < 0.02$). NAC led to reduction of oxidative stress, as evidenced by reduced formation of cholesterol oxidation products in the livers and brains of the NAC-treated mice. Eight-week-old mice treated with 250 mg/kg/d showed a 26% decrease of 7-ketocholesterol ($P < 0.005$). Neuropathological examination for quantification of Purkinje cells is currently underway to determine if NAC improves neuronal survival. We conclude that NAC reduces oxidative stress in the NPC1 mice, ameliorating the severity of the neurologic symptoms and improving survival. NAC, which is approved for human use, may prove useful for treatment of human NPC1 disease.

P298 Phylogenetic Analysis of *Myobia musculi* Using Ribosomal Gene Sequence

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Our objective was to perform molecular phylogenetic analysis of the furmite *Myobia musculi* using specimens collected during infestation of a single sentinel in one of our vivaria. We used high fidelity PCR to amplify 2 overlapping regions of the ribosomal gene complex (rRNA) from *M. musculi*. The amplicons were cloned, sequenced, and a consensus nucleotide (nt) sequence derived encompassing a large portion of the mite's ribosomal gene complex. The rRNA sequence we determined spanned 3,128 nt comprising the entire 18S rRNA, internal transcribed spacer (ITS) 1, 5.8S rRNA, ITS2 and a small portion of the 5′-end of the 28S rRNA. Multiple sequence alignment was performed using 1,524 nt of *M. musculi* 18S rRNA, homologous sequences from 42 prostigmatid mites in the NIH GenBank and *Dermacentor andersoni*. We used a statistical program to determine the nucleotide substitution model to incorporate in subsequent computation phylogenetics program and Bayesian inference methods of analyses. The resulting phylograms were rooted to the divergent tick *D. andersoni*. Both phylograms were in agreement regarding terminal, secondary, and some tertiary relationships among mites. Bayesian inference discriminated infraordinal relationships between Eleutherengona and Parasitogona amongst the members of the suborder Anystina. Basal relationships between Anystina and Eupodina were less well supported. *M. musculi* is in the Suborder Anystina, Infraorder Eleutherenona and Superfamily Cheyletoidea. We identified the 5.8S rRNA sequence using conservation of rRNA secondary structure since primary sequence was not conserved, after which we inferred the ITS1 and ITS2 regions. Our molecular data supports previous classification of *M. musculi* and contributes DNA sequence information and molecular analysis previously unknown to the laboratory animal scientific community.

P299 Yucatan Miniature Swine Surgical Glaucoma Model Development and Response to Therapy

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The purpose of this project was to develop a model of glaucoma via surgical induction of increased intraocular pressure (IOP) in Yucatan miniature swine. Three pigs (2 female, one male) had bilateral IOP measurements performed prior to surgical intervention to establish a baseline IOP for each animal from which future changes in IOP could be identified. IOP measurements were taken with a tonometer. In order to reduce venous drainage from the eyes, episcleral veins were scarified by cauterization in each eye. IOP were periodically measured for several weeks after cauterization surgery. Pharmacologic intervention was then instituted with a commercially available synthetic prostamide analog with ocular hypotensive activity. Drops were applied once daily, and IOP continued to be measured. After 7 wk of daily treatment, eye drops were discontinued, and IOP measurements continued to be obtained. All animals presented with significant increases in IOP measurements after surgical intervention and significant decreases in IOP with pharmacological therapy. IOP means were as follows ± SD: pretreatment, 19 ± 4 (65 readings); postsurgery, 24 ± 5 (124 readings); post treatment, 18 ± 4 (75 readings); recovery, 20 ± 4 (80 readings). Therefore, the Yucatan miniature swine could be considered a viable model for surgically induced glaucoma. It has also been shown that the miniature swine eye is responsive to pharmacological therapy to reduce IOP and as such could be a potential model for future pharmacological research.

P300 Decreased Inflammation Following Dental Prophylaxis in Diabetic Cynomolgus Macaques

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Diabetes mellitus (DM) is a widespread health concern. Poor oral health is a concern in diabetics with a "bidirectional" relationship between periodontal disease and DM; with both conditions increasing inflammation and release of proinflammatory cytokines. We hypothesized that dental scaling would decrease both markers of inflammation and insulin requirements after treatment, when compared with the pre treatment levels. The objective was to assess inflammation and cytokine levels as well as glucose and insulin parameters following periodontal treatment (including basic scaling and cleaning, and antibiotic therapy with clindamycin initiated 2 d before scaling for a total of 6 d). Twenty-two cynomolgus macaques

with naturally occurring type 2 DM and various degrees of periodontal disease were used in the study. The animals were evaluated for CBC and chemistry, inflammation (cytokine levels), and insulin requirements pre- and postdental cleaning. A significant decrease in total WBC ($P = 0.02$) and lymphocyte ($P = 0.01$) counts after treatment was found. A significant increase was noted in the hematocrit levels after treatment ($P = 0.03$). Although there was a decline in the cholesterol and triglyceride levels after treatment these were not significant. A significant decrease ($P = 0.003$) in glucose concentrations was observed between baseline (302.05 mg/dL) concentrations and after treatment (226.53 mg/dL) with a nonsignificant decrease in fructosamine concentrations, while insulin requirements remained unchanged. A significant decline IL6 ($P = 0.002$), IL1β ($P < 0.0001$), and C-reactive protein ($P = 0.04$) concentrations were noted between baseline and after treatment. Although not significant, a similar trend was observed in the TNFα levels. This study demonstrated the benefits of treatment of periodontal disease in diabetic animals, not only in terms of decreased inflammation but also decreased glucose concentrations. Thus, cynomolgus macaques may be a useful model for the study of the effects of periodontal disease on diabetes and vice-versa. Finally, inflammation can be a significant variable in research studies and based on the results here, regular dental evaluation and prophylactic dental cleaning should be considered.

P301 Destabilization of the Medial Meniscus Mediated Osteoarthritis Lesion Formation in C57BL/6 Mice

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Age and gender are important variables to eliminate when creating a surgically induced osteoarthritis lesion. Destabilization of the medial meniscus (DMM) in C57BL/6 mice results in the formation of an osteoarthritis lesion on the tibial plateau and femoral condyle. Determining the optimal age and gender of C57BL/6 mouse for the creation of this lesion, via DMM surgery, can provide significant refinements in the surgical procedure and reduce the animal numbers required to obtain statistical significance by eliminating lesion variability. The best age and gender of C57BL/6 should create a consistent lesion resulting from the DMM surgery, without lesion formation on the control leg and without severe lesion formation on the surgical leg that might significantly impact mobility or require pain management (all animals were provided buprenex 0.1 mg/kg SC perioperatively). For this study, 16 male and 16 female, 8-wk-old C57BL/6 mice were used to create the young animal cohort, and 16 male and 16 female, 32-wk-old C57BL/6 mice were used to create the aged animal cohort. A stereoscope was used to locate the medial menisco-tibial ligament, and visually confirm full transaction at time of surgery. Eight weeks following DMM surgery, all animals were euthanized and both knees were taken for histologic evaluation. The 2 histologic scoring systems used were a modified Chambers score and the Mankin scoring system. When the histology scores were reviewed, the 8-mo-old males had the most severe lesion on the surgical knee, as well as increased spontaneous lesion development on the control knee. From the results provided it was determined that the model with the most consistent formation of a moderate lesion on the surgical leg without development of lesions on the control leg were the 8-wk-old male C57BL/6 mice. Each of the female cohorts used had some formation of osteoarthritis lesions on the control knee consistent with spontaneous osteoarthritis development in this mouse strain. This study strongly indicates that young male mice will likely make the best osteoarthritis model using the DMM technique, while having the lowest chance of variability arising from lesion formation on the control knee.

