Platform Sessions

PS01 Appendectomy at an Early Age Is Not Protective against Typhlocolitis in Helicobacter hepaticus-Infected B6.129 TCRα−/− or TCRβ−/− Mice

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The potential for appendectomy at an early age to lower the risk for ulcerative colitis (UC) or increase risk for Crohn’s disease is controversial in human medicine. This paradigm has been modeled in conventionally maintained TCRα−/− mice that were protected against UC-like disease by appendectomy performed between 3 and 5 weeks of age. Typhlocolitis spontaneously develops in conventionally maintained TCRα−/− or TCRβ−/− mice and there is evidence from germ-free studies that disease results from an aberrant host response to intestinal flora. Experimental infection of helicobacter-free TCRα−/− or TCRβ−/− mice with Helicobacter hepaticus has been shown to reproducibly induce typhlocolitis. We tested the hypothesis that appendectomy in weanling mice (4 weeks of age) would decrease the severity of typhlocolitis in B6.129 TCRα−/− or TCRβ−/− mice naturally infected with H. hepaticus. Mice born to helicobacter-free or H. hepaticus-infected dams were randomized to experimental groups that underwent appendectomy at 4 or 12 weeks of age or did not have surgery (n = 10 per group). Maintenance of helicobacter-free status or horizontal transmission of H. hepaticus from experimentally infected dams to pups were confirmed by PCR of feces. Necropsy at 6 months of age revealed that H. hepaticus infection induced severe typhlocolitis in both TCRα−/− and TCRβ−/− mice. Gross changes included thickening of the cecal-colic junction and proximal colon along with enlarged mesenteric lymph nodes and proctitis. Histologically, there was severe chronic inflammation and hyperplasia. Appendectomy had no protective effect in H. hepaticus-infected mice and no adverse effect in helicobacter-free mice. These results suggest that early appendectomy is not protective against IBD-like disease in the TCRα−/− or TCRβ−/− mouse model when a known enterohepatic pathogen is used to exacerbate onset and persistence of inflammation.

PS02 Helicobacter hepaticus Infection Induces Regulatory T Cells and Prevents Inflammatory Bowel Disease in mdr1a−/− Mice

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Mdr1a−/− mice lack the membrane efflux transporter, p-glycoprotein, and develop spontaneous inflammatory bowel disease (IBD) (JI 161:5733, 1998). We have shown that onset of disease in mdr1a−/− mice can be accelerated by Helicobacter bilih infection and H. hepaticus can delay the development of IBD in this model (AJP 160:739, 2002). We hypothesized that H. hepaticus may be preventing or reducing severity of IBD in these mice via induction of regulatory T cells as has been shown in a cytokine-deficient mouse model of IBD (JEM 196:505, 2002). To test this hypothesis, 3- to 4-week-old female mdr1a−/− mice were infected by oral gavage with H. hepaticus (2 × 107; ATCC 51448) or broth. Four weeks after infection, CD4+CD45RB− mesenteric lymph node T cells (5 × 106) from either H. hepaticus-infected or broth-treated mice were transferred (day 0) into 4- to 7-week-old female recipient mdr1a−/− mice. Recipient mice were infected with H. bilih (day 2) to trigger IBD. Sixty percent (3/5) of the mice receiving H. hepaticus-induced CD4+CD45RB− T cells were completely protected from disease by both clinical and histopathological assessment at 8 weeks postinfection (P = 0.016 when compared to mice receiving broth-induced CD4+CD45RB− T cells). In a second experiment, regulatory T cell phenotype was further defined by examining whether CD4+CD45RB− CD25+ (1 × 106) or CD4+CD45RB− CD25− (3 × 106) T cells were most efficient at preventing disease. Small numbers of cells transferred in this experiment did not provide the complete protection noted with larger cell transfers. However, it was evident clinically (weight loss, hydration, diarrhea) that the mice receiving H. hepaticus-induced CD4+CD45RB− CD25+ T cells were less affected than those receiving H. hepaticus-induced CD4+CD45RB− CD25− T cells. Our studies indicate that H. hepaticus can induce regulatory T cells that protect immunocompetent mice from IBD. All studies were approved by the IACUC.

PS03 Transcriptional Profiling in Enterohepatic Helicobacter spp.-Infected C57I Mice: A Gene Expression Pattern Consistent with Cholesterol Gallstones and Biliary Cancer Risk

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In humans, cholesterol gallstones are a common and costly disease caused by interaction of multiple environmental factors and susceptibility loci. Moreover, cholesterol gallstones increase the risk for all biliary cancers four- to fivefold. Recently, we demonstrated that colonization with enterohepatic Helicobacter spp. played a significant role in cholelithogenesis in the diet-induced C57L mouse model. When fed a lithogenic diet, Helicobacter spp.-infected mice (n = 9) developed cholesterol gallstones at 78% prevalence, whereas uninfected mice (n = 9) did not develop gallstones (P < 0.005). To elucidate
the pathophysiological mechanisms underlying this phenotype, we performed Affymetrix microarray analysis on livers from Helicobacter spp.-infected and uninfected mice consuming the lithogenic diet. Approximately 175 of 6000 genes were differentially expressed (>1.8-fold difference) in infected mice. Of particular note were genes encoding proteins involved in cholesterol homeostasis including Lss (lanosterol synthase, > 3-fold increase), Apob (apolipoprotein B 100/48, > 2.5-fold increase), and Cyp7a1 (cholesterol 7α hydroxylase—the rate limiting enzyme in bile salt synthesis, > 2.3-fold increase). Therapeutic inhibition of lanosterol synthase, an enzyme involved in de novo cholesterol synthesis, significantly decreases the prevalence of cholesterol gallstones in C57L mice. Consistent with upregulation of Apob, increased hepatic clearance of cholesterol occurs in gallstone-susceptible mice and polymorphisms in APOB in humans are associated with cholesterol gallstones. Increased expression of CYP7A1 occurs in humans with cholesterol gallstones and is likely a result of positive feedback inhibition from decreased enterohepatic cycling of bile acids, presumably from increased loss of conjugated bile acids. Also of interest are Hpqg2 and Hmgat1 (perlecain and high-mobility group protein, each > 2.3-fold increase), which are upregulated in human livers with cholangiocarcinoma but not hepatocellular carcinoma. Consistent with these findings, gallbladders of infected mice demonstrated premalignant biliary lesions including hyalinosis, intestinal-type mucous metaplasia, hyperplasia, and dysplasia. This study begins to elucidate a mechanism for Helicobacter spp. inducing cholelithogenesis, and is the first to raise the possibility that cholesterol and Helicobacter spp. may interact to induce biliary cancers.

PS05 Immune-Mediated Cholangitis in T eff Mice: A Potential New Model of Primary Biliary Cirrhosis

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Colorectal cancer is believed to result from irreversible genetic changes culminating in uncontrolled growth and neoplastic invasion. We have previously shown that anti-inflammatory CD4+CD25+ regulatory lymphocytes disrupt progression of epithelial tumors in the lower bowel of H. hepaticus-infected 129/SvEv Rag2−/− mice by an IL10-dependent mechanism. This suggested that these adaptive immune cells and their cytokines play an important role in regulating colon cancer growth. Here we show that adoptive transfer with CD4+CD25+ regulatory lymphocytes induced regression of neoplastic invasion in Rag2-deficient mice with established mucinous carcinoma (n = 12). Further, intraperitoneal treatment with IL10-Ig fusion protein alone was sufficient for complete reversion to normal mucosal morphology in these mice (n = 10). It remains to be determined whether regulatory lymphocytes, and IL10 in particular, abrogate neoplastic invasion by inhibiting host inflammatory response or through other mechanisms. These studies provide further evidence at immune-modulated regression of neoplastic invasion and suggest novel immune-mediated mechanisms of induction, prevention, and treatment of colon cancer.

PS06 CD4+CD25+ T Cells Exacerbate Helicobacter hepaticus-Induced Colon Cancer in RAG-Deficient Mice with Concomitant Increase in Nitric Oxide (NO) Production

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Colon cancer risk is significantly higher in humans with inflammatory bowel disease (IBD) including ulcerative colitis and Crohn’s disease. Several studies in this field have shown that both innate and acquired immunity are important in pathogenesis and prevention of IBD and colon cancer. Recent studies in our laboratory have established a mouse model to study roles for innate and adaptive immune factors in progression of IBD and colon cancer. Experimental infection of Rag-deficient 129/SvEv mice with a widespread natural mouse pathogen, H. hepaticus, leads to colitis-associated colon cancer. Previously we have shown that development of IBD and colon cancer in these mice can be prevented by adoptive transfer of CD25+ T cells, a subset of regulatory T lymphocytes with characterized anti-inflammatory functions, by an IL10-dependent mechanism. Here
we report that adoptive transfer of CD4+CD45RBhigh T cells (effector T cells) into H. hepatis-infected syngenic recipients (6 male and 6 female mice, of 6-8 weeks of age) precipitates rapid onset of severe IBD and colon cancer. Adoptive transfer of effector T cells at the time of infection with H. hepatis resulted in increased inflammation and a higher frequency of colon cancer in recipient mice in comparison to age-matched infected and untreated controls. Mucinous carcinoma characterized by locally invasive malignant epithelia was disproportionately increased in mice that received effector T cells. Interestingly, rapid development of IBD in mice injected with effector T cells paralleled higher levels of systemic nitric oxide (NO) production. In contrast, animals (4 male and 4 female mice, of 6-8 weeks of age) that underwent co-transfer with protective CD25+ subset showed reduced or no IBD or cancer, and no increase in NO levels. Further studies are necessary to determine specific roles for NO in microbially induced colon carcinogenesis.

PS07 Helicobacter bilis Infection to Elucidate the Role of MHC Class II and Dendritic Cells in Inflammatory Bowel Disease in CD11c Transgenic/MHCII/Rag2 Deficient (CD11cTgRII) Mice

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Inflammatory bowel disease is a complex immune mediated disease characterized by chronic enteritis. The CD4+CD45RBhigh adoptive transfer (AT) model of colitis and Helicobacter based on our previous work has been used to understand the role of T cells and bacteria in this process. To dissect pathways associated with MHC Class II antigen processing in IBD, we generated a transgenic model in which Class II expression is directed to dendritic cells via the CD11c promoter on a Class II-/- genetic background. CD11cTgClass II-/- mice were backcrossed onto Rag2-/- mice to eliminate the presence of regulatory T or B cells and these CD11cTgRII mice were used as recipients in an AT assay using H. bilis to trigger disease. Two replicate experiments were done using 3- to 5-month-old male and female CD11cTgRII and nontransgenic (nTg) mice with AT cells derived from spleens of C57BL/6 mice. Mice were orally given Hb (2×10^6) or broth 1 week prior to AT of 2×10^5 CD4+CD45RBhigh cells (intraperitoneal injection). Experimental groups (n = 5-13) comprised a) CD11cTgRII mice+Hb+AT, b) CD11cTgRII+Hb, c) CD11cTgRII +AT, d) nTg+Hb+AT, e) nTg+Hb, f) nTg+AT and g) Rag2-/- +Hb+AT. Mice were housed under SPF conditions, monitored for diarrhea and weight loss, and euthanized by CO₂ at 2-4 weeks post-AT. Large bowel was graded histopathologically with a maximal pathology score of 80. CD11cTgRII mice infected with Hb+AT had the most severe colitis (mean score = 57.7) and were similar to positive control Rag2-/- mice (mean score = 55.3). CD11cTgRII mice given AT alone (mean score 8.8) or Hb alone (mean 19.6) had colitis that was significantly less severe (P = 0.004 and P = 0.005, respectively). These studies demonstrate the role of dendritic cells, Class II, T cells, and bacteria in the pathogenesis of IBD. Interestingly, Hb was able to trigger mild colitis (mean scores = 10.8 and 11.8) in nTg CD11cTgRII mice in the absence or presence of AT cells, demonstrating that Helicobacters can trigger disease in animals lacking both T and B cells, and Class II expression. These findings suggest that Helicobacters can cause large bowel disease by multiple pathways that involve innate and/or adaptive immune responses. Studies were funded by NIH R01 DK056204-07.

PS08 Serological and Nucleic Acid Sequence Characterization of a Newly Identified Mouse Parvovirus Strain

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The murine parvoviruses, including mouse parvovirus (MPV) and mice minute virus (MMV, also known as MVM), remain a significant concern to those working with laboratory animals because of their disruptive influence on experimental results. In the course of developing a recombinant antigen ELISA based upon the virus capsid protein VP2 from an isolate of MPV, significant differences in VP2 gene sequences were seen between the MPV-1 type strain and the isolate, which has been given the designation MPV-2 (isolate MPV-2a). To determine the prevalence of MPV-2, three specific ELISAs using recombinant VP2 antigens from MPV-1, MPV-2, and MMV were used to screen 2,345 mouse serum samples. A total of 28 samples tested positive by these assays: 19 (68%) by both the MPV-1 and MPV-2 ELISAs; 4 (14%) by MPV-1 alone; 4 (14%) by MPV-2 alone; and 2 (7%) by MMV in combination with one or both MPVs. A generic parvovirus ELISA using the nonstructural protein NS1 (which is highly conserved between strains) from MPV-1 as antigen tested positive for 24 (86%) of the 28 samples. In addition to the serological assays, three strain-specific fluorigenic PCR assays were developed to target the VP2 genes of MPV-1, MPV-2, and MMV. Archived samples which had previously tested positive for murine parvoviruses by a generic PCR assay targeting the conserved NS1 gene were retested by the strain-specific assays. Of 211 samples, 109 (52%) were positive for MPV-1, 36 (17%) for MPV-2, 59 (28%) for MMV, and 19 samples (9%) tested positive by more than one PCR assay. These preliminary results suggest that MPV-2 is distinct from other mouse parvoviruses both serologically and at the nucleic acid sequence level, and that it may not be detected by current serological assays targeting MPV-1 VP2 or NS1 antigens. The VP2 gene sequence of MPV-2a has been submitted to GenBank and is available as accession AY567965.