P302 Assessing Immunomodulatory Effects of a Novel Compound on the Acute Inflammatory Response with Cantharadin Dermal Exposure in Cynomolgus Macaques

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Exposure to cantharadin has been used to investigate the acute inflammatory response in vivo in humans, by assessing neutrophil influx to the exposure site, a key component of the early response to inflammatory stimulus. Compounds that alter neutrophil influx could provide new therapeutics for a variety of inflammatory-based disease states. Our aim was to develop a nonhuman primate model of neutrophil influx by adapting a cantharadin dermal exposure technique used in humans to the cynomolgus macaque (*Macaca fasicularis*) to assess the immunomodulatory effects of a novel compound and provide a model that would be directly translatable to clinical testing in humans. All studies were conducted after review by the IACUC and in accordance with the our Policy on the Care, Welfare and Treatment of Laboratory Animals. On day 0, monkeys ($n = 3$ per group) were either dosed intravenously at 10 mg/kg with a control compound or a novel immunomodulatory compound. On day 1, monkeys (under ketamine anesthesia) had 2 filter paper discs placed on each forearm and 100 µL of 1% cantharadin to the discs. The exposure sites where bandaged and the monkeys returned to their cage. On day 2, the bandages were removed and accumulated fluid at the exposure site where a small blister formed was harvested. Animals were observed for healing over approximately a 21-d period and dosing and cantharadin exposure was repeated in a crossover fashion. Fluid from the cantharadin exposures was analyzed for total protein, white blood cell count, and differential to assess compound related changes in the acute inflammatory response. Total protein, as a measure of exposure site permeability and consistently of inciting response, was not significantly different between control and treated (mean ± SEM, control: 3.76 ± 0.29 g/dL, treated: 4.08 ± 0.21 g/dL), while neutrophil influx was significantly ($P < 0.05$) reduced with the immunomodulator (control: 2.00 ± 0.44 × 103 cells/µL, treated: 0.40 ± 0.13 × 103 cells/µL). The immunomodulator compounds inhibited an important early response of the immune system in a model that is already in use in humans, providing a translational opportunity by using this model in both preclinical and clinical research.

P303 Validation of Portable Blood Glucose Monitoring Systems for Use in Rats

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Our objective was to evaluate 3 different blood glucose monitoring systems, currently on the market for use in humans, for potential use in the measurement of blood glucose levels in laboratory rats. Current methods of measuring plasma glucose in rats require relatively large blood sample volumes, higher numbers of animals, as well as more lab supplies and personnel. Use of a glucose monitoring system would significantly reduce each of these areas, while also providing immediate results. Twelve untreated ZDF/Crl-Leprfa/fa diabetic rats, and 20 Crl:CD(SD) rats were used for this study. The diabetic rats and 10 of the SD rats were untreated, while the other 10 SD rats were treated with a marketed agent for the treatment of diabetes which, in effect, would cause hypoglycemia. Blood samples were collected from SD rats prior to dosing and approximately 1 to 2 h after dose, and once from ZDF rats and untreated SD rats for measurement of plasma glucose as well as glucose monitor evaluations. Each rat was sampled using each of 3 marketed glucose monitors, and results were compared with plasma glucose data. Whole blood glucose values were converted to their plasma equivalent. Glucose monitors 1 and 2 demonstrated acceptable performance in rats, with intraassay precision being similar at all glucose levels. Monitor 1 was more accurate than monitor 2 at low glucose values when compared with plasma results, but the difference between values was not considered biologically significant. Monitor 3 demonstrated poorer performance in rats particularly in the hyperglycemic range and had a high negative bias. Therefore, monitors 1 and 2 were considered acceptable for use in the measurement of blood glucose in rats, depending on study objectives, investigator needs, and anticipated glucose levels.

P304 Alternatively Activated Macrophage Targeting in Breast Cancer Mouse Model

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Tumor cells often polarize macrophages into alternatively activated type (M2) in the tumor microenvironment. These M2 macrophages inhibit antitumor immune responses as well as secrete several molecules that can enhance tumor cell motility, induce angiogenesis and finally promote metastasis. COX2 inhibitors are empirically known to prevent breast cancer metastasis. Thus, we hypothesize that specific COX2 inhibition could polarize M2 macrophages into classically activated type (M1). Syngeneic Balb/c mice were implanted with 4T1 breast cancer cells orthotopically and fed with 500 ppm

etodolac, specific COX2 inhibitor-containing feed during experimental periods, 4 wk. In vitro 20 μM etodolac was treated into human blood monocytes isolated using magnetic beads negative selection by MACS. Primary tumor sizes did not changed but metastatic lung nodules were significantly inhibited both in their sizes and numbers in etodolac-treated group. Spleen and tumor associated macrophages from etodolac treated group expressed only 37% of M2 macrophage marker CD163 on their cell surface compared with control in FACS analysis. In vitro study resulted that etodolac inhibited M-CSF induced M2 polarization of macrophages below to 13% compared with DMSO treated control group. LPS/IFNγ stimulation induced much more IL1β, TNFα, IL6 in etodolac treated macrophages and these cells produced less VEGF and TGFβ than original M2 macrophages. VEGF expression levels were recovered by forskolin-stimulatd cyclic AMP production in etodolac-treated macrophages, suggesting that M2 macrophages have enhanced COX2-PKA-VEGF signaling pathway. In summary, redirecting M2 macrophages into M1 type by NSAID drug, etodolac may help preventing breast cancer metastasis.

P305 Antitumour Effects of Monoclonal Antibodies in a Mouse Model Engrafted with CD5-Expressing B-Cell Malignancy

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The recent availability of active monoclonal antibodies (mAbs), such as rituximab and alemtuzumab, enables innovative therapeutic approaches of patients with B chronic lymphocytic leukemia (CLL). Although neither of them is curative, these 2 mAbs are largely used to treat such individuals, because they have substantially improved their outcome. There is, however, a need for mAbs newly engineered and directed to other surface antigens on CLL B lymphocytes. Among these structures, CD5 presents as an attractive target for mAbs, owing to its high expression levels on malignant B cells, compared with normal B lymphocytes. Unfortunately, given the lack of human CD5-expressing B CLL animal model, preclinical studies on antiCD5 reagents are limited. To clear it out, we have established a new B cell line, named JOK1-5.3, expressing the human CD5 gene and developed an animal model for CLL by engrafting these lymphocytes into severe combined immunodeficient mice. We analyzed in our mouse model the biologic and therapeutic properties of mAbs directed against the CD5, CD71 or HLA-DR molecules highly expressed on B-CLL cells compared with rituximab.

P306 Evaluation of Blood Glucose Levels Using a Handheld Glucometer as Compared with a Clinical Chemistry Analyzer in BBDP/Wor and BBZDR/Wor Rats

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Historically, our facilities have been using a clinical chemistry analyzer (CCA) to determined serum glucose levels for our proprietary diabetic-prone rat models. This method is an operationally time-consuming process. Additionally, a whole blood glucose analysis via a handheld glucometer is a less expensive and faster method for evaluation of glycemic state. Portable glucometers have been developed and used for whole blood glucose analysis in human patients and are increasingly used to monitor diabetic animals. Moreover, their sensitivity and accuracy has been continuously improved. Therefore, we evaluated the accuracy of whole blood glucose levels measured by a handheld glucometer as compared with serum glucose levels (SGL) measured by CCA in BBDP/Wor and BBZDR/Wor rats. Cohorts of rats that exhibited chronic diabetes were used in this study. Type 1 diabetic BBDP/Wor rats were tested for glucose levels twice daily, prior to and 3 to 4 h after insulin administration. Type 2 diabetic BBZDR/Wor rats were tested once daily during the nonfed state. At each time point blood was measured by CCA and glucometer. Our data show that no statistically significant difference was detected between CCA or glucometer measurements in BBZDR/Wor rats. However, glucose levels measured by a handheld glucometer were significantly lower than the levels measured by CCA in BBDP/Wor rat. Moreover, when categorizing the readouts into subgroups according to SGL > 500 mg/dL, 200 to 500 mg/dL, or < 200 mg/dL, glucose levels measured by a glucometer were significantly lower than the levels measured by CCA in all 3 subcategories. In summary, using a handheld glucometer for measuring blood glucose levels in BBZDR/Wor is equally accurate to the use of a clinical chemistry analyzer. However, the BBDP/Wor rat's poor hydration state may contribute to the higher glucose values measured by CCA in comparison to the values measured by a glucometer.