PS09 Virus-Like Particles as Antigen for Serologic Detection of Rat Parvovirus Antibodies

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Recombinant rat parvovirus structural protein (RPV VP2) was produced using a baculovirus expression vector system (BEVS). The protein RPV-VP2 was produced using expresSF+ insect cells in serum free media and wave bioreactor. The recombinant baculovirus expressed protein self-aggregates to form virus-like particles (VLPs) and resembles in structure rat parvovirus (RPV) capsids. The RPV VLPs were found to be present in both cells and culture supernatants. Cell lysate and/or culture supernatants were concentrated and washed with PBS-sarkosyl using hollow fibers. Further processing and purification of the VLPs was done using a combination of ultracentrifugation, enzyme treatment, detergent washing and Cesium chloride gradient purification techniques. Purified VLPs were analyzed by enzyme linked immunosorbent assay (ELISA), polyacrylamide gel (SDS-PAGE), electron microscopy and western blot techniques. Qualified VLPs were used as ELISA antigen for serologic detection of RPV VP2 antibodies. RPV serol-
ogy was done using sera from rats experimentally infected with RPV virus and field sera from serology. RPV VLP ELISA picked up seroconversion on day 7, much earlier than day 28 for non-structural protein (NS-1) ELISA. Sera samples from an outbreak of RPV in a rat colony were tested by RPV VLP ELISA and NS-1 ELISA on three different weeks; of these, 7/8, 12/16 and 31/32 samples tested positive by RPV VLP ELISA. Comparatively, only 0/8, 5/16 and 15/32 sera samples tested positive by NS-1 ELISA. Results from the field trials suggest that ELISA using RPV VP2 VLPs is highly sensitive and specific in detecting RPV VP2 antibodies in rat sera.

PS10 Use of Polymerase Chain Reaction in a Rodent Health Monitoring Program
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Disease prevention and monitoring programs for rodents historically use serological tests to detect antibody to specific viruses in sentinel or colony rodents, and animal inoculation tests as indirect tests for murine viruses in biologicals for in vivo use. During investigations of positive serological tests for mouse parvovirus (MPV) or mouse minute virus (MMV) in sentinel mice, polymerase chain reaction (PCR) to detect viral DNA was evaluated. Mouse antibody production (MAP) tests plus PCR for MPV/MMV DNA were used to screen 59 biologicals. Two were negative for MPV and MMV on MAP tests, and positive on PCR tests. One had a “nearly positive” lactic dehydrogenase virus on MAP and negative on PCR. Sentinel mice occasionally gave positive or equivocal serological tests for MPV or MMV. Follow-up included room quarantine, re-bleed positive or equivocal sentinel, bleed cage mate, and harvest mesenteric lymph node (MLN). Sera were tested in parvovirus profile and MLNs were tested by PCR. Several mice with equivocal serological parvovirus profiles were positive by PCR. To determine if MPV/MMV DNA was present in the environment of areas where positive mice were housed or their tissues used, sterile swabs were dipped in sterile saline, wiped across “high-risk” environmental surfaces, and sent to commercial diagnostic laboratory to test for MPV/MMV DNA using PCR. Forty-eight swabs were taken in the mouse room and contiguous procedure rooms, and 13 swabs were taken from surfaces in the investigator’s laboratory. Four swabs (computer keyboard, door handle, procedure room floor, and floor/casters in animal room) from the animal facility were positive. No swabs from the laboratory were positive. Biologicals for in vivo use are now screened for a panel of murine viruses using PCR. PCR is faster and cost effective compared to MAP test. PCR tests are also a useful tool in investigation of positive serological tests from sentinel mice.

PS11 Molecular Epidemiologic Analysis of Murine Parvovirus Field Strains
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High-fidelity PCR amplification of a hypervariable region of the viral capsid gene with subsequent restriction fragment length polymorphism (RFLP) analysis was used to distinguish strain variants of minute mouse virus (MMV) and mouse parvovirus-1 (MPV-1). Evaluated were tissue or fecal samples obtained from naturally infected mice (n > 200) from numerous institutional sources. Approximately 85% of these samples were MPV PCR-positive, ~20% were MMV PCR-positive, and ~5% were PCR-positive for both MMV and MPV. RFLP analysis of MMV PCR-positive samples indicates the existence of an MMV strain distinct from the well-characterized prototype (MMVp), immunosuppressive (MMVi), and Cutter (MMVc) strains. Alignment of the predicted VP1 amino acid sequence indicates that this MMV variant has similar relatedness to MMVp, MMVi, and MMVc at 97.0%, 96.8%, and 96.7% amino acid identity, respectively. The vast majority of MMV PCR-positive samples displayed an RFLP banding pattern consistent with this novel MMV variant, with only a few samples displaying a banding pattern consistent with MMVc. RFLP analysis of MPV PCR-positive samples indicates the existence of an MPV strain distinct from MPV-1. Approximately one-third of the genome of this MPV variant has been sequenced, and when compared with characterized rodent parviruses, the sequence of the novel variant is most closely related to hamster parvovirus (96.1% sequence homology) and MPV-1 (95.4%). The majority of MPV PCR-positive samples displayed a banding pattern consistent with MPV-1, with approximately 25% of the samples displaying a banding pattern consistent with the novel MPV variant. These studies indicate that, although MPV-1 appears to be the predominant strain of murine parvovirus circulating in contemporary laboratory mouse colonies, there exists at least two other murine parvovirus field strains circulating that are distinct from the well-characterized murine parviruses.

PS12 A Comparison of Surgical Techniques for Telemetry-Based Measurement of Respiration Rate in Canines and Nonhuman Primates
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Assessment of respiration rate (RR) in conscious dogs and nonhuman primates (NHP) has been achieved by implantation of a pressure catheter to detect pleural pressure (PP) changes either beneath the serosal surface of the esophagus within the thoracic cavity via the abdomen (in the canine and NHP) or intercostally between a rib and costal pleura (in the NHP). This study describes techniques for esophageal catheter placement in the canine and NHP and compares surgical methods and changes in PP to those obtained using a modified intercostal approach in the NHP. In the NHP, the transmitter was implanted IM in the flank region. Esophageally, the catheter was passed from the transmitter to an incision made on the neck. An incision was made in the serosal layer of the esophagus and the catheter was advanced caudally beneath the serosal layer into the thoracic cavity then secured to the esophageal wall. Intercostally, the catheter was passed to an incision made on the thoracic wall over the seventh-eighth intercostal space, positioned beneath the eighth rib and periosteum (against the costal pleura), and secured to intercostal muscle. Through 30 weeks post-surgery, data from both catheter placements verified detectable changes in PP in the NHP (during inspiration) allowing respiratory quantifications. In the canine, the transmitter was implanted in the abdomen via midline laparotomy. An incision was made in the serosal layer of the esophagus at the level of the cardiac sphincter, and the catheter was advanced cranially beneath the serosal layer into the thoracic cavity. The catheter was then secured to the esophageal wall. Through 12 months post-surgery, data from the esophageal placement verified detectable changes in PP in the canine (during inspiration) allowing for the quantification of respiratory data. These methods describe relatively simple procedures that permit quantifiable data to be collected for determi-
nation of RR in conscious dogs and monkeys. Further evaluation of this data in these models may provide further justification for integrated cardiovascular and respiratory safety endpoints that can be assessed by telemetry in conscious dogs and nonhuman primates.

PS13 An Automated Blood Sampler for Rat Pharmacokinetic Studies

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Pharmacokinetic studies typically involve labor-intensive and time-constrained sampling of small volumes of blood from rodents. Manpower needed for timed sample collection and limitations on sampling time points are two common problems faced in traditional pharmacokinetic studies. In a side-by-side study, blood samples were collected using tethered manual sampling compared to an automated sampler. Rats were carotid catheterized for both methods and blood samples collected just prior to and at 1 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 12 h, 18 h and/or 24 h post-compound administration. All withdrawn blood volumes were replaced with sterile heparinized saline for both methods. The automated blood sampler was used to collect blood from freely moving rats and samples were stored in a refrigerated fraction collector until used. The automated sampling method was evaluated for manpower savings, time point flexibility, functionality and sample compound analysis. Results of this study indicate automated blood sampling technology can be used to streamline traditional pharmacokinetic studies by providing manpower savings and access to sampling time points when technical staff is not available while maintaining functionality and sample stability.

PS14 Practical Experiences with Library-Style Rack Systems in Rodent Housing: Better Cost Efficiency by Increased Caging Density

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During the past 15 years, individually ventilated caging systems (IVCS) have found steadily increasing use in rodent housing, particularly for genetically engineered mice. Recent technical developments in IVCS have aimed at achieving higher stocking densities. A major step in this direction has been the development of library-style rack systems (LSRS) which, as the name implies, function in a manner analogous to book storage systems in libraries. LSRS significantly increase cage capacity by 30-70% depending on the cage type used (about 23 to 30 cages per square meter floor area). This requires larger animal rooms (about 60-80 m²) to provide enough space for moving the racks sideways apart and together during animal care. The rooms are equipped with units consisting of two double-sided racks connected to a blower, which are fixed to wall guides. At the German Research Center for Biotechnology, we have been operating a mouse-housing unit equipped with LSRS since October of last year. The unit consists of one room, with dimensions of 15 × 6.5 m (97 m²), outfitted with nine caging units or a total of 1,728 cages (size 365 × 207 × 140 mm).

The following characteristics and advantages of LSRS have been observed:

- Improved space utilization enables the housing of more mice in smaller animal facilities
- Savings in building, energy and technical maintenance costs
- Well accepted by the care staff
- Stimulates teamwork during cage service
- Good access to the cage and easier rack movement within the room
- No differences in breeding performance observed with different mouse lines

In conclusion, the use of LSRS, which are ergonomically designed and easy to handle, maximize cage density in the mouse house, resulting in a better cost efficiency (about 10% lower per diem rates) for the research institution. No problems regarding animal care or animal well-being have been observed.

PS15 Evaluating the Potential Impact of Nonhuman Primate Sound on Rodent Behavioral Studies in a Multi-Species Neuroscience Facility

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Exposure of laboratory animals to sound has well-documented effects on physiology and behavior. At a minimum, uncontrolled sound affects the validity and reproducibility of research data and in many cases has been shown to be deleterious to behavioral, reproductive and other studies. This consideration became a critical factor in the recent design and construction of a behavioral science facility. The building architects were able to identify several sound transmission pathways and recommended remediation for most of these pathways. The largest concern related to the type of sounds generated by macaques and their possible effects on rodent (rat) behavioral testing. A unique series of controlled acoustic recordings was conducted for Sound Pressure Level and Frequency (4-100,000 Hz) during the early phase of the new facility construction project to determine any potential impacts. These surveys measured sound in five locations for various activities in a similar room configuration of an existing facility. It was found that the most significant nonhuman primate (NHP) vocalization occurred between 30 and 20,000 Hz at an average amplitude of 30 dB above background noise in the housing room. The vocalization passed to the anteroom at frequencies between 500-10,000 Hz with an average increase of 11 dB from the background noise. No vocalization passed to the corridor or any adjacent areas. This series of testing was then replicated during the qualification phase for the behavioral study building. Similar testing results were achieved, and it could be demonstrated that NHP sounds would not affect testing activities within the building. The authors concluded that sound transmission beyond the NHP housing room was below the most sensitive range for rodents (12-25 kHz) and no types of sound would travel beyond the anteroom. This also confirmed the effectiveness of the facility design criteria and actual construction.

PS16 Initial Application of RFID Technology in the Vivarium

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Maintaining mice in a common housing room serving multiple protocols and investigators requires a reliable means to ensure that
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Ohio proficiency scores demonstrate that elementary and middle-school students struggle with basic science literacy. In both 2001 and 2002, only 43% of Ohio’s fourth and sixth graders passed the science portion of the state proficiency exam. Without knowledge of the basic fundamentals of science and healthcare, children cannot make informed decisions about bioscience issues including the use of animals in research. Animal rights advocates have historically relied on children’s lack of awareness and understanding of basic facts about health, science, and biomedical research in order to gain recruits for their agendas and activities. In 1999, the People for the Ethical Treatment of Animals (PETA) spent over $3 million on public outreach and education, enrolling more than 4,125,700 students into its programs and reaching over 170,000 teachers and schools with its anti-research message. Pre- and post-questionnaires were administered to the students. The questionnaires were designed to measure both factual and attitudinal impact resulting from the five-week Playbook curriculum. A comparative analysis of the pre- and post-test results will be presented in this paper. Data will be presented consisting of the summarization and analysis of pre- and post-survey responses to questionnaires from school children participating in the "Playbook . . . to better health" curriculum. Overall descriptive summaries of each question will be presented with a focus on questions pertaining to animal research.

PS18 Establishing aPTT and PT Reference Ranges for Coagulation Time in Rhesus Macaques Enabling On-Site Coagulopathy Recognition

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The objective of this study was to obtain activated partial thromboplastin time (aPTT) and prothrombin time (PT) values in normal healthy rhesus macaques using a point-of-care-coagulation analyzer (PCCA). These values were later used to compile a normal range table for aPTT and PT tests in this particular species. Rhesus macaques of Indian origin (n = 78) were divided into two groups: A) randomly chosen normal healthy animals (n = 67), and B) catheterized healthy animals (n = 11). There was no significant difference between aPTT or PT values within group A animals based on age or gender. The aPTT and PT mean times of this control group were 54.9 ± 4.6 sec, and 19.2 ± 1.6 sec, respectively. Group B was tested during routine preventative health care procedures. The maintenance of their catheters consisted of a daily heparinized saline flush (195 µl/ml). The mean aPTT time of this group was 89.9 ± 15.0 sec and the mean time of the PT test was 33.1 ± 7.3 sec. These coagulation times illustrated significant prolongation compared to those of the controls, P < 0.001. This study indicates that chronic administration of heparin to catheterized rhesus macaques significantly increases coagulation time as measured by the aPTT and PT tests. In addition, it validates the use of a point-of-care-coagulation analyzer for the rapid detection of potential coagulopathies in the rhesus macaque.

PS19 Long-Term Administration of Alcohol to Rhesus Macaques (Macaca mulatta): A Jacket-and-Tether System for Intragastric Catheterization

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At the Tulane National Primate Research Center (TNPRC) we developed a system for long-term administration of alcohol to rhesus macaques (Macaca mulatta) by intragastric catheterization. We developed this system by the request of investigators to study the effect of alcohol on the pathogenesis of Simian Immunodeficiency Virus (SIV) as part of the AIDS research program at the TNPRC. Here we describe and discuss advantages and disadvantages of the system.

The system consists of a surgically placed silastic intragastric catheter that is tunneled subcutaneously to exit the skin on the upper back of animal. All monkeys are fitted with a specially designed primate jacket to prevent the monkeys from disturbing the catheter. The catheter is then connected to Tygon tubing that runs within a stainless steel tether. The tether attaches at one end to the primate jacket and the opposite end to a cage-mounted swivel.

This system has been effective in the long-term administration of alcohol to rhesus macaques at the TNPRC. With the intragastric catheter, a precise amount of alcohol can be administered, allowing for tight control of blood alcohol levels in monkeys. In general, with proper acclimatization, monkeys tolerate the jacket and tether well.