P307 Optimization of Insulin Treatment for Spontaneous Type 1 Diabetes in BBDP/Wor Rats

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New long-acting types of insulin capable of maintaining blood glucose levels within a near-normal range are in great demand for use in rodent models of T1D. However, standardized protocols (SP) for maintaining normoglycemia in preclinical diabetic rodent models are lacking. Therefore, we evaluated the efficacy of new long acting insulins for maintaining glycemic levels in our spontaneous T1D BBDR/Wor rat colony. Factors considered in the development of this SP included both the type of insulin and treatment regimen relative to fed state. Cohorts of BBDP/Wor rats that exhibited chronic diabetes were used in these studies. Four types of insulins were evaluated by a single subcutaneous injection to maintain normoglycemia within a 24-h period: INS1 (protamine zinc; 90% beef, 10% pork), INS2 (glargine, rDNA), INS3 (detemir, rDNA), or INS4 (protamine zinc, recombinant human). Next, the optimum timing of insulin administration for glycemic control was compared when therapies were administered during the nonfed or fed state by measuring blood glucose levels every 2 to 4 h over a 24-h period. Our data show that the once daily injection of INS1 targeted to 0.9 U/100 g body weight was superior for maintaining daily glycemic control in diabetic rats to both INS2 and INS3 when administered at the same dose. Interestingly, a comparable dose of INS4 exhibited superior duration of normoglycemia compared with INS1. Moreover, similar to human treatment regimens, insulin administration just before fed-state with either INS1 or INS4 provided safer and longer daily glycemic control compared with an early treatment at the nonfed state. In summary, once daily subcutaneously injection of either INS4 or INS1insulins, administered just before fed-state, are effective at maintaining daily glycemic levels in spontaneously diabetic BBDP/Wor rats. This knowledge will allow for more accurate mimicking of that expected in human patients in our preclinical efficacy trials.

P308 Optimization of the Rederivation Process through Ultrasound

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Rederivation of a mouse line requires that multiple embryo transfers be performed over a given period of time, depending on the number of blastocysts obtained, the number of fosters available, and the success of the transfer. Multiple embryo transfers are planned and performed on a given mouse line within several weeks. If successful, this can lead to multiple pregnancies, multiple litters, and an investigator not wanting or needing all created animals. This is not only wasteful in terms of the pups produced, but the fosters that underwent surgery or donated embryos as well as the time and labor afforded the project. When performing embryo transfer surgery, there is an approximate waiting period of 10 to 15 d for a visual observation and detection of pregnancy. We began to explore whether it was possible to detect pregnancy earlier than 10 to 15 d. If detection were possible, then fewer surgeries would be performed. This, in turn would optimize our rederivation process by decreasing the number of donors, decrease the number of animals exposed to surgery, which cause pain and distress, and decrease the wait time for confirmed pregnancies. Since embryos can be detected by ultrasound as early as E5.5 we decided to try this method. We began to ultrasound CD1 foster females 4 to 9 d after embryo transfers, which had various gestational ages between E5.5 to E11.5. Conclusively we found that we were able to detect pregnancies as early as E6.5 and determine an approximate number of pups in the uterus. Using ultrasound for early detection of pregnancy following rederivation is a perfect example of adhering to the 3Rs, because we were able to replace palpation or visualization with a modern day imaging modality, refine the project by reducing the number of donors, fosters used for surgery, and total number of animals created.

P309 Blood Sampling Methodologies Employed in the Conduct of Rodent Pharmacokinetic Studies Affect Determination of Drug Clearance

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The impact of anesthesia on physiologies affecting drug clearance (for example, blood flow) is generally recognized, meaning that routine pharmacokinetic (PK) studies in rodents are commonly performed using conscious animals. With automated blood sampling (ABS) technology, serial collections can be performed on catheterized animals in a lower stress environment with limited human intervention. Alternatively, blood may be manually sampled directly from the tail or jugular vein, although this additional manipulation adds to the stress burden on the animals during the PK assessment and may lead to altered physiology. In the present study, the PK of 4 commercially available compounds known to be low, moderate, or high clearance (CL) in rat were investigated under study conditions ranging from: full ABS automation, manual sampling of catheterized animals, and full manual design (sampling via jugular stick). The primary goal was to assess how such factors may influence PK outcomes, particularly clearance and steady-state distribution volume (Vss). Results for antipyrine, a low CL compound, indicated no statistical difference in CL or Vss between groups by ANOVA ($P > 0.05$). Data for acetaminophen, a moderate CL compound, showed that Vss was unaffected by sampling method; however, CL for the fully manual group was lower (2 times) compared with the ABS group ($P < 0.05$). Results for high CL compounds, propranolol and midazolam, were even more striking than acetaminophen. In 2 separate studies with propranolol, animals sampled manually showed 2 to 5 times lower CL and 2 to 3 times lower Vss compared with the fully automated study ($P < 0.001$ and $P < 0.01$ for CL and Vss, respectively). For midazolam, both CL and Vss were 3 times lower in the fully manual group compared with ABS group ($P < 0.001$ for both CL and Vss). These results demonstrate that high CL compounds may be particularly sensitive to sampling methodology employed in rodent PK studies. In the drug discovery setting, this could lead to erroneous or variable assessments of lead molecules with clinical potential.

P310 A Method for the Evaluation of Barriers to Bioavailability and Drug Absorption in the Cannulated Dog Model

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The measure of drug bioavailability depends on metabolism as well as absorption of the drug at different locations along the gastrointestinal tract. To determine the bioavailability of a drug, including the fraction of drug escaping the liver and gut, the test compound oseltamivir was evaluated in a unique canine model. Three healthy male beagle dogs had chronic intraduodenal access ports (IDP) and portal vein cannulas (PVC) placed. Dogs were administered oseltamivir at 0.5 and 2.5 mg/kg. The dosing and sample regimen was as follows: intravenous (IV) administration (0.5 mg/kg) and cephalic vein (CV) blood sampling, intraportal vein (IPV) administration (0.5 mg/Kg) and CV sampling, intraduodenal administration (ID) (2.5 mg/Kg) and CV sampling, and ID administration and portal vein (PV) blood sampling. The dose normalized area under the curve (DNACU) was 1258, 959, 933, and 1761 h × kg × ng/mL/mg for the administration/sampling routes, respectively. Bioavailability was calculated at 74% and the fraction escaping the gut was calculated at 97% (ID administration/CV sampling compared with IV administration/CV sampling and ID administration/CV sampling compared with IPV administration/CV sampling, respectively). However, the fraction escaping the liver varied from 76% to 53% depending on the method used for the calculations (method 1 = IPV administration/CV sampling compared with IV administration/CV sampling or method 2 = ID administration/CV sampling compared with ID administration/PV sampling, respectively). The preferred calculation method for determination of hepatic contribution is to compare DNAUC after IPV administration/CV sampling compared with IV administration/CV sampling. The alternative method is biased towards an overestimation due to dilution and distribution of test compound prior to cephalic sampling (that is, post ID dosing and when compared with PV sampling/ID dosing). In conclusion, the hierarchy of barriers (hepatic > intestinal) obtained from this study are consistent with human data available for this compound, and therefore, illustrate the potential of this unique cannulated dog model.

P311 Comparison of In Vivo, Ex Vivo, and a Novel In Vitro Method (Corneal Orbs) for the Assessment of Corneal Drug Absorption

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Traditional corneal drug absorption methods use either in vivo systems, such as rabbits, or ex vivo methods, such as excised corneal tissue (human and rabbit). However, traditional ex vivo methods suffer from limited availability or compromised quality. We compared in vivo and ex vivo techniques to a new in vitro alternative: hollow, fluid-filled spheres (corneal orbs) produced from cultured, human embryonic stem cells using a set of 10 test compounds with diverse ocular indications. The orbs, approximately 10 mm in diameter, were incubated at 37 °C in wells containing the compounds (10 µM concentration) for 30 min followed by measuring accumulation inside the sphere. For the ex vivo experiments, drug transport across corneas was evaluated in vertical Ussing chambers employing mounted excised rabbit and human corneas. Donor and receiver concentration measurements were made up to 2 h at 37 °C. For the in vivo studies, the compounds were topically applied to the eyes of laboratory rabbits. Thirty minutes after dosing, the animals were euthanized and aqueous humor, vitreous humor, and retina/choroid were collected. Compound concentrations for all 3 methods were assessed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The in vitro methods demonstrated high correlations with a correlation coefficient (r^2) of 0.93 for orbs compared with ex vivo human corneal tissue; and 0.91 compared with ex vivo Dutch belted rabbit corneal tissue. High permeability measured in corneal orbs also showed good correlation with aqueous humor test compound accumulation in vivo. The results of these studies demonstrate that human corneal orbs may represent an ideal in vitro model for testing corneal drug permeability, which circumvents the limited availability of healthy human corneal tissue and potentially reduces the use of rabbits for preclinical testing of ocular drugs.