We have faced several challenges in the implementation of this system. The most frequently occurring are catheter-associated problems such as infection and malfunction of other equipment. We have found that advantages of this system outweigh the difficulties associated with its complexity.
PS20 Use of Fresh Embryo Imports to Establish a New Animal Facility

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Institute of Animal Technology (IAT)

The establishment of the animal facility at the Wellcome Trust Sanger Institute would initially involve the transfer of mouse strains from the Baylor College of Medicine, Houston, Texas to our facility just outside Cambridge in the UK. Various options in moving the mice from the USA to the UK were considered including the import of live animals and frozen embryo transfer. Devastating floods in Houston that unfortunately included Baylor College resulted in a poor health status of the mouse colonies and made transfer of live animals a non-viable option. Frozen embryo transfer was discounted due to high cost associated with lack of in-house expertise. The decision, therefore, was taken to import the various mouse strains by fresh embryo transfer. Embryos were transported in 1ml cryovials containing a transport medium of Dulbecco Modified Eagle’s Medium plus 10% Foetal Calf Serum. The vials were placed between packs of wet ice in a polystyrene carrier which in turn was placed in side a cardboard transport box. Over a period of a year, 19 strains involving some 900 embryos were imported with an average recovery of at least 48%. Results of the technique employed have shown that with correct synchronisation of the time matings for both donor and recipient mice in the USA and UK respectively, fresh embryo transfer is a very viable way of importing mouse strains, utilising many less embryos than that required for frozen embryo transfers. This presentation will discuss how these fresh embryo imports were set up and the results that were achieved.

PS21 Intraoviductal Introduction of Plasmid DNA and Subsequent In Vivo Electroporation as a Possible Tool for Manipulation of Preimplantation Mammalian Embryo

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It has been known that humoral factors secreted from embryo itself and oviductal epithelial cells affect proliferation and differentiation of preimplantation mammalian embryos. Of these factors, TGF-b is a well-known factor providing beneficial effects on preimplantation embryos. However, the function of other known factors remains unclear at preimplantation stage. In this study, we developed in vivo evaluation system of genes of interest by in vivo electroporation (EP)-mediated gene transfer to oviductal epithelium. We directly introduced one ml of solution containing enhanced green fluorescent protein (EGFP)-expression plasmid (0.3-0.5 mg) and 0.05% trypsin blue into ampulla of the eCG-hCG-treated B6C3F1 females at E 0.5. The entire oviducts were then electropermed with tweezer type electrodes attached to the T820 electroporator (BTX Co. Ltd.) with 8 square pulses of 50 V in strength and 50 ms in wave length. On E 3.5, embryos at morula/early blastocyst stages were collected and their number, morphology and EGFP-derived fluorescence recorded. Furthermore, fluorescence in oviducts was also examined. In some cases, these fluorescent oviducts were subjected to cryostat sectioning. Strong fluorescence was observed in some of the oviductal epithelia. It was also observed in the lumen of the oviduct when cryostat sections were inspected. The number and morphology of the collected embryos were not affected by electroporation. Some embryos possessed fluorescence in their blastocoel, but not in their cytoplasm, suggesting incorporation of EGFP present in the oviductal lumen.

PS22 Ectromelia Virus in a Research Institution: A New Report and Follow-Up Investigations

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Mousepox is a disease caused by ectromelia virus that causes significant morbidity and mortality in laboratory mice. A recent diagnosis of mousepox in a research institution was linked to commercial mouse serum contaminated with ectromelia virus and used for cell culture and subsequent injection into mice. Clinical signs in affected mice were mild but included skin lesions on the rear feet and tails. The spleens and livers of some affected mice showed gross lesions, chiefly organomegaly and evidence of multifocal necrosis. Histologic lesions were seen in numerous organs and generally consisted of multifocal to coalescing necrosis with eosinophilic intracytoplasmic inclusion bodies. After eliminating the affected animals and avoiding an outbreak, we reproduced the disease using the suspected contaminated serum. Fifty-six mice (BALB/c, C57BL/6) were exposed to three different products by intraperitoneal, subcutaneous (footpad) or intranasal inoculation. Administered products included serum produced obtained from the same source as the recent incident, cell culture medium made with the suspected contaminated serum product, and cell cultures grown with the suspected contaminated medium. Mice were euthanized when clinical signs developed or at the conclusion of the study. Post-mortems were performed on all mice. Only mice injected with cell cultures and their cage contacts had antibodies to ectromelia virus and developed disease/lesions. Mice used as sentinels and exposed to contaminated bedding seroconverted, but did not develop disease/lesions. Presence of ectromelia virus was detected by polymerase chain reaction analyses in spleens of mice that had developed disease. Clinical signs and lesions in affected mice included lethargy, and ocular discharge. These findings corroborate and complement previous reports on the extent and distribution of lesions caused by ectromelia virus infection in mice, and highlight the limitations of Mouse Antibody Production (MAP) testing in the screening of ectromelia virus.

PS23 Simian T-Lymphotropic Virus-I (STLV-I) Proviral DNA in the Blood and Breast Milk of Lactating Female Rhesus Macaques (Macaca mulatta)

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Simian T-lymphotropic virus-I (STLV-I) is a retrovirus of the C-type oncornavirus subgroup that is a nonhuman primate (NHP) counterpart to human T-lymphotropic virus (HTLV). There are three well-described mechanisms by which HTLV is transmitted: 1) vertically and horizontally from mother to child, 2) venereally, and 3) via infected blood or blood products. Venereal transmission of STLV has been documented among captive Japanese macaques; however, other mechanisms of STLV transmission remain
undescribed. For this study, breast milk and blood samples were collected from a group of eight lactating Rhesus macaques that were seropositive for STLV-I by enzyme-linked immunosorbent assay (ELISA). Of these samples, 8/8 blood samples and 7/8 breast milk samples were positive for the presence of STLV-I proviral DNA by nested polymerase chain reaction (PCR) analysis. Similar samples from animals that were ELISA negative for STLV-I were uniformly negative by nested PCR analysis. This data presents possible mechanism(s) by which the perinatal vertical or horizontal transmission of STLV-I could occur from dam to offspring. Additional longitudinal tracking studies and PCR analysis on the offspring of seropositive dams are needed to establish these mechanism(s) of STLV-I transmission.

PS24 Assessment of a Rhesus MPTP Model of Parkinson’s Disease Using PET Imaging and Behavioral Analysis
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Parkinson’s disease is a degenerative neurological malady resulting from the loss of dopamine neurons in the substantia nigra. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is highly toxic to dopamine neurons of the substantia nigra and produces clinical features of Parkinson’s disease when administered to nonhuman primates. We developed a colony of rhesus monkeys which mimic the symptoms of various stages of Parkinson’s disease by using methods of MPTP dosing including: intracarotid (ICA), IV and ICA followed by IV. PET imaging was used to examine the relationship between dopamine metabolism and the level of MPTP-induced Parkinson’s symptoms in these monkeys. A staircase performance test was used to measure hand speed while retrieving small rewards and additional measurements of motor deficits were performed using a video tracking system for locomotor activity. Initial results suggest that the clinical stage of MPTP-induced parkinsonism correlated to some extent with the degree of PET-determined dopaminergic terminal degeneration and reductions in staircase performance and locomotor activity. These studies will prove useful in further refining this animal model and provide independent methods to assess the efficacy of novel palliative treatments at different clinical stages of parkinsonism in rhesus.

PS25 Testing on Hepatitis E Virus (HEV) Infection in Nonhuman Primates
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Careful consideration must be taken for the risk of hepatitis E virus (HEV) infection in animal caretakers and investigators working with laboratory animals. As appropriate measures must be taken from the occupational health and safety point of view, it is necessary to grasp the actual conditions of HEV contamination in laboratory animals. Blood samples from nonhuman primates maintained in our facility (63 samples from *Macaca fuscata*, 11 from *M. mulatta* and 2 from *Cercopithecus aethiops*) and sera (plasmas) having been served for joint studies (72 samples from *M. mulatta*, 57 from *M. fascicularis* and 1 from *M. nemestrina*) were used as test materials. These samples were kept at -30°C until testing. Determination of HEV antibody was made by using a commercial ELISA kit according to the manufacturer’s instructions.

Among the 63 samples from *M. fuscata* and 11 samples from *M. mulatta* introduced and maintained at our facility, 1 and 3 samples were positive for HEV antibody, respectively. Regarding the blood samples for the joint studies, 1 and 5 samples were positive from 3 samples of *M. fuscata* and 72 samples of *M. mulatta*, respectively. From the above results, it became clear that HEV antibody existed in the plasmas from *M. fuscata* and *M. mulatta* being used for experiments in our facility. Therefore, we conclude that attention must be paid to the HEV infection of humans when nonhuman primates are used for experiments.

PS26 Neoplasms in Inbred and Outbred Rats with Intra-Abdominal Radiotelemetry Devices
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Refinements in surgical technique, peri-operative care and advances in radiotelemetry equipment have enabled the long-term maintenance of instrumented models for repeat investigations in drug discovery projects. We report an incidence of neoplastic lesions of mesenchymal origin in Spontaneously Hypertensive (SHR/NCI:BR) and Sprague Dawley (Crl: CD1® (SD) IGS BR) male rats, which had previously been surgically prepared at 8 weeks of age, with telemetry devices positioned inside the abdominal cavity for pressure measurements. Clinical presentation was typically at 18 months of age; at necropsy, multiple firm yellow nodules (0.5-40 mm) attached to the parietal and visceral peritoneum, abdominal fat and/or abdominal organs were found in 9 rats out of 62 examined over a 12-month period. One tumor was identified as an intestinal leiomyosarcoma in an SHR; the remaining eight were malignant fibrous histiocytomas (1 SHR and 7 Sprague-Dawley rats). There has been no tumor incidence of mesenchymal origin in non-instrumented rats. An incidence of 1.1% has been reported for spontaneous, age-related, non-lymphoid hematopoietic neoplasms (i.e., histiocytic sarcoma) in male and female Sprague Dawley rats. The incidences of lesions observed in these instrumented animals are higher than reported in the literature; therefore, we postulate that neoplastic lesions may be associated with intra-abdominally placed radiotelemetry devices. Induction of malignant tumors by foreign body material is well documented for rodents and humans. Rats with radiotelemetry devices on long-term studies should be carefully monitored and routine post-mortem examinations performed. Tumor development could affect animal welfare or decrease our confidence in the animal model. We recommend that tumor incidence should be considered a potential adverse event in instrumented rats when the model is successfully maintained in long-term investigations.

PS27 A Telemetry Model to Monitor ECG and Blood Pressure in Conscious Rabbits
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A rabbit telemetry model for simultaneous monitoring of heart rate and blood pressure using implantable radio transmitters from DSI (Data Sciences Inc.) have been set up in our laboratory. In this
model, male and female New Zealand White rabbits (3-3.5 kg) were implanted with DSI C50-PXT transmitters. A 15 cm blood pressure catheter and two ECG leads were implanted subcutaneously at the left side abdominal area. The blood pressure catheter was cannulated into the left femoral artery and extended 8 cm to ensure that the tip of the catheter was reaching the descending aorta. The two ECG leads were guided by a trocar through subcutaneous tunnels to the left and right side chest areas. Three weeks after the surgery, the rabbit was placed in a cage equipped with a Saiv infusion system. A needle catheter was cannulated into the ear marginal vein, secured with tape, and connected to the infusion system and an infusion pump. This set-up enabled continuous infusion of drugs to the conscious rabbit without additional manipulation of the animal during the experiment. Furthermore, the dosing rate could easily be changed without causing the stressful stimulation observed when administering IV, IM or PO serial doses. Hence, this rabbit model enables monitoring of heart rate and blood pressure unaffected by co-administration of anaesthetics or by multiple dosing.

**PS28 Refined Breeding Colony Management Scheme for the Han:SPRD-Pkd1 Rat Model of Polycystic Kidney Disease**

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The Han:SPRD-Pkd1 rat is a model of polycystic kidney disease (PKD) that is lethal at weaning age in homozygous rats but is slowly progressive in heterozygous rats. The gene is unknown for the PKD (PKD) that is lethal at weaning age in homozygous rats but is slowly progressive in heterozygous rats. The earliest change seen on abdominal ultrasound of heterozygous rats was a hyperechoic pattern, and cysts became evident by 9 weeks of age for males and by 12 weeks of age for females. The purpose of this study was to determine if the carrier status of the Han:SPRD-Pkd1 rats could be confirmed by other methods. Abdominal ultrasound, creatinine and blood urea nitrogen (BUN) levels were assessed at 4, 6, 9, and 12 weeks of age for 12 offspring. Necropsy was performed at the end of the study to determine carrier status. The earliest change seen on abdominal ultrasound of heterozygous rats was a hyperechoic pattern, and cysts became evident by 9 weeks of age for males and by 12 weeks of age for females. Creatinine levels in both sexes and BUN levels in females where not elevated for heterozygous rats at any of the ages examined. BUN became elevated at 9 weeks of age in heterozygous male rats (mean = 44 mg/dL) compared to wild-type males (mean = 17 mg/dL). These findings have allowed us to change the breeding scheme of this colony from a random breeding scheme to a selective breeding scheme in which all of the litters generated can be used. Currently we maintain the breeding colony by testing males at 9 weeks of age for elevated BUN levels. An elevated BUN level indicates that a male is heterozygous for the PKD mutation and these males can be mated to either wild-type or heterozygous females to produce litters that carry the PKD mutation.

**PS29 Clinical Linoleic Acid Deficiency in Dahl Salt Sensitive (SS/Jr) Rats**

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Male SS/Jr rats were placed on a specially formulated, high-cholesterol/low-sodium diet at 3 weeks of age. In one group, 40/50 animals developed skin lesions that were noted as early as 18 days following introduction to the diet. Lesions ranged from focal areas of alopecia to diffuse areas of moist dermatitis on the head, face, ear pinnae and neck. Similar lesions were later observed in 17/36 SS/Jr rats in a second study group. Clinical rule-outs included parasitic disease, fungal infection, and a dietary deficiency (e.g., zinc or fatty acid). Histopathological findings from two affected animals revealed diffuse, hyperplastic, ulcerative dermatitis, with bacterial colonies of cocci in superficial crusts, as well as chronic hepatic inflammation with hepatocellular glycerogen and sinusoidal macrophage aggregates suggestive of lipidosis. Bacterial and fungal cultures revealed a light growth of Staphylococcus sp. and an Aspergillus sp., respectively. Treatment of the lesions initially included betadine cleansing and application of topical triple-antibiotic ointment. Later, topical zinc oxide was added to the regimen. Two animals treated with ivermectin made no significant improvement. Results of a fatty acid profile taken from affected rats showed serum linoleic acid levels of 931-1566 µmol/L. Serum zinc levels in affected animals ranged from 1.0-1.9 ppm. Control (SS/Jr) samples ranged from 2711-3145 µmol/L. Linoleic acid and 1.2-1.4 ppm zinc. Dietary analysis of the specially formulated diet showed that it contained only 0.225% linoleic acid, below the recommended 0.3-0.6% (ILAR. Nutrient Requirements of Laboratory Animals. 4th Edition. National Academic Press. 1995.). Based on the clinical and dietary findings, a diagnosis of linoleic acid deficiency was made. The food manufacturer revised its dietary formulation to increase the linoleic acid content to 1.05% and no further cases of dermatitis were observed in any subsequent groups of rats maintained under the same study protocol.

**PS30 Waste Anesthetic Gas During Canine Anesthesia: Comparison of Isoflurane Levels Emitted under High- and Low-Flow Carrier Gas Rates**

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Waste anesthetic gas has been associated with a wide variety of human health problems (reviewed in Contemp. Topics Lab. Anim. Sci., 41: 10-17, 2002). In the past decade, anesthetic regimens utilizing variable fresh gas flow rates in veterinary medicine have been adopted, at least in part, to reduce anesthetic pollution. In the present study, we assessed whether or not this rationale was correct by comparing waste isoflurane levels emitted during canine anesthesia protocols employing low-flow and high-flow conditions. Adult, spayed female beagle dogs (n = 10, 6.2-10.7 kg) were premedicated SC with a combination of butorphanol (0.50 mg/kg), atropine (0.50 mg/kg) and acepromazine (0.25 mg/kg). After 15 min, general anesthesia was induced with an IV solution containing diazepam (0.50 mg/kg) and ketamine (10 mg/kg). Dogs were intubated with a cuffed endotracheal tube of appropriate size (7-8 mm o.d.) and then connected to a conventional circle isoflurane delivery system. Isoflurane (2%) was delivered in oxygen at either 100 mls/kg/minute (high flow rate) or 4-6 mls/kg/minute (low flow rate). Waste gas emissions were...
assessed by real-time spectrophotometry with the probe placed at the animal’s oral cavity (AOC, a point 2 cm from the margin of the lips), the breathing zones of the anesthetist (BZ-A, a point 45 cm from the dog’s nose) and surgeon (BZ-S, a point 65 cm from the dog’s nose), and the exhaust port of the passive gas-scavenging canister (a point 100 cm from the dog’s nose). Atmospheric isoflurane levels measured for the high flow rate were: AOC, 20.7 ± 9.3; BZ-A, 5.9 ± 1.7; BZ-S, 3.5 ± 1.1, and canister, 1.5 ± 0.5. Isoflurane concentrations detected under the low flow rate were AOC, 6.7 ± 2.0; BZ-A, 2.2 ± 0.9; BZ-S, 0.8 ± 0.4, and canister, 0.3 ± 0.1. Emissions at the BZ-A and canister measured under high-flow conditions were significantly higher (P ≤ 0.05) than those obtained for the low flow situation. These data confirm that using a low carrier gas flow rate will substantially reduce anesthetic pollution, particularly in the breathing zones of the anesthetist and surgeon.

PS31 The Soleus Flap: A Therapeutic Option for Mid-Tibial Diaphyseal Osteomyelitis Defects in a Goat Model

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A variety of reconstructive methods for the management of tibia defects have enhanced the outcomes and expediency of wound closure. Transfer of local and regional tissue for coverage of proximal tibia defects has been performed using gastrocnemius muscle flaps and other local tissue. This muscle, however, has a limited reach due to the small size and proximal location of its muscle belly, limiting the use of this highly vascular tissue to coverage of proximal tibia defects. The purpose of this study was to evaluate the effectiveness of the soleus muscle transfer in the eradication of tibial diaphyseal osteomyelitis after debridement in a goat model. Thirteen goats were inoculated in their left proximal tibial metaphysis with a consistent concentration of Staphylococcus aureus and followed clinically and radiographically for 12 weeks. A chronic osteomyelitis model was confirmed both radiographically and histologically. The osseous defects created after debridement were then reconstructed in a single stage with an ipsilateral soleus muscle transfer in all goats. Donor site was evaluated by the surgeon, veterinarian and surgical technician evaluating weight-bearing status and wound appearance daily, and radiographs were assessed monthly in the postoperative period for 6 months. Radiographs were analyzed in a blinded fashion for presence of lucency, periosteal reaction, sclerosis, and sequestrum (indicators of recurrent osteomyelitis). The dominant blood supply to the soleus muscle is via branches from the caudal tibial artery arising from the popliteal artery. Flap dissection proceeded from distal to proximal through a medial incision with preservation of the surrounding muscles and their innervation beginning at the common calcaneal tendon and separating the muscle from the superficial gastrocnemius muscle. All soleus muscle flaps survived and provided adequate blood supply and soft tissue bulk to treat defects of the mid third of the tibia. Treatment of mid-shaft defects of the tibia of goats was successfully performed using the soleus muscle from the superficial posterior compartment of the hind limb. This method has not been described previously in this animal model. With minimal functional impairment and successful wound coverage, the soleus muscle flap provides an ideal flap for the treatment of wounds of the mid tibia in goats. There were no recurrences of osteomyelitis both clinically and radiographically, and all animals were fully weight bearing approximatively 1 week after surgery. The soleus muscle has a reliable blood supply, is easy to harvest, provides added vascularity to ischemic tissue, and has adequate tissue volume for the reconstruction of tibial diaphyseal osteomyelitis defects.

PS32 Evaluation of Antiangiogenic-Targeted Therapy in an Atherosclerotic Rabbit Model Using Paramagnetic αβ3-Targeted Nanoparticles Incorporating Fumagillin

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Background: Angiogenesis is a critical feature of plaque development in atherosclerosis and may play a key role in both the initiation and later rupture of plaques leading to myocardial infarction and stroke. Disruption of the neovascularization with targeted antiangiogenic therapy may stabilize atheroma and promote plaque regression.

Methods and Results: Atherosclerosis was induced in New Zealand White rabbits fed 0.5% cholesterol for ~80 days. αβ3-integrin-targeted paramagnetic nanoparticles with (n = 5) and without fumagillin (n = 6) were injected intravenously and provided specific detection of the neovascularisation using routine magnetic resonance imaging (MRI) at a clinically relevant field strength (1.5T). A third group of hyperlipidemic rabbits received non-targeted paramagnetic nanoparticles incorporating fumagillin (n = 6). Increased angiogenesis was detected as a 16.7% ± 1.1% enhancement in MRI signal averaged throughout the abdominal aorta wall among rabbits receiving αβ3-targeted paramagnetic nanoparticles either with or without drug. One week later, molecular imaging with αβ3-targeted paramagnetic nanoparticles revealed a dramatic decrease in angiogenesis (2.9% ± 1.6%) among rabbits receiving targeted fumagillin therapy and no significant change in rabbits treated with non-targeted fumagillin (12.4% ± 0.9%) or targeted nanoparticles without drug (18.1% ± 2.1%). Immunohistology for angiogenic vasculature expressing αβ3-integrin corroborated the MRI findings.

Conclusions: This combined molecular imaging and drug delivery platform may provide a novel approach to define, treat, and monitor the atherosclerotic burden and therapeutic responses of susceptible individuals.

PS33 Development of a Hybrid Individually Ventilated Caging System for In Situ Euthanasia of Mice

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Carbon dioxide (CO2) overdose is the most commonly employed euthanasia technique in mice; however, dependent on the method employed, the technique may be stressful and is labor intensive when euthanizing large numbers of animals. An individually ventilated caging system was developed, which adhered to the AVMA Panel on Euthanasia Report (2000) for euthanasia with CO2, to enable in situ (home cage) CO2 euthanasia to reduce animal stress and labor. The automated system ventilates cages with either room air or CO2. Euthanasia is conducted in three phases controlled by a programmable logic...
controller (PLC): gas insufflation, soak, and purge. Indicator lights on the control panel reveal the cycle phase and completion. Upon key activation, the PLC turns off the supply blower which provides cages with HEPA-filtered room air, activates closure of a gate damper isolating the supply blower, and opens a solenoid valve allowing CO₂ to enter the supply plenum and the cages. The exhaust blower remains on during the insufflation phase to aid in gas distribution into the cages. At the end of this phase, the PLC turns off the exhaust blower and the solenoid valve immediately thereafter, ending CO₂ inflow. Mice are exposed to CO₂ during the subsequent soak phase, ensuring sufficient time for euthanasia. At the end of this phase, the gate damper is opened, and exhaust and supply blowers activated to purge the cages. The system is ducted to the building’s HVAC system through a thimble connection to prevent CO₂ release into the facility. The PLC can be programmed with up to 6 cycles, permitting changes in phase duration and number. A 2-min insufflation and 15-min soak cycle were found to be highly effective for mice greater than 7 days of age. Neonates are not sacrificed in the system as the time needed for euthanasia requires an excessive amount of CO₂. The system incorporates a variety of features to ensure both animal and personnel safety.

PS34 An Enhanced Method for Ovarian Transplantation in the Mouse Model

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Ovarian transplantation in the mouse model has proved to be an invaluable procedure in the production of mutant mouse strains in which reproductive ability in the homozygous female is compromised. However, the procedure has been proven to be technically difficult and relatively unknown by all but the few institutions in which it plays a critical role. The micro-surgical skills required to access the ovary of the recipient while maintaining the integrity of the bursa housing the newly transplanted ovary is the most challenging aspect of the procedure. Also, after removing the ovary from the recipient and replacing it with the ovary of the donor, there is a tendency for the newly transplanted ovary to become dislodged when the uterus and the ovary are replaced into the peritoneal cavity. The traditional procedure was performed on 15 8-week-old C57BL/6 females with no success. Admittedly, this lack of success is due to the technical difficulty of the procedure.

Fortunately, we found that physiological changes occurring 12-36 h after copulation renders both the ovary and the bursa housing the ovary in an ideal condition to perform this procedure. The increase in size of both the ovary and bursa makes the procedure less difficult to perform. The immense expansion of the bursa as compared to the ovarian size increase creates an excellent pocket to both excise the recipient’s ovary and transplant the donor ovary in its place. Hyperplasia of the bursa membrane during this period increases the integrity and ability of the bursa to retain the newly transplanted ovarian tissue, even during the replacement of organs into the peritoneal cavity. Therefore, breeding ovarian recipients to vasectomized male mice to induce a state of pseudopregnancy significantly enhanced the ease and success of the procedure. The procedure was performed on 15 pseudopregnant C57BL/6 females; 10 of the recipients which received B6 agouti ovarian transplants produced agouti pups. We feel this procedure might prove to be an invaluable tool for the production reproducitively challenged strains of mice.

PS35 Normal Flora Contamination of Water in Mice Receiving Acidified and Autoclaved Water

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A common husbandry practice for transgenic mice colonies is to provide water acidified to a pH of less than 3.0 to prevent bacterial contamination. Previous studies have demonstrated that mice drinking acidified water consume less, have slower growth and can weigh less than age-matched mice on non-acidified water. The use of sterile, autoclaved water would eliminate these husbandry concerns without ill effect on the mice. The purpose of this study was to demonstrate that mice receiving autoclaved water were not exposed to bacteria that are not considered normal flora and any bacterial contamination in the water would have originated from the mice. Six groups of five cages housing four C57BL/6 mice received non-treated deionized water, acidified deionized water (pH of 2.55) or autoclaved deionized water for 5 and 7 days. Water was autoclaved for 30 min at 121°C. Autoclave efficacy was tested with SporeAmpules and indicator strips, and water sterility was confirmed by culture. After receiving treated water for 5 or 7 days, the water was cultured for bacterial colonization and two adult mice were euthanized and respiratory and enteric cultures taken. The culture results demonstrated that the bacterial contaminants cultured from the water were also found in either the oropharynx or cecum of the mice in the corresponding cages, including Staphylococcus sp. and Enterobacter cloaceae from deionized and autoclaved water treatment groups, and Staphylococcus sp. from the acidified water treatment group. These findings suggest that the bacterial contamination is minimal when using autoclaved water and does not necessarily expose the mice to bacteria other than normal flora, and using autoclaved water avoids the potential side effects of acidifying water.

PS36 “Flash” Sterilization for Surface Decontamination of Irradiated Feed Bags

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When maintaining strict biosecurity for pathogen-free rodents, all materials, including feed, are processed to prevent introduction of unwanted biological agents. Historically, feed was routinely steam sterilized. Validated steam sterilization, performed at the site of utilization, ensures that both the feed and bag are sterile. Although effective, shortcomings with this process include nutritional content degradation, alteration of pellet hardness, adherence of pellets, and variability both within an individual feed bag and different bags within a sterilization load. Gamma-irradiated rodent feed was introduced in 1984 as an alternative to steam sterilization. Although dependent on exposure dose, feed irradiation performed offsite requires transport to the point of use and thus the possibility of feed bag surface contamination. Conventional methods to address this risk involve either removing the paper cover from the underlying plastic liner or spraying the bag with an appropriate disinfectant or sterilant. However, these methods are labor intensive and dependent on staff performance. We hypothesized that a “flash” sterilization cycle could...
be employed to sterilize the outer paper bag surface while protecting the nutritional quality of the feed. We evaluated various “flash” autoclave cycles on the surface contamination of feed bags inoculated at separate sites with *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacterium in a thioglycollate broth standardized at 10^5 CFU/ml. Gravity autoclave cycles at 100°C with zero sterilization time resulting in bag surface exposures above 82°C for 2 min achieved surface sterilization. To ensure that these cycle characteristics had minimal effect on feed quality, nutritional analysis was performed on autoclaved samples. To confirm that there was no contamination of feed due to heating the plastic liner, gas chromatography mass spectrometry was performed on autoclaved feed samples. We have subsequently employed this process in our three largest vivaria.

**PS37 Developing and Implementing Training Course in Basic Surgical/Microsurgical Techniques in Rodents**

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Adequate personnel training is a very important and fundamental issue in the research community to ensure consistent application of the “3Rs” (replacement, reduction, refinement). To further ensure quality and animal welfare, we decided to go one step beyond the standard lecture and “wet” lab to develop a new course, bringing our training to a more sophisticated level. This new program teaches research staff the basic surgical techniques and procedures performed on rodents. This program is offered as two-day intensive hands-on course. Participants are researchers and technical personnel that are involved in surgical protocols for rodents. The CD-ROM lecture covers the main aspects on anesthesia, preparation for surgery and post-op care, regulation and requirements. Hands-on sessions include developing a working knowledge of using surgical microscope, microsuturing and instrumentation, basic surgical aseptic techniques, dissection and cannulation of major vessels (jugular vein, carotid artery, and femoral vessels), laparotomy, wound closure and wound management. Each procedure is performed on rat and mouse model and each student is evaluated individually by the instructor. A ratio of only three students and one instructor per class is an essential part for the investigators and their staff covering topics such as proper conduct in a barrier facility, aseptic technique, and rodent peri-operative care.