P312 Development of a Rat Model to Differentiate Absorption and First-Pass Effect for Poorly Bioavailable Drugs

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Our objective was to develop a rat model to differentiate the roles of absorption and metabolism in limiting the oral bioavailability of certain drugs and to discern differences in first-pass metabolism in the gut compared with the liver. Rats were dosed with testosterone, dextromethorphan, atenolol, and fexofenadine as a cassette intravenously (1 to 10 mg/kg) or orally (3 to 30 mg/kg), with or without pretreatment with 1-Aminobenzotriazole (ABT) (50 mg/kg IV) or (100 mg/kg PO). ABT is a suicide cytochrome P450 (CYP) inhibitor that blocks the liver from metabolizing many drugs and toxic chemicals. Pharmacokinetic parameters were estimated based on plasma samples collected from the portal and jugular veins. Following oral dosing, the systemic exposure of testosterone and dextromethorphan increased 32- and 38-fold, respectively, in rats pretreated orally with ABT. Following intravenous dosing, the systemic exposure of dextromethorphan did not change significantly in rats pretreated with ABT orally or intravenously. These results suggest that testosterone undergoes first-pass metabolism in both the gut and liver and dextromethorphan in the liver only. There was no significant change in the rate and extent of absorption or metabolism of atenolol or fexofenadine in ABT pretreated compared with untreated rats. Low systemic exposure in ABT pretreated rats implies that systemic exposure is limited by absorption, whereas large differences in exposure between ABT pretreated and untreated rats suggest that first-pass metabolism is the limiting factor.

P313 Composition of Microorganism and Seasonal Fluctuation in Respiratory Organs and Intestine of Mice and Rats in Korea

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When primary microbiology contamination break out in massive

laboratory animal production facilities, the secondary contamination can affect research results and experiments more seriously by causing adverse immune responses. This study aimed to survey the status of laboratory animal quality for major suppliers and research institutes on a national scale, and also the distribution of respiratory and intestinal organs with the season changes. We monitored 738 mice and rats in 34 major laboratory animal facilities in Korea, collecting a total of 1616 identified isolates including 857 isolates (94.90%) and 759 isolates (97.31%) from 447 mice and 291 rats, respectively. *E. cloacae*, *K. kirstinae*, *P. mirabilis*, *P. vulgaris*, and *S. lentus* were much higher in mice than in rats. The microbial flora which belongs to same species and genus also distributed higher in autumn than in spring. *P. vulgaris* was found mainly in spring, while *S. paucimobilis*, *M. luteus*, *L. adecarboxylate*, and *K. rosea* in autumn. *P. polymyxa*, *G. elegans*, *G. adiacens*, *E. cloacae*, *B. choshinensis*, *B. firmus*, *B. circulans*, *A. baumannii*, *A. denitrificans* were specifically distributed in spring, and *S. pneumoniae*, *S. thalpophilum*, *P. canis*, *M. lylae*, *B. meltensis*, and *Salmonella* spp. were found in autumn. These results indicate that it is necessary to study how to minimize the contamination of laboratory animals, and suggest the composition and seasonal fluctuation of bacteria could serve as useful information for animal facility management.

P314 Cutaneous Exposure to GD and VX: Timing Pretreatment and Antidotes

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Treatment of cutaneous exposure to organophosphates presents unique problems due to delayed absorption. This delay affects whether and when to pretreat with pyridostigmine (26 µg/kg) and when the antidotes 2-PAM (25 mg/kg) and atropine (16 mg/kg) should be given. The purpose of these experiments was to address timing of pretreatment and antidotes for clipped, anesthetized guinea pigs receiving either neat soman (GD) or VX on their sides. Since pyridostigmine is known to enhance 2-PAM/atropine therapy when GD is administered as a subcutaneous injection, in the first phase of this work we pretreated our animals with pyridostigmine 30 min prior to agent exposure. When a single injection of 2-PAM/atropine was given 1 min after agent exposure a protective ratio (PR) of 5.5 for GD and 2.0 for VX was observed. This poor PR for VX was observed if a single antidote injection was given at any time, and to increase the PR 2 antidote injections were required. When 2 injections of 2-PAM/atropine were given at 1 min and 3 h after VX exposure the PR = 4.0. When 3 injections of 2-PAM/atropine were given at 1, 4, and 7 h after VX exposure the PR = 6.4. We then sought to examine the effect of removing pyridostigmine pretreatment completely (PR = 6.7), administering pyridostigmine 1 h post exposure (PR = 7.5) or adding a second dose of pyridostigmine at 7 hs post exposure (7.2). None of these variations were statistically different from each other in enhancing the effects of a 3-injection 2-PAM/atropine therapy. These studies demonstrate not only the need for prolonged antidote 2-PAM/atropine therapy after cutaneous exposure to VX, but also that administering pyridostigmine as either a pretreatment or a therapy for cutaneous VX applications provides no benefit.

P315 Prognostic Markers of Radiation-Induced Neurocognitive Impairment in the CSF of Wistar Rats

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The deep space missions planned by NASA will result in astronauts being exposed to galactic cosmic radiation (GCR). We have previously reported that low doses (20 cGy) of GCR, specifically 1 GeV 56Fe, leads to significant impairment of spatial memory performance at 90 d post irradiation. We hypothesized that because the cerebrospinal fluid (CSF) "bathes" the brain, it will concentrate protein from the brain which will be an ideal source to identify prognostic biomarkers for a susceptibility to develop GCR-induced spatial memory impairment. Such biomarkers could be used to screen astronauts prior to a deep space mission to identify "at risk" individuals. CSF was collected from 1-mo-old rats prior GCR-exposure using a 26.5-gauge needle introduced percutaneously into the cisternal magna orthogonally to the neck of the rat. The rats were then exposed to 20 cGy of 1 GeV 56Fe irradiation. At 90 d post exposure, the spatial memory of the rats was assessed in the Barnes maze. Once the rats had been tested, the CSF samples from 6 unirradiated rats, 6 irradiated rats that performed badly in the Barnes maze, and 6 rats that performed well were recovered and subjected to proteomic analysis. Briefly, the biologic fluids undergo WCX-bead enrichment, followed by digestion with trypsin, and are then analyzed on a linear ion trap mass spectrometer (LIOMS). The LIOMS-generated data was subjected to analysis using a Venn Diagram program that helps to identify proteins whose expression is specific to a certain population, in this case, severe or no HISMI. We identified 4 proteins that were uniquely expressed in the pretreatment CSF samples of rats that did not develop spatial memory impairment following 20 cGy 1 GeV 56Fe irradiation: serotransferrin, triosephosphate isomerase, polyamine-modulated factor 1-binding protein, and vimentin. These proteins would thus appear to contribute to an enhanced resistance of the hippocampus to the deleterious effects of 20 cGy 1 GeV 56Fe irradiation, and could potentially be used to screen for astronauts who would have a low risk of developing radiation-induced cognitive impairments.

P316 Dried Bloodspot Comparison Study: Comparison of 2 Blood Collection Sites and 2 Blood Collection Methods after a Single-Dose of Acetaminophen in Male Rats

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Dried bloodspot (DBS) technology has been available for many decades but only in the last 5 y has it been considered for routine bioanalysis of blood samples collected on preclinical and clinical studies as part of a drug development program. Advantages to using DBS compared with typical plasma samples include smaller blood volumes, less processing of the samples (for example, no centrifugation), and no requirement for storing or shipping of the samples at frozen temperatures. The current study compared blood concentrations (AUC0-t and Cmax) from rats given an oral dose of acetaminophen (APAP) using 2 different sampling sites (caudal venipuncture compared with tail snip), 2 different collection methods (3 separate 15-µL EDTA-coated capillary tubes compared with an EDTA-integrated capillary blood collection system), and variability between blood spots on one card. There were no noteworthy differences (that is, 2-fold or greater) in blood concentrations of acetaminophen using the different sites or methods. Furthermore, comparisons of the APAP blood concentrations in the original spot to a duplicate bloodspot from the same bloodspot card were within 12% of the original concentration.