**PS38 Developing Standard Operating Procedure Training Videos for a Laboratory Animal Facility**

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In order to help standardize on-the-job training and assure more uniform application of the Standard Operating Procedures (SOPs) in the University of Illinois at Chicago’s (UIC) animal care program, a training program was developed using digital technology to present key SOPs in a user-friendly video format. To accomplish this task, a student from the UIC Biomedical Visualization Program was hired. A digital video camera and an audio/video editing package (Pinnacle Studio SE) was purchased to create short film segments. A digital camera was used for still pictures, and the institution’s Instructional Technology Lab was queried for information on streaming video from a streaming server, video editing, and setting up a secure web site for instructional use (Blackboard Learning System®). Once these capabilities were in place, members of the animal care staff were videotaped performing various husbandry SOPs and the images were edited and placed in a Microsoft PowerPoint® presentation that could be accessed by the animal care staff via the secure instructional web page. The editing equipment can be easily installed on a computer, is user friendly, inexpensive, and has considerable versatility saving, reusing, and editing files. During this presentation, the process for creating and editing custom training videos for facility SOPs will be described. This will include the equipment and software used, the editing process, and the process used to construct a streaming video containing the SOPs. Setting up a “virtual classroom” will also be described. This is an easy process, which can be done at most academic institutions and allows one to track an employee’s progression through the training material. Using institution-specific images in conjunction with written SOPs is an excellent way to train staff and assure that SOPs are being learned correctly. The initial success of this program has exceeded original expectations. The Biologic Resources Laboratory is developing similar videos for the investigators and their staff covering topics such as proper conduct in a barrier facility, aseptic technique, and rodent peri-operative care.

**PS39 Management of Veterinary Care and Post-Operative Monitoring of Rats in an Academic Research Facility**

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An animal care and use program must provide veterinary oversight for post-operative management of experimental surgical animals according to the *Guide for the Care and Use of Laboratory Animals*. While centralized facilities for large animal surgery allow close monitoring by qualified veterinary staff during the post-operative period, rodent post-operative monitoring usually becomes the responsibility of the research staff following surgical procedures performed in individual laboratories. In our facility, rat survival surgeries include experimental procedures such as intracerebral cannula implants, heart and lung transplants, coronary artery ligations, stroke induction, intramedullary wire placement, hepatectomies and manipulations of other major organs. Because of the invasiveness of these procedures, there is a need for more veterinary oversight regarding post-operative monitoring. As a result, our department developed a comprehensive program to monitor rats in a centralized area of conventional housing for a minimum of 72 h post operatively. No other species are housed in this room. Following recovery in the laboratory or procedure room, the investigator relocates the animal in its home cage to a designated rack in the room and places a standardized monitoring form under each cage for veterinary inspection during rounds. This form indicates that the investigator has provided heat, fluids, and analgesics during the immediate post-operative period until the animal is fully conscious. The investigator continues to
make observations and notations on the form twice a day for the minimum time stated in the approved protocol. This new standard operating procedure requires minimal effort by the research team yet it has enhanced record-keeping and compliance for annual IACUC protocol renewals, has established a strong communication between the surgeons and the veterinary staff, has reduced the incidence of untreated adverse effects, and has decreased morbidity, resulting in an overall improvement in post-operative care and treatment of rats.

**PS40 Use of Ex Vivo Models in Surgical Skills Training**

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Introduction of operative skills to medical students and surgical residents has traditionally employed large animal models. Societal concerns for animal welfare and costs for maintenance of animal facilities have necessitated alternative teaching methods. Although non-animal models such as mannequins, video simulators and virtual reality trainers are available, their costs may be prohibitive to many institutions. We describe the models used in our Surgical Skills Laboratory that reduce the need for live animals and minimize operative costs. Knot boards and commercially available pigs’ feet are used to teach knot tying and suturing/stapling. Techniques of sutured and stapled bowel anastomoses use latex bowels and ex vivo porcine intestines. Vascular anastomoses are taught with GoreTex® grafts and ex vivo porcine aortas. Video trainers allow development and refinement of all laparoscopic skill levels. Basic inanimate laparoscopic drills include pegboard, cup drop, rope pass, pattern cutting, and placement of Endo™ Clips and Loops. Intermediate skills of laparoscopic knot tying and bowel anastomosis use cloth and latex models, respectively. Advanced laparoscopic procedures of cholecystectomy and Nissen fundoplication are taught in ex vivo porcine livers and stomachs. In conclusion, the use of inanimate laboratory teaching models, such as synthetic materials and ex vivo organs, can reduce the need for live animals.

**PS41 A Novel Approach to Improving Compliance within a Laboratory Animal Facility**

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Personnel often become “sign blind” from constant exposure to posted messages alerting them to important requirements upon entering an animal facility. A two-pronged approach to the problem, incorporating both visual and audio media, can be more effective for posting facility alerts. A battery-powered, self-contained audio enunciation system using off-the-shelf electronics is described. The components include an integrated circuit (IC) chip that stores digitally recorded messages, a microphone, an amplifier, a volume control, batteries, and a speaker all assembled into a fracture-resistant plastic container (15.5 x 9.3 x 3.5 mm), at a cost of approximately $100. This system can be affixed by various means (magnets, hook-and-eye fasteners such as Velcro®, 3M® removable fastener system) to a doormframe or any other surface. The system can be activated by various switching mechanisms (door switch, touch plate switch, motion detection switch, magnetic switch, or electric eye controlled switch). The system has the capacity for recording messages of up to 16 sec in length. IC chips are available with capacity for recording of up to 120 sec in length. The recorded message can be easily changed by recording over the previous message, so the audio message can be frequently tailored to a particular need. The system can be used for delineating requirements for entry into an animal facility or specific areas of a facility such as quarantine areas or other areas where entry is restricted or where proper Personal Protective Equipment (PPE) is critical to disease control or occupational safety. The system can also be used to alert personnel of status change within an animal facility including facility entrance alert, quarantine entrance alert, or restricted area alert. When the system was posted on the doorframe of a room quarantined for mouse parvovirus, personnel entering the room activated the message playback by tripping a door switch and were thereby reminded of the requirements for special gowning upon entry. Personnel observed entering the room were 100% compliant with posted signs during the periods the system was in place.

**PS42 Comparing the Effectiveness of Mandibular Versus Retro-orbital Blood Collection**

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The goal of this study is to compare two bleeding techniques and to determine the efficiency of these two techniques in mice at various weight ranges. The mandibular approach is appealing due to the fact that anesthesia is not required, whereas anesthesia is necessary for retro-orbital bleeding. Our hypothesis is that mandibular bleeding is quicker, easier and will yield a similar volume of blood as retro-orbital bleeding regardless of weight range. Five groups with 10 animals per group of different weight ranges were bled using both techniques, under an animal study proposal approved by our IACUC. We used 20-gauge needles for mandibular bleeds, while micro-hematocrit tubes were used for retro-orbital bleeds. Blood volume was measured using a graduated tube. Healthy immuno-competent colony animals of various strains and stocks were used. Technicians were trained and became skilled at these bleeding methods prior to this study. Results from retro-orbital bleeding suggest that this technique could be used on all weight ranges safely, yielding an average of 0.4 ml of blood per animal with a 99% success rate. Mandibular approach for blood collection is very safe and effective when using animals above 20 g, with 97% success rate yielding an average of 0.2 ml per animal. Collecting an adequate volume of blood (mandibular technique) from animals of lower weight ranges may jeopardize their health. Care must be taken with either approach to not damage the eye; if using the mandibular approach, damage to the ear canal, esophagus or trachea should be avoided. The information obtained in this experiment suggests that the mandibular bleeding approach is faster and therefore an acceptable alternative to collecting a blood sample via the retro-orbital plexus, depending on the volume needed and the animals’ weight.
PS43 Low-Intensity Light Exposure During Animal Room Dark Phase and Alterations in Hepatoma and Human Breast Tumor Lipid Metabolism in Rats: A Dose-Response Study

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Controlled lighting in the animal facility is of critical importance to both biomedical researchers and animal care personnel alike, significantly affecting the very chronobiologic nature and outcome of scientific investigations. Previous investigations from our laboratory revealed that dark phase “light contamination” with as little as 0.25 lux (0.08 μW/cm²) stimulated tumor growth and metabolism in rats by suppressing the nighttime production of the circadian neurohormonate melatonin. Recent studies demonstrated that growth of both the hepatoma 7288CTC and MCF-7 human breast tumor, implanted and grown as “tissue-isolated” tumors, are dependent upon linoleic acid (LA) and its metabolism to the mitogenic agent 13-hydroxyoctadecadienoic acid (13-HODE). At nighttime, with increased nutrient consumption, blood levels of LA increase; levels of blood melatonin also increase, effectively blocking LA uptake and 13-HODE production. Here we investigated the dose-response effects of different light intensities on the tumor lipid profiles and melatonin levels in rodents on a 12L:12D photoperiod. Light-during-dark-phase regimens were: 12L:12D (no nocturnal light), 0.02 μW/cm² (II), 0.05 μW/cm² (III), 0.06 μW/cm² (IV), 0.08 μW/cm² (V), Constant Light (345 μW/cm², VI). Fatty acids in total tumor lipids (mean ± 1 SD) for hepatoma 7288CTC/MCF-7 human breast tumors for groups I-VI were 17.5 ± 1.8/3.3 ± 0.6, 18.6 ± 2.9/3.9 ± 1.8, 42.4 ± 2.4/12.2 ± 1.8, 74.5 ± 6.2/16.2 ± 2.5, 111.1 ± 4.0/24.5 ± 1.1, and 118.1 ± 1.6/18.7 ± 1.8 mg/g wet weight tumor, respectively. Fatty acids in tumor phospholipids and cholesterol esters were not significantly different for either the hepatoma or the MCF-7 human breast tumors of the various lighting regimens. However, tumor triglyceride fractions in the experimental groups I-VI in hepatoma 7288CTC/MCF-7 human breast xenografts were, respectively, 7.4 ± 1.5/3.3 ± 0.3, 8.7 ± 1.7/3.7 ± 0.2, 33.2 ± 1.6/7.2 ± 0.8, 66.0 ± 6.3/11.9 ± 1.8, 103.6 ± 3.0/15.2 ± 2.2, and 107.6 ± 11.8/16.4 ± 1.7 mg/g tumor. Tumor triglycerides, representing the principal lipid component, increased markedly in both the hepatomas 7288CTC and the MCF-7 human breast tumors grown in animals subjected to increased light intensities during dark-phase. Nocturnal melatonin levels in the rodent blood decreased with increasing dark-phase light intensities, as expected. This is the first evidence demonstrating that elevated tumor triglyceride levels, associated with increased rates of tumor growth and metabolism, increase as light intensity during the dark-phase increases and melatonin levels decrease.

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PS44 An Improved Technique for Tailcuff Blood Pressure Measurements with Dark-Tailed Mice

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Study of the genetics of hypertension has been greatly facilitated recently by modifying genes affecting blood pressure in mice. A current successful method for measuring blood pressure relies on detection of light passing through the tail to determine the pressure in a tail cuff necessary to stop pulsed flow. Success in obtaining reliable blood pressure measurements in light-tailed strains of mice such as the C57BL/6 has been good; however, mice having highly pigmented tails, including 129 SVEV, have yielded inconsistent measurements. This problem is of concern because the 129 SVEV strain is the parent strain of ES cell lines from which many genetic mouse models have been derived. We report here that inserting four layers of Saran Wrap between the tail and the optical flow detection sensor greatly reduces the problem. The small amount of light leaking around the tail results in a more consistent detection of flow changes in the tail and facilitates the measurements. The consequence is an increased frequency of successful measurements and reduced variability in the measured blood pressures of the 129 SVEV mice. Two separate experiments comparing male with female 129 SVEV and female 129 SVEV with female C57BL/6 show the number of successful measurements improved by 50% for the dark-tailed mice; the standard deviation was reduced by a factor of 1/3 and there was no effect on the daily mean blood pressure. Measurements with C57BL/6 mice were not significantly affected by the procedure.

These findings have two important implications. First, studies planned in strains with high tail pigmentation can now be completed more effectively; and second, the ease of measurement can be improved with reduced variation, allowing subtle changes to be detected with fewer mice.

PS45 Sensitivity of Bedding Transfer as a Means of Detecting Viral Infection in Mice

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Serological monitoring of sentinel mice after transfer of soiled bedding is a common method to detect infections in mice. Because protocols for bedding transfer vary, the sensitivity of this method has not been adequately documented. This study examined the efficacy of bedding transfer at various times during acute infections with mouse parvovirus (MPV) or mouse hepatitis virus (MHV). A preliminary study demonstrated that 150 ID₅₀ of MPV (stable) or 3000 ID₅₀ of MHV (labile) added to autoclaved corncob bedding elicited seroconversion in contact-exposed mice. Next, 6-week-old female Swiss Webster (SW) index mice were inoculated oronasally with 30 ID₅₀ of MPV or 300 ID₅₀ of MHV. Bedding (25, 50, or 100 ml, 2 mice per “dose”) was transferred to sentinel cages of SW mice 3, 8, and 15 days post-inoculation (dpi). Sentinels were tested for seroconversion after 14 days by indirect immunofluorescence assay. Viral shedding by index mice also was determined by fecal PCR at each time point. MPV shedding by index mice was detected...
at dpi 3 (11/12), 8 (11/11), and 15 (11/12), One of two sentinels exposed to 100 ml of soiled bedding on dpi 3 seroconverted. All sentinels (6/6) from the dpi 8 transfers seroconverted, but none (0/6) from the dpi 15 transfers did so. Shedding of MVH from index mice was detected at dpi 3 (11/12) and 8 (9/12), but not dpi 15 (0/12). All sentinels (6/6) from the dpi 3 transfers seroconverted, whereas no seroconversion (0/6) was detected in sentinels from the dpi 8 transfers. Nevertheless, 2/2 sentinels exposed to 100 ml of soiled bedding on dpi 15 seroconverted. These findings suggest that transfer of soiled bedding detects infection reliably only during peak shedding of MPV (dpi 8) and MVH (dpi 3).

PS46 Comparison of Mouse Oral Flora to the Bacteria Found in the Biofilm of Automatic Watering Systems

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We previously assessed the bacterial species that colonized biofilm within the piping of automatic watering manifolds. Bacteria found in biofilm samples were a mixture of Gram-positive and Gram-negative organisms. We then assessed if the bacteria found in the biofilm was consistent with the oral flora of mice, and whether oral flora could survive in a low-nutrient medium (to simulate water piping conditions). Our hypothesis was that bacteria found in biofilm were derived from the source water, and not from the oral flora of the mice. We sampled eight groups of five mice each. Groups represented several vendors, strains, and ages. Each group was sampled upon removal from the shipping container. A sterile swab was used to sample the oral mucosal surfaces; swabs were placed in broth for 24 h, then transferred to R2A and blood agar plates. The R2A agar plates were maintained at room temperature (22°C) for 7 days. The blood agar plates were incubated at 37°C for 5 days. All 40 samples yielded bacterial growth. The most frequently cultured bacteria were Staphylococcus spp. (20/40), Escherichia coli (13/40), and Enterococcus faecalis (13/40). Most bacteria cultured from the oral flora grew on R2A agar, indicating that these species could survive in a low-nutrient, biofilm environment. Staphylococci were identified in some biofilm samples; however, E. coli was not found, and no coliform species were ever identified in the biofilm. We concluded that biofilm bacteria are likely derived from the source water, since municipal water treatment eliminates coliform species, as per the definition of ’potable water.’ Since oral flora bacteria were not reasonably represented in biofilm populations, they are probably not significant contributors to the biofilm found in automatic watering manifolds.

PS47 Variations in Phytoestrogen Content Between Different Mill Dates of the Same Diet Produces Significant Differences in the Time of Vaginal Opening in F344 Rats

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The selection of the PMI certified rodent diet #5002 by the Organization for Economical Cooperative Development (OECD) and the Environmental Protection Agency (EPA) as the test diet for the conduct of uterotrophic assays in rats for the evaluation of the hormonal activity of endocrine disruptor compounds (EDCs) is the subject of current debate. We have shown that the variation in the phytoestrogen content of different mill dates of the #5002 diet produces significant differences in the time of vaginal opening (VO) in CD-1 mice. This report describes the effects of a fourfold variation of the phytoestrogen content of two different mill dates of the PMI #5002 diet on the time of VO in F344 rats. Dams with standardized litters, 10 female pups born on the same day, were received when the pups were 8 days old. Each dam and 10 pups were housed in a polypropylene cage containing autoclaved hardwood bedding in an AAALAC, International-accredited animal facility. The dams were fed the Zeigler 5412-01 phytoestrogen-reduced diet containing <10 µg daidzein and genistein (D&G)/g diet and given RO/DI water ad libitum. The pups were weaned at post-natal day (PND) 19 and were randomly assigned to cages (4 pups/cage). Cages were randomly assigned to the PMI #5002 test diets or PMI #5K96 phytoestrogen reduced diet (at least 16 pups/diet). The weaned pups were fed the test diets and VO was recorded daily from PND 19 to PND 46. Body weights were determined at weaning and at weekly intervals until PND 46. The average time of VO (PND 32.6 ± 0.4 days) in rats fed the #5002 diet containing 431 µg of D&G/g diet, was significantly (P < 0.05) advanced when compared to the average time of VO (38.2 ± 0.5 days) in rats fed the PMI #5K96 phytoestrogen-reduced diet (< 10 µg of D&G/g). The average times of VO PND 32.6 ± 0.4 days and PND 36.0 ± 0.6 days were significantly (P < 0.05) different in rats fed different mill dates of the PMI #5002 diet containing 431 µg/g or 98 µg/g of D&G/g diet, respectively. No significant differences in body weights were observed between the groups of rats. It was concluded that the PMI #5002 diet containing 431 µg/g of D & G demonstrated strong estrogenic effects. These estrogenic effects were not due to total metabolizable energy (ME) since the ME was essentially the same in these diets. The effects of the variation in D&G content in different mill dates of the #5002 could be eliminated by using a phytoestrogen reduced diet (< 10 µg of D&G/g diet). These results support the use of phytoestrogen-reduced diets in studies evaluating the hormonal activity of EDCs.

PS48 Age-Related Disruptions in Rat Sleep Architecture

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In humans, aging is associated with disruptions in the quality of sleep including a decrease in delta and REM sleep, an increase in number and duration of awakenings during sleep, and a decrease in daytime activity. In this study, we used radiotelemetry to examine how aging can affect rat sleep architecture. Adult (n = 9, 8 months old) and aged (n = 8, 20-24 months old) male SD rats were subcutaneously implanted with radiotelemetry physiological transmitters which allowed for simultaneous recording of electrocorticographic (ECoG) and electromyographic (EMG) activities. Data were collected and then scored with automated sleep stage analysis software. Sleep stages and active wake were assigned based upon a combination of ECoG frequencies, EMG activity, and gross locomotor activity. The data for each group of adult or aged rats were averaged over each 30-min. period based upon a Student’s two-tailed t-test. Quantification of entries into wake state revealed statistically significant differences between the light and dark phases of the circadian cycle (12:12 L:D) for adult rats (entries into wake 169 ± 19 light
versus 398 ± 11 dark; P < 0.001) but not for aged rats (entries 216 ± 18 light versus 295 ± 30 dark; P = 0.214). The circadian selectivity for mean duration in wake was also present in adult rats (duration wake 66.1 ± 14.8 min. versus 153.8 ± 15.2 min. dark; P < 0.0001) but not for aged rats (140.5 ± 14.4 light versus 167.2 ± 25.5 in dark; P = 0.713). Our results show that aged rats do not exhibit the normal circadian wake cycle that is seen in adult rats. Further, when sleep stage data were analyzed over 30 min. periods, statistically significant (Student’s t test, P < 0.05) reductions in light sleep and REM sleep could be seen over multiple 30-min. time periods during the later half of the inactive period of aged rats. This animal model allows for examination of age-related changes in the architecture of sleep and may provide for investigation of therapies for age-related sleep disorders.

**PS49 Comparative Analysis of the Immune Response in B6x129 and DBA1/lacJ Mice**

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The immune response in inbred strain of mice varies from strain to strain due to genetic variability of the histocompatibility complex and other factors that determine immune response. The goal of the study was to compare two strains of mice, the hybrid B6x129 and DBA1/lacJ, used in gene targeting experiments in their ability to generate immune response. The analyzed mice performed similarly in several immunological assays and experimental models of inflammation. However, the B6x129 hybrids were not sensitive in the monoclonal antibodies-induced arthritis (MIA) model, while the DBA1/lacJ mice had a poorer innate immune response. The number of monocytes migrated to the site of inflammation was significantly (P < 0.0001) reduced in the DBA1/lacJ mice (n = 40) compared to the age- and sex-matched B6x129 hybrids (n = 59). In addition, the levels of tumor necrosis factor-α in the peripheral blood after lipopolysaccharide (LPS) injection were significantly (P < 0.0001) lower in the DBA1/lacJ mice (n = 51) than in the hybrids (n = 69). The differential analysis of white blood cells from the peripheral blood revealed a significantly (n = 69). The differential analysis of white blood cells from the peripheral blood compared to the hybrids. In conclusion, the LacJ mice are likely to be due to the lower number of monocytes in the site of inflammation and lower response to the LPS in the DBA1/lacJ mice (n = 41) compared to the hybrids (n = 42). Thus, the reduced number of monocytes in the peripheral blood compared to the hybrids. In conclusion, the B6x129 hybrids provide a better genetic background for detecting potential impairments of the innate immune system but are a poor choice for the MIA model. On the contrary, the DBA1/lacJ mice are sensitive to the MIA but are weaker responders in the assays for the innate immunity.

**PS51 Biological Activity of Human Recombinant Erythropoietin in Normal Healthy Miniature Swine**

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Aim: Human recombinant erythropoietin (hrEpo) is an amino acid glycoprotein used to treat non-regenerative anemia in human patients with chronic renal failure. Previous studies have shown an 80-82% homology of amino acid sequences of mature epo proteins between humans and swine. Swine are increasingly used as pre-clinical models of human disease, in particular that of both allo and xen renal transplantation. However, to our knowledge, there is no study of the effects of epo on non-regenerative anemia in swine. Therefore, we conducted a study to determine the biological response of miniature swine to human recombinant erythropoietin.

Method: Three normal juvenile miniature swine had bilateral, indwelling external jugular catheters surgically placed. Baseline hematology was monitored three times per week (TIIW) for 4 weeks before and after treatment. The animals were treated with 75 iu/kg of hrEpo IV TIIW (n = 2) or 0.3 ml normal saline (n = 1) for 4 weeks. Hematologic testing was continued for an additional 4 weeks post-treatment.

Results: The mean packed cell volume % (pcv) of the experimental animals (n = 2) pre-treatment was 38.5%. This rose approximately 10% to a mean of 42.8% (P < 0.001) during treatment with hrEpo, a mean pcv of 37.6% was recorded for the 4 weeks post-treatment. The results for hemoglobin before, during, and after treatment were 11.85 g/dl, 13.45 g/dl, and 11.15 g/dl respectively. Throughout the study, no significant changes in hematology were observed in the control animal (n = 1). A noticeable drop in mean reticulocyte count was observed approximately 2 weeks post-hrEpo initiation. The reticulocyte decline continued into the post treatment era. This drop may be suggestive of an immune-mediated response to the exogenous Epo.

Conclusion: These data indicate that hrEpo has biological activity in a miniature porcine breed. Thus, hrEpo may be considered for...
medical support of valuable preclinical models, such as renal transplantation, using miniature swine. Further study to investigate the development of anti-epo antibody with prolonged administration is under investigation.

PS52 Leukotriene Inhibition on Premature Rat Pup Lungs Exposed to Hyperoxia

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Bronchopulmonary dysplasia (BPD) is a major source of morbidity in preterm human newborns, requiring mechanical ventilation. Hyperoxia contributes to this significant lung injury, often accompanied by elevated leukotriene levels. Two leukotriene-receptor antagonists, Zileuton and Zafirlukast, were investigated in a rat pup model of hyperoxic lung injury. We hypothesized that this novel class of anti-asthmatic drugs would provide a better clinical outcome than previous published reports of treatments. Rat pups at 21 days of age were exposed to constant 95% oxygen for 7 days using a hyperoxic chamber as well as control groups in room air. Zileuton, Zafirlukast or saline was administered to the rats by oral gavage. The animals were divided into one of six groups; air/saline, air/zileuton, air/zafirlukast, oxygen/saline, oxygen/zileuton, and oxygen/zafirlukast. Lung tissue was collected from 39 rat pups at 28 days of age and examined by both light and electron microscopy. Pathology lesions were graded on a numerical score. Statistical significance was seen with zileuton only in protecting against hyperoxia-induced hemorrhage ($P = 0.037$) and type II pneumocyte hyperplasia ($P = 0.027$). While hemorrhage is considered to be a feature of hyperoxia-induced lung injury, type II pneumocyte hyperplasia is not typically observed with hyperoxic lung insult. These findings present new information on the mechanisms of anti-asthmatic drug in their protective effects from hyperoxia lung injury. More work is needed to fully characterize this paradigm.

Posters

P001 Evaluation of Dexamethasone for the Treatment of Intracerebral Hemorrhage Using a Collagenase-Induced Intracerebral Hematoma Model in Rats

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Dexamethasone, an anti-inflammatory drug used mainly in large animals in veterinary medicine, was evaluated for the treatment of intracerebral hemorrhage using rat model of cerebral hematoma induced by the intracerebral (IC) injection of collagenase. Positive controls ($n = 6$) received an IC collagenase injection followed by intraperitoneal (IP) saline at 1 and 24 h following surgery. Negative controls ($n = 6$) were sham operated animals receiving saline (IC, IP) in an similar fashion to hematoma controls. The treatment group ($n = 6$) consisted of hematoma-induced rats receiving 1 mg/kg of dexamethasone IP at 1 and 24 h following surgery. Prior to, and at 24 h and 48 h following surgery, all animals were evaluated on a neurological exam (battery of 7 tests). Following behavioral evaluations, animals were deeply anesthetised and brains were quickly removed following perfusions for selected histological and GFAP immunocytochemistry. Behavioral scores were significantly improved in dexamethasone-treated animals ($P < 0.0001$). At 48 h the hematoma zone consisted a mosaic-like structure composed of cell debris, neutrophils, scattered clusters of erythrocytes and necrotic parenchyma. Edema, seen as clear spaces within the nervous tissue, was localized surrounding the penumbral region in the ipsilateral hemisphere as well as all along the corpus callosum and in the contralateral caudoputamen area. The hematoma volume was significantly smaller ($P < 0.02$) and neutrophils as well as astrocytes were less numerous in the hematoma of dexamethasone-treated animals ($P < 0.001$); however, the number of necrotic neurons in the penumbral was not affected. The number of necrotic neurons in the cerebral cortex was fewer in treated as opposed to non-treated animals (not significant). Non-treated animals had many vascular pathological changes, including necrotic endothelium and fibrin deposits, compared to treated animals. These results suggest that dexamethasone administered shortly after an intracerebral hematoma at a dose of 1 mg/kg is beneficial for the treatment of this condition.

P002 Genetic and Behavioral Diversity of Mouse Strains and Substrains

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Sixteen strains and substrains of mice commonly used in transgenics and physiology were compared with regard to genetic background and behavior. Those strains included C57BL/6J, C57BL/6NTac, 129P3/J (formerly named 129/J), 129S6/SvEvTac (formerly named 129/SvEvTac), FVB/NTac, BALB/CAnNTac, and a number of other strains (in total, $n = 593$ mice of 16 strains). We compared 342 microsatellite markers in selected strains, and performance in the behavior tests, rotorod, habituation to an open field, and contextual and cued fear conditioning was determined. Results will form a basis for the planning and evaluation of transgenic and knock-out models of neurodegenerative diseases. Two substrains, C57BL/6J and C57BL/6NTac, were found to be very close to each other genetically. Only 12 microsatellite polymorphisms were found, and marker sizes varied by only two or four base pairs. This is consistent with the two strains being true substrains that have diverged by point mutation or DNA polymerase slippage only, and with the genetic divergence being very small. Given the data on the genetic background, one might predict that the two B6 substrains should be very similar behaviorally. To test this hypothesis, behavior testing was performed. Behavioral differences among the mouse strains generally followed the genetic differences. There were no significant differences between C57BL/6J and C57BL/6NTac. Contrary to literature reports on other 129 group strains, 129S6/SvEvTac often performed as well as B6 strains. On the rotorod between day 1 and day 3, latency to fall increased 110% in female 129S6, 100% in female C57BL6/NTac, but only 25% in female FVB/NTac. FVB/NTac mice showed only weak habituation of horizontal activity on the 4th daily trial in the open field, and low responses to context and cue after fear conditioning. Therefore, both 129S6/SvEvTac and C57BL6/6NTac can be recommended to be used as background strains in the three behavior tests.