P317 Characterization of Fecal Corticosterone Levels in RCAN1 Mutant Mice Neurologic Mouse Models: An Indicator of Anxiolytic Behavior

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Regulator of calcineurin1 (RCAN1) is related to the expression human neurologic disorders such as Down syndrome (DS), Alzheimer disease (AD), and chromosome 21q deletion syndrome. Recently, we found that RCAN1 knockout (KO) mice exhibit reduced innate anxiety. To examine the role of stress in this phenotype, we sampled blood cortisol levels from male RCAN1 KO mice at 2 time points spaced 12 h apart. Additionally, we characterized fecal corticosterone (CORT) levels in male wildtype and RCAN1 KO mice and in transgenic mice of both genders neuronally overexpressing RCAN1 (Tg-RCAN1tg). Using blood sampling, we found differences in CORT levels between RCAN1 KO and their wildtype littermates. However, using less invasive fecal sampling, we found no difference in CORT levels between these genotypes. As expected, we found differences in shed corticosterone levels between the genders, but also found higher levels of shed CORT in Tg-RCAN1tg females compared with female wildtype mice Tg-RCAN1wt. This is interesting for 2 reasons, first because RCAN1 was only overexpressed in the forebrains of these mice and secondly, we did not observe this increase in male tg-RCAN1 mice. Our data indicate that circadian-mediated CORT production in RCAN1 mutant mice is normal and do not suggest a causal role in either the cognitive or anxiety phenotypes exhibited

by RCAN1 KO mice. To our knowledge, this is the first assessment of glucocorticoid expression during a normal 24-h period in mouse models manipulating RCAN1 function.

P318 Effect of Rat Collagen on Bone Formation in Calvarial Defect in Sprague–Dawley Rat

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Our objective was to evaluate the effect of collagen on bone formation in calvarial defect model. A total of 21 adult Sprague–Dawley rats were divided into 3 groups for different treatments: rat collagen group, commercial collagen group, and negative control group. An 8-mm diameter calvarial defect was surgically created in each animal. The bone defects were filled by 2.4 mg of rat collagen, 2.4 mg of commercial collagen, or not filled in the negative control group. All of the animals were euthanized at 1 or 3 mo after surgery for radiography and histology analysis. The results of the 1-mo group showed in the radiograph that the defects were filled with new bone (% area). The rat collagen group had a statistically greater filling than the negative control group (43.63% ± 12.63% and 17.88% ± 2.22%, respectively; $P = 0.006$), and there was no significant difference with the commercial collagen group (31.80% ± 8.35%; $P = 0.127$). The results of the 3-mo group showed that the rat collagen group was statistically greater than the negative control group (53.83% ± 5.73% and 32.50% ± 3.48%, respectively; $P = 0.025$). In conclusion, we found a much greater bone formation in calvarial defect the in the rat collagen group than in the negative control group, with no significant difference to the commercial collagen group in the 1- and 3-mo groups.

P319 Establishing a Program of Effective Isoflurane Scavenging in the Mouse Anesthetic Environment

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Establishing a program that effectively scavenges waste anesthetic gases (WAG), limits personnel exposure, trains personnel to reduce WAG, and monitors exposure to WAG requires an understanding of waste gases in the mouse anesthetic environment (MAE). Unfortunately, practices proposed are often extrapolations of those used in human anesthetic areas, which ignore the logistical, physical, and practical differences of the MAE. Further, scant documentation exists regarding WAG in the MAE, efficacy of scavenging methods used in the MAE, and whether WAG detected using personal monitoring badges correlate with those measured using infrared (IR) spectroscopy. To establish MAE practices that ensure WAG are below the National Institute for Occupational Safety and Health (NIOSH)-recommended 2-ppm threshold, studies were undertaken to document WAG using IR at the mouse interface (MI), operator's vicinity (OV), and room environment during isoflurane induction and maintenance using passive or active scavenging techniques. Absence of scavenging invariably resulted in WAG > 2 ppm in the OV. During chamber induction, continuous passive scavenging produced levels greater than 2 ppm in the OV, as did active scavenging initiated after completion of induction. Meanwhile, continuous active scavenging maintained levels at less than 2 ppm. During maintenance anesthesia using Bain circuitry (> 5 min), passive scavenging resulted in WAG > 2 ppm in the OV. Active scavenging introduced around the operative field produced levels greater than 2 ppm at the MI but maintained levels at less than 2 ppm in the OV, while active scavenging introduced via the Bain circuitry maintained WAG < 2 ppm at both the MI and OV. WAG detected during ultrasound, MRI, and bioluminescence imaging reflected those documented for the anesthetic and scavenging methods used. WAG measured using the monitoring badges correlated with those measured by IR. These observations contribute to the potential development of substantiated departmental and/or institutional guidelines that ensure a safe MAE.

P320 Early Tetraploid Microinjection with a Single Embryonic Stem Cell for the Generation of Targeted Mutant Mice

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Targeted mutant mice have been generated using a variety of techniques and at various embryo stages. Classic tetraploid (4n) microinjection with embryonic stem (ES) cells from natural fertilization into embryos at the blastocyst stage generates mice, which are exclusively ES cell derived. Only one other study describing early, or preblastocyst tetraploid microinjection has been published, which used 10 to 20 somatic cell nuclear transfer ES cells per embryo. In the present study, we evaluated fetus and pup viability after microinjection of a single ES cell into a preblastocyst tetraploid embryo as an alternative to classic tetraploid microinjection. Two-cell diploid murine embryos (C57BL/6NTac) underwent electrofusion and were monitored until they reached the 2-cell tetraploid stage. Embryos were then subjected to laser-assisted microinjection with a single ES cell and either transferred to recipients the same day or cultured until the blastocyst stage and transferred. Examination of recipient female reproductive tracts was performed midgestation and the number implantation sites and viable fetuses counted. A total of 44.1% of transferred embryos implanted and underwent early to midgestational resorption, and 3.4% of transferred embryos generated viable fetuses. Our preliminary results suggest that this technique is comparable to classic tetraploid microinjection. Follow-up experiments will evaluate the viability of offspring. To our knowledge, this experiment is the first to evaluate the efficiency of early tetraploid microinjection with a single ES cell to contribute to formation of the embryo proper of a 4n embryo.

P321 Distinct Distribution of Tensin Family in the Mouse Kidney

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The tensin family members are cytoplasmic proteins that confer bidirectional links between the extracellular matrix and the cytoskeleton, and are thereby implicated in cytoskeletal organization, cell migration, and proliferation. Mammals have 4 tensin paralogs, Tns1 to Tns4. Tns 1, 2, and 3 proteins structurally resemble each other and act similarly. Absence of Tns2 is associated with glomerulosclerosis, a different pathologic phenotype from the polycystic kidney disease in Tns1 null mouse. However, there is little information between tensin expressions and the disease symptoms in kidney. Thus, we have compared tissue and cellular distributions of Tns family in mouse kidney. Quantitative RT-PCR analysis revealed that Tns1 was highly expressed in tubule-rich fraction and was not expressed in glomeruli fraction, in contrast to Tns3. Tns2 was expressed almost equally in both fractions and Tns4 was not expressed in kidney. Furthermore, immunofluorescence staining revealed that Tns1 was expressed in mesangial area, basement membrane of Bowman capsules and tubuli, whereas Tns2 and Tns3 were strongly expressed in podocytes. Tns2 was also expressed in mesangial area. Interestingly, Tns2 and Tns3 were localized in intracellular junctions in a part of tubuli, which has previously been implicated in integrin-mediated cell-basement membrane junctions in vitro. Tns1, 2, and 3 expression do not completely overlap each other, suggesting independent function. These findings suggest that diverse function and subcellular location of Tns family members in kidney for the first time.

P322 Histologic Changes and Seminiferous Tubule Staging in Yucatan Boars at Various Ages during Sexual Maturation

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Preclinical guidelines often specify the use of prepubertal, pubertal, or sexually mature animals. However, puberty and, consequently, sexual maturity can be defined a number of different ways (all of which reflect androgen production), beginning with mounting behavior/erection and followed by ejaculation of sperm and a threshold portion of seminiferous tubules engaged in spermatogenesis with epididymal sperm. Yucatan boars have been reported to reach puberty as early as 12 wk or as late as 16 to 20 wk of age, but, regardless of how stages of sexual development are defined, it is critical to know what is happening histologically in the testes at these various ages. Modified Davidson-fixed and PAS-stained testicular and epididymal sections were evaluated from 12-, 14-, 16-, 18-, 20-, 22-, and 24-wk-

old Yucatan boars (n = minimum of 4 per age group). Approximately 200 seminiferous tubules were evaluated per testis for the presence of round spermatids only (immature tubules), as well as for species-specific cellular associations (stages) involving round and/or elongate spermatids ("mature" tubules). The proportion of the total number of seminiferous tubules represented by "mature tubules" was calculated. The presence of sperm in the cauda epididymidis was also noted. Round spermatids began to appear at 12 wk of age, and a majority of 14-wk-old boars had seminiferous tubules containing both round and elongate spermatids. While sparse numbers of sperm appeared in the epididymides of one boar at 14 wk of age, at least half of the 16- and 18-wk-old boars exhibited some spermiation with sperm in the excurrent duct system. By 20 wk of age almost all seminiferous tubules were "mature", with sperm present in the epididymides.

P323 Zoonotic and Infectious Disease Surveillance for *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and *Dirofilaria immitis* in Dogs in Ecuador

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Vector-borne diseases (VBD) make up a large number of emerging infectious and zoonotic diseases. Ticks, fleas, and mosquitoes are effective vectors parasitizing canines, making dogs adequate reservoirs for zoonosis. The US military deploys personnel and government-owned animals around the world with possible risk of exposure to VBD. Canine VBD have veterinary and public health significance for the host nations as well as for the US troops and its working animals, giving disease surveillance a great importance. The objective of this work was to survey canines from the cities of Manta and Guayaquil in Ecuador to determine prevalence of heartworm disease (*D. immitis*), ehrlichiosis (*E. canis*), Lyme disease (*B. burgdorferi*), and anaplasmosis (*A. phagocytophilum*). Canine blood samples (1 to 3 mL) from the cities of Manta (n = 50) and Guayaquil (n = 50) were tested on site using a test kit. Prevalence for single or multiple disease status was calculated for each city. In the city of Manta the overall prevalence of diseases was 78%, 52% for *E. canis* alone, and 26% for coinfection with *E. canis* and *A. phagocytophilum*. The overall prevalence for the city of Guayaquil was 88%, 40% for *E. canis* alone, 22% for *A. phagocytophilum* alone, and 26% for coinfection with *E. canis* and *A. phagocytophilum*. Neither heartworm disease nor Lyme disease was detected in any sample. In Conclusion, this study showed the presence of *E. canis* and *A. phagocytophilum* in both cities in Ecuador, emphasizing the value of surveillance for zoonotic diseases to determine disease prevalence and risk assessments, as well as to implement control measures.

P324 Development of a Safe Protocol of Leukophoresis for Nonhuman Primates and Miniature Swine

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Leukophoresis is an important technique for collecting peripheral blood stem cells from the donor for hematopoietic transplantation. Priming of the leukophoresis machine and tubing represents one of the main challenges to using this procedure experimentally in small animals (4 to 7 kg) due to the volume required. Small animals have a relatively small blood volume, thus one of the major concerns is the maintenance of physiologic blood pressure during the procedure. Compensation for any blood loss/dilution during the procedure in addition to preventing the consumption of platelets is crucial. Here we present a protocol that was developed for leukophoresing small (4 to 7 kg) miniature swine (*Sus scrofa*) and rhesus monkeys (*Macaca mulatta*). We have developed a safe and effective modified procedure using a peripheral blood mononuclear cells (PBMC) kit without modification of the machine or the tubing kit. In our studies we were unable to use a third party blood donor or other synthetic blood products to prime the machine. We collected 10% of the total circulating blood volume (TCBV) of the stem cell donor 3 to 4 times during the 35 d preceding the procedure. The blood was used to prime the machine or injected into the animal directly to volume expand him (autotransfusion). We found that volume expanding the donor while slowly priming the machine was best for maintaining stable blood pressure. Blood pressure was controlled by monitoring the inlet volume from the COBE machine and assisted by the anesthesia staff through a second intravenous line. Intermittent monitoring of hematocrit values and platelet counts during an effective 4- to 6-h leukapheresis was performed for safety. A total of 14 leukophoreses have been performed in 6 monkeys and 8 miniature swine; all but one of the animals recovered. The total number of PBMC collected have varied between 1 to 6 × 10^9. The average WBC count before and after the leukophoresis was (8000 cell/μL pre and 5000 cell/μL post). An average of 20 × 10^7 CD34+ cells were harvested. Mild/moderate decrease in hematocrit and PLT were observed without life- threatening complications. We document a modified leukapheresis procedure that is safe and can be used for collection of PBMC from a small donor for stem cell transplantation research studies.

P325 Ovine Model of Uterine Transplantation, Implantation, and Birth

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The prevalence of female factor infertility is 40% of the general population where 3% to 5% are attributed to uterine factor infertility. Currently, options to offset uterine factor infertility towards parenthood are limited to gestational surrogacy or adoption. Similar to transplantation of vital organs, immunosuppressive therapy and overall management presents challenges for success of reproductive organs, specifically of uterine transplantation and implantation to result in live birth. We hypothesized that the ovine model for uterus allotransplantation provides a useful experimental preparation to expand this option for human applications. To test this hypothesis, sexually mature, nonrelated and fertile African sheep were used as donor and recipients (Sudanese and Ethiopian: n = 12). Studies were performed where each breed received the uterus from the other. Screening blood tests for parasitic/bacterial infections were performed prior to surgery and used as indicators for tolerance to anesthesia (xylazine/ketamine/isofluorane). Parasitic infections were treated (ivermectine), pre- and postoperative care included controlled nutrition depending upon gestational stage, handling, antibiotic (ceftiofur), analgesic/antiinflammatory (meloxicam), and immunosuppressant therapy (cyclosporine, prednisone). Pregnancy was facilitated by time-release progesterone implant and an estrous synchronization protocol (progesterone, prostaglandin F2α and PMSG). C-section delivery was perfomed at near term (day 138 of 150-d gestation term) to preclude labor and preserve uterine tissues for analysis. The lamb was prophylactically treated with exogenous surfactant and supplemental oxygen due to high altitude challenge. Of the 12 animals, 5 successful allografts were achieved based on macroanatomy, allograft viability, and lack of infection. Three of the uterine allografts progressed to successful implantation with one resulting in a normally developed, near term lamb with age-appropriate gas exchange and lung mechanics. These data demonstrate a successful interdisciplinary protocol to support functional uterine transplantation and successful birth in the sheep. Further study is warranted to extrapolate these findings to the human.

P326 Production of Transgenic Rabbit Models for Human Diseases

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Successful generation of the first transgenic rabbits was reported in 1985. Since then, transgenic rabbits have been generated and used as animal models for a variety of human diseases and as bioreactors for production of pharmaceutical proteins. Although transgenic mice are still dominantly used in biomedical research, it is known that the causative mutations resulting in human diseases do not cause corresponding pathologic changes in mice in some instances. Additionally, a number of techniques are difficult to apply to mouse models due to their small size and phylogenetical features. Alternatively, the rabbit (*Oryctolagus cuniculus*) with an intermediate size between rodents and large farm animals and closer phylogenetical features to primates has attracted more attention in recent years in biomedical and pharmaceutical research. Transgenic rabbits have been most commonly used as models for studies of cardiovascular diseases, such as atherosclerosis, hypertrophic cardiomyopathy, and long QT syndrome, in addition to their use in studies of cancers. Our lab was

one of a few labs in the world that successfully generated some of the earliest transgenic rabbits. Since then, more transgenic rabbit lines have been produced in this lab, including the expression of papillomaviral genes, EJras gene, HLA-A2.1 gene, LQT1 and LQT2 genes, and others. These transgenic rabbits have been used as models for studies of papillomas, squamous cell carcinomas, keratoacanthomas, immune reaction to human papillomavirus infection, and long QT syndromes.