Support contributed by NIH.
**P003 High Prevalence of Helicobacter spp. in Mouse Research Colonies in the United States, Europe and Asia**

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With the discovery of Helicobacter hepaticus and its role in hepatitis and liver carcinoma, and the subsequent discovery of other Helicobacter spp. involved in enterohepatic disease, Helicobacter spp. colonization in mouse colonies has become a major concern for the research community. At MIT, mice from non-approved vendors and gift mice are rederived to eliminate the introduction of pathogens into MIT facilities. From 2001-2003, selected mice received for rederivation were screened for Helicobacter spp. using culture and molecular methods. This included 89 mice from 34 sources: 3 commercial and 19 academic (US) and 1 commercial and 11 academic (Europe/Asia). Unless otherwise noted, 2 animals were screened from each source. One US commercial vendor was helicobacter-free. Another had 2 facilities, one helicobacter-free (3/3 mice) and the other had mice infected simultaneously with H. bilis, H. rodentium, and H. hepaticus (4/4 mice). Vendor 3 had H. bilis (12/12 mice). The European commercial vendor had mice infected with an unnamed helicobacter (MIT 96-1001) and another unidentified Helicobacter spp. In academic US institutions, 1 was helicobacter-free (1/1 mice), 7 had H. hepaticus, 2 had H. bilis, 1 had MIT 96-1001, 1 had ‘H. natalensis’ (4/4 mice), 6 had H. hepaticus and H. typhlonius, and 1 had a novel helicobacter (MIT 03-1614). Two non-US academic institutions had mice with no helicobacters, one had H. rodentium, 3 had H. hepaticus, one had H. muridarum, 2 had H. typhlonius (1/1, and 2/2 mice), 1 had H. hepaticus with another unidentified helicobacter (1/1 mice), and one had H. hepaticus and H. typhlonius infected mice. This survey indicates an 88% prevalence of helicobacter infection in mouse colonies in Europe, Asia and the USA. H. hepaticus was most commonly isolated, followed by H. typhlonius. Mixed infections with different Helicobacter spp. was also common. Thus, helicobacter infection in mice and its potential impact on in vivo experiments continues to be an important issue in biomedical research.

**P004 Optimization of a Rabbit Model of Attenuated Vibrio cholerae Immunogenicity**

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_Vibrio cholerae_ is the causative agent of cholera. AVANT Immunotherapeutics is developing attenuated _V. cholerae_ strains to serve as live oral vaccines for cholera (e.g. Peru-15) or as vectors for eliciting an immune response to other antigens. Efforts to study the biology of _V. cholerae_ have been hampered by the observation that the organism does not typically colonize normal adult animals. This poster describes the modification and optimization of a rabbit model of attenuated _V. cholerae_ immunogenicity. In the optimized protocol, male NZW rabbits were fasted overnight and sedated. Gastric acid secretion was inhibited with 50 mg/kg of cimetidine administered i.v. at time zero. Rabbits were given an acid neutralizing buffer (15 ml of 5.0% NaHCO3) by gastric tube at 35 and 50 min. The animals were then given the attenuated _V. cholerae_ inoculum (approximately 2 × 1010 cfu) in 15 ml of bicarbonate buffer by gastric tube. At 80 min, 5 mg/kg of morphine was given i.p. to inhibit peristalsis. Serum was drawn on approximately days 0, 14, 21, 28, 42, and 56, and anti-vibriocidial immune responses were determined. In optimizing the model we investigated 1) the effect of a second attenuated _V. cholerae_ inoculum given on approximately day 47, 2) the route of morphine administration, and 3) the route of cimetidine and morphine in the model. In conclusion, the rabbit model as described reproducibly elicits the production of high-titered vibriocidial antibodies following a single gastric administration of attenuated _V. cholerae_.

**P005 A 40kb Genomic Deletion Including Transmembrane Inner Ear Protein Gene Causes Deafness, Circling and Head Tossing in Circling Mice**

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Circling (cir) mice manifest profound deafness, head-tossing, and bi-directional circling behavior, which they inherit in an autosomal recessive manner. Histologic examination of the inner ear reveals abnormalities of the region around the organ of Corti, spiral ganglion neurons, and outer hair cells. The cir gene was mapped to a region between D9Mit116/D9Mit15 and D9Mit38 on the mouse chromosome 9 at a site indistinguishable from that of the spinner (sr) mouse. The results of allelism tests between circling and spinner indicated that cir is allelic with sr. Originally the sr allele consists of 40kb genomic deletion including transmembrane inner ear (tmie) protein. In our study of tmie expression analysis demonstrated that the cir allele also has a 40kb defective genomic sequence including the tmie gene. Therefore, the circling mouse demonstrates a new allele of spinner mutation which provides an important source to clarify gene function.

**P006 A Mouse Model to Study the Pathogenesis of Antiretroviral-Induced Lipodystrophy Syndrome: A Pilot Study**

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Many HIV-infected patients receiving highly active antiretroviral therapy (HAART) have developed abnormalities in body fat distribution, glucose homeostasis, and lipid metabolism, which has been termed the lipodystrophy syndrome. Studies investigating the impact on this syndrome of switching from a protease inhibitor (PI) to a non-nucleoside reverse transcriptase inhibitor (NNRTI) have reported partial improvement, but further investigation of NNRTI effects is required. Our study examines the metabolic and histologic impact of the NNRTI efavirenz (EFV).

Methods: C57/BL6 mice were divided into eight groups of eight mice: 1) control group, no intervention (C2); 2) EFV, 2mg daily for 2 weeks (E2); 3) pharmaceutical vehicle daily for 2 weeks (V2); 4) saline daily for 2 weeks (S2); 5) EFV daily for 6 weeks (E6); 6) pharmaceutical vehicle daily for 6 weeks (V6); 7) saline daily for 6 weeks (S6); and 8) ritonavir (PI) daily for 2 weeks (R2). Measurements included weight changes, serum levels of glucose, total cholesterol, triglycerides, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, serum albumin, and total bilirubin. Histologic and biochemical analysis were performed on all groups. Results: The PI group had greater body weights than the control group. The EFV group showed a trend toward lower weight gain compared to the saline group. There were no significant differences in serum biochemistry among the groups.

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phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, insulin, and histopathologic analysis of liver, pancreas, kidney, brain and skeletal muscle.

Results: Interim data are available for 48 mice. Mean cholesterol levels (mg/dl) ± standard deviation (SD) were 216.70 ± 93.27, 119.75 ± 18.52, and 225 ± 85.16 for C2, E2, and E6, respectively. Mean triglyceride levels (mg/dl) ± SD were 319.60 ± 172.26, 126.0 ± 3.37, and 279.29 ± 142.02 for C2, E2, and E6, respectively. Mean insulin levels (ng/ml) ± SD were 0.799 ± 0.748, 0.170 ± 0.03, and 1.086 ± 1.38 for C2, E2, and E6, respectively. At 6 weeks, histopathology in the EFV-treated group revealed hepatocellular hypertrophy compared to saline control.

Conclusion: EFV resulted in biochemical and histologic effects in the absence of HIV infection. These changes suggest that the mouse may serve as an effective model for further study of this syndrome.

P007 Aberrant Expressions of Pathogenic Phenotype in Alzheimer’s Diseased Transgenic Mice Carrying NSE-Controlled APPsw

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Mutations in the APP gene lead to enhanced cleavage by the β- and γ-secretase, and increased Aβ formation, which are tightly associated with Alzheimer’s disease-like neuropathological changes. To examine whether depositions of Aβ by APP mutations are increased, and if this is associated with potential pathogenic phenotypes, the APPsw was expressed in a transgenic line under the control of the neuron-specific enolase (NSE) promoter. A behavioral dysfunction was shown at 12 months, and intensive staining bands, with APP and Aβ-42 antibodies, were visible in the brains of transgenic mice. Of the MAPK family, both JNK and p38 were activated in the brains of transgenic mice, whereas there was no significant activation of the ERK. In parallel, tau phosphorylation was also enhanced in the transgenic relative to the control mice. Moreover, the Cox-2 levels, from western blot and immunostaining, were increased in the brains of transgenic mice. Furthermore, there were significant caspase-3- and TUNEL-stained nuclei in the transgenic line compared to the age-matched control mice. Thus, these results suggest that NSE-controlled APPsw transgenic mice appear to be a more relevant model in neuropathological phenotypes of AD, and could be useful in developing new therapeutic treatments for targeting the aberrant phenotypes that appear in these mice.

P008 Amplification of Mouse Telomeres by Quantitative PCR

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Telomeres are DNA structures located at the ends of eukaryotic chromosomes. In vertebrates they consist of the nucleotide repeats of the unit TTAGGG. Telomeres are important for genome stability and for maintenance of the cell cycle. Telomere length has been correlated with aging, tumor formation, and DNA repair mechanisms. Measurement of telomeres by PCR amplification has been problematic due to the formation of dimers by the primers designed to hybridize to the telomere repeats. Recently, a set of primers that overcome this problem have been created and used to develop an assay to measure human telomeres by real-time quantitative PCR. Our objective was to evaluate these primers for amplification of telomeres in other mammalian species from total genomic DNA, most specifically in mice. Total genomic DNA was extracted from one sample each of bovine, hamster and E. coli cells and from 3 C57BL/6J mice by a standard DNA extraction protocol. A standard 10 µl PCR protocol was used to evaluate the primers for each of the samples in duplicate. Initial results suggest that this set of primers does adequately amplify telomere repeats from mammalian DNA. Also, when total genomic DNA is omitted or bacterial DNA is substituted, no PCR products were produced. This set of primers should be useful to adapt the human assay for telomere measurement to other mammalian species. This will provide a rapid and simple assay for the measurement of telomere length from total genomic DNA. This assay will aid in the study of telomere function and importance in diseases associated with aging and cancer formation.

P009 Development and Application of a Guinea Pig Model of Q Fever Via Aerosol Exposure

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Q fever is a zoonotic disease caused by the obligate intracellular, globally distributed bacterium Coxiella burnetii. Humans with Q fever may experience acute flu-like illness and pneumonia and/or chronic hepatitis or endocarditis. Biochemical markers have grouped isolates from chronic disease patients distinct from acute disease/arthropod/domestic animal isolates. A guinea pig aerosol challenge model was developed using C. burnetii Nine Mile phase I administered using a specialized chamber designed to deliver droplet nuclei directly to the alveolar spaces. Three guinea pigs per group were used to compare 6 log10-increment doses of C. burnetii, vaccine protection against high-dose infection, and PBS negative controls. Clinical signs included fever, inappetance, respiratory difficulty, and death, with degree and duration of response corresponding to the dose of organism delivered; vaccination conferred complete protection against development of clinical signs. Histopathologic evaluation of high-dose infected animals showed coalescing panleukocytic broncho-interstitial pneumonia at 1 week p.i. that resolved to multifocal lymphohistiocytic interstitial pneumonia by 1 month. Clinical signs and pathologic changes noted in these guinea pigs are comparable to those seen in human acute Q fever, making this an accurate and valuable animal model of human disease. This model was used to compare the relative virulence of a selection of 8 isolates of C. burnetii from 4 different genotypic groups. Low, intermediate, and high doses were used with 3 guinea pigs per dose-group for each of the 8 isolates. Severe clinical illness, including death, was seen in guinea pigs infected with acute disease-associated isolates, while no to moderate clinical illness was observed in animals from chronic disease-associated isolate groups. These data suggest that phase 1 C. burnetii isolates have a range of disease potentials and support a distinction in strain virulence between the established genotypic groups.
P010 Echocardiography of a Transgenic Mouse Model Using Isoflurane Anesthesia as an Alternative to Tribromoethanol

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Avertin (2,2,2-tribromoethanol) has been used for over a decade in noninvasive murine cardiovascular studies; however, its non-pharmacological-grade status conflicts with regulatory guidelines. In this study we evaluated isoflurane anesthesia as a pharmacological-grade alternative to tribromoethanol in murine echocardiography. We anesthetized wild-type (7-12 weeks old, n = 3) and transgenic mice (6-15 weeks old, heterozygote n = 4 and homozygote n = 6) overexpressing the sodium calcium exchanger NCX-1 (a trans-sarcolemmal protein that maintains cardiac calcium homeostasis) on a mixed C57BL/6-C3H background with isoflurane (0.8-1.0% in 100% O2 at 0.5L/min) or tribromoethanol (2.5%, 0.01ml/g i.p.) followed by ultrasound imaging. We imaged each group of mice at least twice with each anesthetic regimen over four weeks using 15 MHz, 2-D directed M-mode echocardiography. Scanning time, as a function of anesthetic efficiency, was assessed by measuring time from initial induction to end of a complete scan. Between anesthetic groups at similar heart rates (545 versus 528 beats/min, isoflurane and tribromoethanol) anesthesia yielded comparable (P > 0.05) values for all measured cardiac parameters (isoflurane versus tribromoethanol: ventricular septal thickness = 0.68 versus 0.67 mm; posterior wall thickness = 0.65 versus 0.61 mm; end diastolic dimension = 3.72 versus 3.67 mm; end systolic dimension = 2.42 versus 2.41 mm; aortic ejection time = 52 versus 53 msec; fractional shortening = 35.4 versus 34.6%; velocity fiber shortening = 6.77 versus 6.58 cm/sec; ejection fraction = 71.2 versus 68.9%). Within anesthetic groups, each genotype subgroup also responded with no appreciable anatomical or functional differences (all P > 0.05). Isoflurane anesthetized mice required less total study time than tribromoethanol anesthetized mice (10.08 versus 13.58 min, P < 0.05). These results demonstrate that isoflurane is a suitable alternative to tribromoethanol in echocardiographic studies of NCX-1 transgenic mice because data were more efficiently obtained while conserving cardiac parameters within a defined physiologic range.

P011 Effects of Adriamycin on Urinary Protein Excretion in Mongolian Gerbils

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Adriamycin administration in rats is a model of proteinuria resembling the nephrotic syndrome in humans. The purpose of this study was to evaluate the potential proteinuric effect of adriamycin in the Mongolian gerbil. The gerbil could be an animal model for nephrotoxicity studies because as a desert animal it has very efficient nephrons. This particularity has allowed the development of an experimental model of nephropathy (Am J Pathol 1976; 85: 519-522).

Methods: We used 20 male gerbils (Meriones unguiculatus), aged 4 months and housed in the Central Laboratory Animal Facilities of the University of León. Two groups were defined: a control group (n = 10) and an experimental group (n = 10). The gerbils belonging to the experimental group received a single injection of adriamycin (4mg/kg) into the femoral vein, using a minimally invasive procedure (Lab Anim 2003; 37: 68-71). Blood and 24-h urine (in metabolic cage) were obtained, and plasma urea, creatinine and total proteins and daily urinary protein excretion (Coomassie Brilliant Blue method) were measured. All the animals were sampled the day before the administration of the adriamycin (D1). The first five animals of each group were sampled again on day 3 and day 14; the other five were sampled on days 7 and 21.