P327 Prevalence of *Helicobacter* Species in Mice Received from Noncommercial Sources and Entering Institutional Quarantine

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Enterohepatic *Helicobacter* spp. are enzootic in mouse colonies at most academic institutions. Infection with *Helicobacter* species has been associated with hepatitis, hepatic neoplasia, gastritis, typhlitis, and colitis. Disease is noted in both immunocompromised and immunocompetent mice. Disease expression and severity is strongly dependent upon the interaction of host factors and bacterial virulence. Many institutions struggle with the presence of *Helicobacter* infections within their colonies as well as determining the need for segregation or eradication. The sharing and use of genetically engineered mice is one method of spreading infection. Institutions vary on their acceptance of mice with *Helicobacter* infections. Many institutions have set up *Helicobacter*-free facilities, while others accept its presence. Our institution has set up specific *Helicobacter* spp.-free areas. Mice entering quarantine from noncommercial sources are routinely tested via fecal PCR by commercial diagnostic laboratories for various *Helicobacter* species. Between 2007 and 2010, a total of 883 samples were tested from 405 shipments of animals representing 302 institutions. A total of 833 samples were tested for *H. bilis*, *H. hepaticus*, and *Helicobacter* spp. A subset (n = 627) were also tested for *H. rodentium*, *H. trogontum*, and *H. typhlonius*. Of the 883 samples tested, 533 were positive for *Helicobacter* spp., 351 for *H. hepaticus*, and 66 for *H. bilis*. Of the 627 samples tested, 160 were positive for *H. rodentium*, 2 for *H. trogontum*, and 181 for *H. typhlonius*. *Helicobacter* infection from animals imported to our facility from noncommercial sources was very high, with the most common *Helicobacter* identified as *H. hepaticus*. The high prevalence represents a concern for genetically engineered mice and potentially confounds the interpretation of experimental data.

P328 Effect of Physical Restraint on Glucose Tolerance in Cynomolgus Monkeys

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Physiologic stress has been demonstrated to impair glucose tolerance and insulin action. In the present study, we examined whether glucose tolerance is influenced by restraint stress. Studies were designed using female cynomolgus monkeys (3.2 to 4.6 kg) with normal glucose tolerance and impaired glucose tolerance (IGT), all of which were well trained to the restraint procedures, including chair restraint, hand restraint, and the restraint of animals in a home cage using a squeezing device. Oral glucose tolerance tests (OGTT) were performed by administration of glucose (4 g/kg). Blood was collected at 0, 15, 30, 60, 120, and 180 min after the glucose load. Each group underwent 4 to 5 trials of OGTT and there were at least 7 d between each trial. Since monkeys that were adequately trained to physical restraints were used, behavioral changes such as aggression and agitation were not observed during OGTT. In monkeys with IGT, chair restraint induced a significant increase in plasma glucose following a glucose challenge, and reached maxima of more than 150 mg/dL. The overall glucose excursion in chair-restrained subjects was significantly higher than that in hand-restrained or squeezing device-restrained subjects. Furthermore, chair-restrained subjects had high levels of plasma cortisol, a stress marker, during OGTT compared with hand-restrained subjects. Similar results were obtained in monkeys with normal glucose tolerance; chair-restrained subjects elicited higher elevations of plasma glucose and cortisol compared with squeezing device-restrained subjects. Thus, we found that the responses to a glucose challenge are different among restraint procedures. Given that chair restraint imposes higher stress levels to subjects compared with other restraint procedures, we conclude that the high glucose excursion induced by chair restraint is attributed, at least in part, to the increase in plasma cortisol levels.

P329 Comparison of 2 Different Hamster Models Used for the Determination of Testosterone and Finasteride Activity

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Our goal was to compare the effect of testosterone (T) and finasteride (F) treatments on the diameter of the pigmented spot of hamster's flank organs as well as on the weight of seminal vesicles and prostate glands in 2 different animal models: prepuberal and adult hamsters. Prepuberal and adult male Syrian golden hamsters were gonadectomized. Castrated hamsters were housed in microisolation cages, and after 7 d of maintaining these conditions, prepuberal hamsters were injected daily with T or T plus F for 6 d. Gonadectomized adult hamsters were maintained for 30 d in a room under controlled conditions of temperature, light:dark cycles, and humidity. Food and water were provided ad libitum. After 30 d, adult hamsters were treated in a similar way to the prepuberal hamsters. Following the treatment, hamsters were euthanized with CO_2. The diameter of the pigmented spot of the flank organs from the animals of each group was measured. The weight of prostate and seminal vesicles from the animals of each group was determined. The treatments administrated to prepuberal hamsters, after 7 d of castration and adult hamsters after 30 d of castration showed similar results in the growth of prostate and seminal vesicles. However, the flank organs' response to F from prepuberal hamster was different than that observed for the flank organs from adult hamsters. Daily injections with T plus F in prepuberal castrated hamsters did not decrease the diameter of the flank organs, thus suggesting that the 5α-reductase enzyme is not present in these glands. The flank organs from prepuberal hamsters are a good model for the determination of the androgenic and antiandogenic activity. However, this assay is not suitable for the determination of 5α-reductase inhibitory activity of different steroids in vivo. Furthermore, both adult and prepuberal hamsters' prostates and seminal vesicles are good models for the determination of the androgenic, antiandrogenic, and 5α-reductase inhibitory effects in drugs.

P330 Huntington Disease Model in Transgenic Nonhuman Primates

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Huntington disease (HD) is a neurodegenerative genetic disorder affecting over 15,000 Americans and has no cure. HD is caused by cytosineadenine-guanine (CAG, translated into glutamine) trinucleotide repeats in the first exon of the human gene, huntingtin (HTT). Despite transgenic HD rodent and drosophila models being the common animal models for HD research, it is evident that an animal model such as a nonhuman primate, with physiology, cognitive, and behavioral impairments comparable to humans and similar genetic defects that lead to HD is imperative for elucidating HD pathogenesis and developing cures. They also present HD clinical features such as rigidity, seizure, bradykinesia, and chorea that other models are unable to imitate. In this cited research, the development of a transgenic model of HD in 130 mature rhesus macaque oocytes, their progression from oocyte to birth and validation of the expression of polyglutamine-expanded HTT is documented and reported. Key HD features were observed in the brains of the HD transgenic monkeys. Confirmation of transgenic status of HD monkeys was done by PCR and Southern blot analyses on tissue samples. Immunohistochemistry and Western blot were the techniques used to confirm the expression of the mutant HTT gene. The expression of GFP gene used to illuminate HTT, was visualized under a Sky-blue II epifluorescent light. Motor impairment was evaluated by the HD primate model rating scale. The latest development of transgenic HD primates has unlocked a new era of animal modeling that represents human genetic disorders such as HD more clearly, which will accelerate drug development and production of diagnostic tools, and identify novel biomarkers through studies including gene expression and noninvasive imaging.

P331 Behavioral Tests Differentiate between Transgenic Animal Models of Neurodegeneration

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Patients with neurodegenerative disorders such as Alzheimer disease, Parkinson disease, multiple system atrophy, and tauopathies present with a number of different clinical symptoms including motor and memory dysfunction and olfactory deficits. Transgenic animal models of these disorders have been generated that mimic many of the neuropathological features of the disease. Additionally, these animal models recapitulate many of the behavioral deficits observed in the human disease. We present behavioral data from a number of transgenic animal models of neurodegeneration and examine the relative types of behavioral deficits present in each model. We demonstrate that our inhouse behavioral testing paradigms comprised of tests examining olfaction, motor behavior and memory are able to differentiate between animal models that exhibit motor deficits (models of Parkinson disease and multiple system atrophy) and those that display deficits in learning and memory (model of Alzheimer disease). As a number of models exhibit a particular phenotype, we present the particular deficit relative to nontransgenic controls, allowing the degree of impairment to be assessed.

P332 Animal Model of Parkinson Disease: Unilateral 6-Hydroxydopamine Lesion of the Nigrostriatal Pathway

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The neurotoxin 6-hydroxydopamine (6-OHDA) is administered unilaterally to destroy central dopamine neurons in order to study neurodegenerative processes associated with Parkinson disease. Unilateral destruction of these neurons causes a chemical imbalance of the brain's content of dopamine. The 6-OHDA is delivered surgically into the nigrostriatal pathway of the brain determined by stereotaxic coordinates. Animals are evaluated postoperatively by administering a dopamine agonist, apomorphine (0.2 mg/kg). The apomorphine stimulates intact dopamine neurons in the unaffected brain hemisphere. This manifests clinically into animals that rotate in the direction contralateral to the lesion. At 14 d post lesion, animals are evaluated with apomorphine and are acclimated to the rotational activity for 15 min. The number of rotations is recorded for each animal. A second evaluation with apomorphine is conducted at 21 d post lesion. Rotations are counted, and animals with greater than 180 rotations or multiple 5-min periods of greater than or equal to 6 rotations per minute in 30 min are considered successfully lesioned. Animals with a demonstrable lesion can be used to assess the efficacy of therapeutic agents which may be used in the treatment of Parkinson disease. This animal model also offers similarities in key features of the human condition, including derangements in gait, which may be valuable to investigations into the disease pathogenesis, progression, and treatment.

P333 Strategies to Improve Antiprion Potency and Reduce Toxicity of Polylysine

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Prion diseases are a group of neurodegenerative disorders including Creutzfeldt–Jakob disease in humans, bovine spongiform encephalopathy, sheep scrapie, and chronic wasting disease in cervids. The causative agents, termed prions, are composed of the disease-associated scrapie prion protein (PrP^{Sc}), a misfolded isoform of the normal cellular protein (PrP^{C}). These diseases pose the potential risk to public health; however, there is no cure or treatment for these devastating diseases. In previous research to develop antiprion agents, polymers of lysine (poly-L-lysine; PLK) exhibited inhibitory effects on prion propagation in both cell-based and animal models of disease. To reveal PLK-based compounds with improved potency and reduced toxicity, we attempted to explore 2 independent strategies: application of 1) the stereoisomers of polylysines and 2) PLK modified with polyethylene glycol (PEG). Comparative antiprion activity and cytotoxicity assays were performed in prion-infected cell culture (ScN2a cells) by using Western blotting that measures the level of PrP^{Sc} and the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay that measures cell viability. We found that poly-D-lysine (PDK), the stereoiosomer of PLK, exhibited improved potency to suppress PrP^{Sc} propagation than PLK. Furthermore, complete elimination of PrP^{Sc} in the cells incubated with PLK and PDK was achieved within the noncytotoxic concentration range, although PDK was more toxic than PLK in our assays. We also found that PEG-PLK did decrease cytotoxicity of PLK and still retained the activity to inhibit PrP^{Sc} propagation below the detection limit, although a higher concentration of PEG-PLK was needed to accomplish the same level of antiprion activity of PLK. Our study using PDK and PEG-PLK may offer potential options to consider toward future development of antiprion therapy.

P334 Clinical Scoring System for the Evaluation of Autophagia in Laboratory Rats

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Self-mutilation and autophagia are common research complications following spinal cord and peripheral nerve injuries in laboratory rats. Clinical management of these cases involves regular monitoring and evaluation of affected rats, treatment, and for moderate to severe cases, euthanasia. This evaluation is often performed by both animal care technicians and research personnel, who may have conflicting opinions on the severity and appropriate disposition of cases. We developed a clinical scoring system to give evaluators a list of parameters to assess at each evaluation, to track either disease progression or healing, monitor the effectiveness of treatment plans, and establish a consistent humane endpoint. The scoring system has evaluators assess 5 different parameters: overall condition of the rat, degree of lameness as a result of the autophagia, amount of foot involved, amount of swelling, and integrity of the wound. A score of 0 to 3 is assigned for each parameter. By summing the score from each parameter, a total score is assigned for each animal. If any rat has a total score of 8 or higher or has a score of 3 in at least 2 parameters, euthanasia is recommended. To measure the effectiveness and consistency of the system, we had 3 evaluators score a group of 13 rats with varying degrees of autophagia on 2 different days. The result was that 88% of the scores were within 2 points of each other, with a standard deviation of 0.8. This scoring system resulted in different evaluators being able to consistently evaluate an animal with autophagia to track its progress and measure its response to treatment. This scoring system should enhance animal welfare by establishing humane endpoints that can be clearly described in animal use protocols and used to train animal care and research personnel.

P335 Special Care for Experimental Allergic Encephalomyelitis Mice

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Experimental allergic encephalomyelitis (EAE) is a model for multiple sclerosis (MS). Although the etiology of MS is unknown, the study of EAE animal models and the immunologic assessments of MS patients support the concept that MS is an autoimmune disease mediated by autoreactive CD4 T cells with specificity for myelin antigens. Stress reduces EAE susceptibility in mice by suppressing the immune system. Therefore, during EAE induction, mice should be housed in a quiet environment, without excessive noise or vibration. Excessive handling of mice should be avoided, and mice should be handled gently at all times. EAE can assume an acute, chronic, or relapsing-remitting disease course that is dependent upon the method of induction and the strain of animal used. In general, EAE disease course includes escalating degrees of ascending animal paralysis, dysphagia due to lesions in cranial nerves and lingual paralysis. Dysphagia usually results in mice euthanasia due to excessive body weight loss (> 20% of baseline). In order to prevent animal weight loss and abrupt termination of the experiment, at the onset of limb paresis we measure body weight of EAE mice at least 3 times a week. When the CNS signs such as limb paresis or paralysis observed, we check animals daily for symptoms of tongue paralysis and difficulty in swallowing. Once we determine animals are unable to swallow, we deliver soft food via oral gavage and fluid by subcutaneous saline injections at least 2 times a day. The care of paralyzed EAE mice with ability to eat and drink includes: placing food on the floor, providing water bottles with long sipper tubes, and expressing urinary bladder

2 times a day if necessary. The special care of EAE mice is crucial not only for animal welfare but also for unnecessary termination of the study due to excessive body weight loss.

P336 Beagle Model of Alzheimer Disease: Neurodegenerative Disorder

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The genetics of Alzheimer disease are linked to the deposition of amyloid β-protein in the brain of people. A beagle model of Alzheimer disease was developed by the somatic cell nuclear transfer (SCNT) in combination with transgenic technology that allows insights of human genetic diseases through genetic manipulation. Alzheimer disease (AD) is a common age-related neurodegenerative disorder, which is mainly caused by mutations in the amyloid precursor protein gene (APP) associated with beta amyloid (Aβ) plaque deposition as well as neurofibrillary tangles in brain tissue. Amplified dog Thy1 promoter and mutated human APP gene were ligated into basic pGL3-Basic vector linked with the fluorescent protein with (EGFP serving as a marker). This combined genetic construct was transfected into a primary canine fibroblast cell line. After several passages, continuous EGFP expressing cells were confirmed by PCR and frozen until nuclear transfer. A total of 332 in vivo matured canine oocytes were surgically collected from 29 dogs and recombined with a transgenic donor cell. The reconstructed embryos were surgically transferred into the fallopian tube of naturally synchronized surrogate beagles. Four dogs became pregnant and resulted in giving birth to 6 healthy live puppies. Five of the 6 live puppies were successful with this genetic transfer as expressed green fluorescence on their nails, toes, and white hairs. RNA samples from various parenchymal organs expressed canine APP mRNA in multiple parenchymal organs. These transgenic puppies serve a new model of Alzheimer Disease for behavioral and genetic influences of this human neurodegenerative disorder.

P337 A Canine Lumbar Cerebrospinal Fluid Collection Model to Study Biomarkers in Alzheimer Disease

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Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by excessive production of amyloid plaques and neurofibrillary tangles in the brain. Additional hallmarks include synaptic damage, and neuronal and brain atrophy leading to memory and cognitive impairments. Studies have shown that tau and amyloid β (Aβ) protein are altered in the cerebrospinal fluid (CSF) of patients with AD. To support the investigation of CSF biomarkers, a reliable, reproducible chronic system was investigated to collect lumbar CSF from conscious dogs. This model had to simulate human studies where biomarkers are routinely measured in lumbar CSF and plasma to support AD clinical programs. Several procedures have been published for accessing lumbar CSF, including use of lumbar taps, percutaneous indwelling lumbar catheters, and cisterna magna catheters advanced to lumbar region. We elected to use a lumbar catheter/vascular access port model to collect lumbar CSF. Although the surgical model is not novel, it is difficult to perform and maintain. We evaluated modifications to the procedure and maintenance to increase patency. Different catheter materials were evaluated, including catheter sizes, tips, fenestration, and entry into the lumbar subarchnoid spaces. For our purposes, a 3.5-Fr open ended, nonfenestrated polyurethane catheter was inserted into the L3 subarchnoid space and advanced to the L1 region to collect lumbar CSF. With our final modified surgical procedure, 10 of 13 animals (77%) were successfully catheterized. A reported average surgical success rate for various surgical models is 60%. At this writing, our model's patency average is over 150 d, with reported lengths of patency averaging 90 to 120 d. The longest length of patency of our model at this time is over 180 d. Few minor complications were encountered. This included temporary monoparesis and transient blood-tinged CSF. The catheter failure rate was low (30% compared with reported 40% to 50%). The model was characterized and validated by collecting serial lumbar CSF and blood samples after compound dosing for analysis of Aβ, tau, and other CSF biomarkers. Based on the results of the proof of concept studies, our model proved to be useful for pharmacokinetic studies in a search for effective AD treatment.