Results: Plasma urea and creatinine levels were stable along the whole sampling. Proteinuria increased significantly on D3 (average values of 0.71 ± 0.17 mg/day compared to 0.49 ± 0.07 mg/day on D1) and remained in values over 0.60 mg/day during the rest of the experimental period. Plasma total protein diminished significantly (P < 0.05) on D3 and soon returned to normal.

Conclusions: Adriamycin administration in the gerbil leads to a certain proteinuria, more moderate than in the rat, which tends to return to normal in time. This fact reveals the minor glomerular susceptibility of gerbil’s kidney to the action of this drug.

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P012 Effects of Halothane and Isoflurane Anesthesia on the Glucocorticoid Levels in NZW Rabbits

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To determine the effects of two commonly used volatile anesthetics, halothane and isoflurane, on the serum corticosterone and cortisol levels, 20 NZW rabbits maintained under conventional conditions (12/12 L/D; 20-22°C; 50-55% relative humidity; 10-15 air changes/h) were assigned to 2 treatments groups (n = 10): group H (halothane-oxygen) and group I (isoflurane-oxygen). Anaesthesia was induced by face mask (3.5% halothane and 4.5 % isoflurane in oxygen); the animals were then intubated for anesthetic maintenance for 30 min (1.5% halothane and 2.5% isoflurane in oxygen). Blood samples were obtained at 9 time points: before induction, and 1, 10, 30, 60, and 120 min and 24, 48 and 72 h after intubation. Serum corticosterone and cortisol levels were measured by competitive enzyne immunoassay. We observed a significant increase of the serum corticosterone concentrations from 1-30 min after intubation in the halothane anaesthesia when compared with the isoflurane group (from 14.06 ± 1.61 ng/ml in the group I to 27.23 ± 2.36 ng/ml in the group H), whereas there were no significant changes (P > 0.05) in the serum cortisol levels between halothane and isoflurane groups. Both anesthetics increases serum glucocorticoids levels during the maintenance of anaesthesia when compared with the baseline levels. However, isoflurane could block the stimulation of the HPA axis through the GABA receptor. Therefore, halothane seems to stimulate the stress response more than isoflurane in rabbits.

P013 Effects of the Fentanyl-Droperidol Treatment on Select Plasma Biochemical Parameters in NZW Rabbits

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To determine the effects of two commonly used volatile anesthetics, halothane and isoflurane, on the serum corticosterone and cortisol levels, 20 NZW rabbits maintained under conventional conditions (12/12 L/D; 20-22°C; 50-55% relative humidity; 10-15 air changes/h) were assigned to 2 treatments groups (n = 10): group H (halothane-oxygen) and group I (isoflurane-oxygen). Anaesthesia was induced by face mask (3.5% halothane and 4.5 % isoflurane in oxygen); the animals were then intubated for anesthetic maintenance for 30 min (1.5% halothane and 2.5% isoflurane in oxygen). Blood samples were obtained at 9 time points: before induction, and 1, 10, 30, 60, and 120 min and 24, 48 and 72 h after intubation. Serum corticosterone and cortisol levels were measured by competitive enzyne immunoassay. We observed a significant increase of the serum corticosterone concentrations from 1-30 min after intubation in the halothane anaesthesia when compared with the isoflurane group (from 14.06 ± 1.61 ng/ml in the group I to 27.23 ± 2.36 ng/ml in the group H), whereas there were no significant changes (P > 0.05) in the serum cortisol levels between halothane and isoflurane groups. Both anesthetics increases serum glucocorticoids levels during the maintenance of anaesthesia when compared with the baseline levels. However, isoflurane could block the stimulation of the HPA axis through the GABA receptor. Therefore, halothane seems to stimulate the stress response more than isoflurane in rabbits.
In order to obtain blood samples for measuring plasma biochemical parameters, animals must be quiet or anesthetized. Under these circumstances, possible changes due to the treatment used must be considered. To assess the response of plasma hepatic and renal biochemical parameters to anesthesia, 20 NZW rabbits maintained under conventional conditions (12/12 L/D; 20-22°C; 50-55% relative humidity; 10-15 air changes/h), were assigned to 2 treatment groups (n = 10): control (IV saline solution) and fentanyl-droperidol (0.4 ml/kg SC of “Thalamon” solution: 2.5 mg/ml droperidol, 0.05 mg/ml fentanyl). Blood samples were obtained from the central ear artery at 6 time-points: before injection and at 10, 30, 60, and 120 min and 24 h after injection of the tranquilizer/saline. Plasma ALT, AST, ALP, GGT, BUN, creatinine, phosphate and potassium levels were measured by the Hitachi 747 autoanalyzer. Reflexes, heart and respiratory rates were also recorded. We observed a significant decrease of heart and respiratory rate. The only significant change in plasma was observed in the fentanyl-droperidol group, with an increase of BUN levels 120 min after injection (from 11 ± 1.7 of control group to 20.6 ± 2.0 IU/dL). The other plasma biochemical parameters remained within the normal range values of the species. We conclude that use of the fentanyl-droperidol mixture had little effect on the plasma biochemical parameters evaluated, and is therefore recommended for use as a tranquilizer in rabbits.

**P014 Gender Responses to Stress by Continuous Heart Rate Monitoring in the Conscious Mouse**

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The use of radio-frequency (RF) telemetry has recently allowed for continuous electrocardiogram (ECG) monitoring on conscious unrestrained mice. However, the physiological effect of stressors, such as those encountered during routine operations in an animal facility, remains to be evaluated. This project sought to evaluate the effect on heart rate (HR) and gender responses of a single short-duration stress stimulus. Normal adult CD-1 mice (11 male and 10 female) were instrumented with miniaturized ECG RF telemetry implants and allowed to recover for 10 days prior to study. Brief (<10 sec) handling while cage changing was chosen as the stress stimulus. Individual continuous ECG recordings were obtained (i) 15 min prior to stimulus (baseline), (ii) during cage change (stress stimulus), and (iii) 75 min after stimulus (recovery). HR data was obtained from 3- and 5-sec samples extrapolated from the ECG trace and averaged into minute segments for trend analysis. The resulting data showed a higher average baseline HR in females versus males (614.6 ± 49.5 versus 516.4 ± 56.9; P = 0.0025). During the stress stimulus, significant increases in HR (731.3 ± 44.5 males, 736.6 ± 33.0 females; P < 0.0001 versus baseline) were observed in both genders. Males HR increased by 43 ± 16%, remaining at peak for 30 min and dropping to 16 ± 17% above baseline after 75 min. In contrast, HR in females increased only by 20 ± 10% (P = 0.0045 versus males), recovering quicker and dropping to 8 ± 12% (P = 0.0696 versus males) above baseline after 75 min. We conclude that male CD-1 mice: 1) respond with a greater elevation in HR in response to stress stimulus; 2) remain at peak HR longer; and 3) recover slower than female mice. Also, the average HR of both genders does not completely return to baseline levels 75 min after stress, suggesting a prolonged influence of sympathetic output in the conscious mouse.

**P015 Gentamicin Nephrotoxicity in a Gerbil Model**

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Objective: Several species have been used for the development of experimental models of chronic renal failure induced by means of gentamicin administration. In this study we have tried to characterize the renal insufficiency caused by gentamicin administration in the gerbil.

Methods: We used 20 conventional male gerbils (Meriones unguiculatus), aged 4 months and housed in the Central Laboratory Animal Facilities of the University of León. Two groups were defined: a control group (n = 10) and an experimental group (n = 10). The gerbils of the experimental group were treated with a dose of 80 mg/kg of gentamicin (subcutaneous administration) daily for 9 weeks. Samples of blood (to evaluate urea and creatinine) and 24-h urine in metabolic cage (to measure the creatinine) were obtained. From 0 to 9 weeks, half of the animals in every group were sampled alternately. At the end of the experiment, the kidneys were removed for microscopic study.

Results: Animals suffered from an important weight loss, mainly during the first third of the experimental protocol. Plasma urea and creatinine levels showed a progressive increase up to the end of the experiment (average values of urea: week 0 = 41.39 ± 3.84 mg/dl; week 9 = 98.35 ± 18.28 mg/dl) (average values of creatinine: week 0 = 0.45 ± 0.04 mg/dl; week 9 = 0.59 ± 0.06 mg/dl). At the same time, the endogenous creatinine clearance diminished on a gradual basis during all the experience (week 0 = 3.07 ± 0.26 ml/min/kg; week 9 = 2.21 ± 0.23 ml/min/kg). Microscopic evidence of tubulonephrosis were observed.

Conclusions: Our study allows us to state that the chronic renal failure induced by the administration of gentamicin in the gerbil could be a new and interesting model of renal insufficiency.

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**P016 Histomorphologic Phenotype of HLA-B27 Line 133-1 Rats**

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Rats that are transgenic for HLA-B27 develop spontaneous multiorgan inflammatory diseases, including spondyloarthropathies, and inflammatory gastrointestinal disease similar to those of humans that have the HLA-B27 allele. One line of HLA-B27 rats designated 133-1 develops a disease phenotype that includes enteritis, arthritis, and orchitis. The objective of this study was to establish a histomorphologic phenotype of the 133-1 line that had been rederived to remove Pasteurella pneumotropica, pinworms, TMEV, and a Helicobacter sp. Four homozygous 133-1 HLA-B27 rats, four hemizygous 133-1 HLA-B27 rats and four HLA-B7 transgenic control rats (different transgene, no disease expression) were evaluated by histomorphologic examination of multiple organs at 8 weeks (young adult) and 29-36 weeks (aged adult) of age. No evidence of intestinal or arthritic disease was found in any of the rats examined.
Testicular degeneration with testicular and epididymal granulomas were present in all homozygous and hemizygous aged adult rats; no testicular lesions were seen in young adult rats. The lack of intestinal and joint disease in the rats examined suggests that environment (in this case rederivation) may have influenced development of spontaneous disease, reinforcing data obtained from other lines of HLA-B27 rats. The finding of testicular and epididymal disease also confirmed that the latter inflammatory condition occurs independent of rederivation. Previous reports have indicated that pre-rederivation arthritis occurred in greater than 20% of the examined animals, diarrhea was observed in 54% of the males and 100% of the females, and colonic lesions were observed in 4/4 rats of the HLA-B27 133-1 line. Moreover, previous reports indicated that line 133-1 HLA-B27 rats hemizygous for the transgene did not develop intestinal or joint disease. The finding of testicular/epididymal disease in hemizygous rats in this study suggests that genetic requirements for the manifestation of orchitis and epididymitis differ from those needed for the development of arthritis and enteritis in the 133-1 line.

P017 Housing Conditions Influence Rat Sleep Architecture

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Environmental conditions may have pervasive effects on sleep and waking behaviors of laboratory animals. In this study, we used subcutaneously implanted radiotelemetry transmitters to examine how altering housing conditions affects rat sleep architecture. Specific-pathogen-free, 300-600 g adult male SD rats were obtained and housed in static microisolators. Health monitoring information available from quarterly health reports supplied by the vendor indicated that animals were free of the following organisms: Sendai virus, pneumonia virus of mice, rat coronavirus/sialodacryoadenitis virus, Kilham’s rat virus, Toolan’s H-1 virus, Hantaan virus, reovirus type 3, encephalomyelitis virus, mouse adenovirus, rat parvovirus, Helicobacter sp., Mycoplasma pulmonis, Encephalitozoon cuniculi, cilia-associated respiratory bacillus, other pathogenic bacteria and fungi, endoparasites and ectoparasites. Eight rats were subcutaneously implanted with telemetric physiologic monitors that simultaneously recorded electrocorticogram (ECoG), electromyogram (EMG), and gross locomotor activities and allowed to recover 7-10 days before being placed on study. In a three-way cross-over study design, rats were housed in standard, unadorned polycarbonate caging, provided with a section of closed-ended PVC pipe to enter, or housed in polycarbonate caging with one corner blackened externally with paint. Signals were collected simultaneously from all animals and in the PVC pipe or a blackened area within the cage led to significant effects on the architecture of rat sleep. Specifically, a PVC pipe allowed the rat to enter sleep earlier in the inactive “lights on” period. During the first hour following lights on, wake entries and wake duration were significantly reduced compared to standard housing conditions ($P < 0.05$, $n = 8$ animals, 5 nights each condition). In addition, statistically significant increases ($P < 0.05$) in REM sleep occurred during the first 1.5 h of the inactive period of the rat circadian cycle, with further significant increases ($P < 0.05$) at later time points for the animals housed with a PVC pipe. Finally, both the PVC pipe and blackened-corner conditions led to significant modifications in light and delta sleep in the hour prior to and following lights on ($P < 0.05$; $n = 8$ animals, 5 nights both conditions versus standard housing). These data demonstrate that minor changes in housing conditions have a significant impact on rat sleep architecture that may affect the outcomes of experimental studies.

P018 Recombinant Frequency of Each Microsatellite Locus in Congenic Strains, and Suitable Numbers of the Markers for Checking of the Speed Congenic Strain

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Congenic mice have been used for analysis of the effect of genetic background on established mutations. The “speed congenic technique” is a powerful tool to obtain congenic mice rapidly. To identify recipient contribution, each microsatellite locus on each chromosome (Ch.) was monitored. In this study, we clarified recombinant frequency of each locus on Ch. 1 (longest) and Ch. 19 (shortest), and determined how many microsatellite loci on the chromosome should be monitored.

Recombinant frequency of 58 microsatellite loci, including 37 microsatellite loci on Ch.1 and 21 microsatellite loci on Ch.19, were examined using 19 N2:{{(BALB/c×C57BL/6J)×C57BL/6J}×C57BL/6J} mice. These markers were spaced at 0.2-15.9 cM. In each locus, 7-14 out of the 19 individuals on Ch.1 (36.8- 57.9%), and 7-11 out of the 19 individuals on Ch.19 (36.8- 57.9%) were homozygous (B6/B6 type). No significant difference in recombinant frequency on each locus was observed ($P > 0.05$). On the basis of these results, the suitable number of markers to check the background of speed congenic mice was estimated. Various densities of markers, 10, 8 and 6 markers in total, were selected from Ch.1 and Ch.19. These markers were spaced at approximately 20, 25, and 33 cM, respectively. A mouse that revealed highest percentage of the loci showing the B6/B6 type by 10, 8, or 6 markers also revealed the highest percentage by 58 microsatellite markers. However in other mice with lower percentages using the 58 markers, the level of recombinant frequency was different depending on the density of the markers. The results suggest that recombinations occur randomly between C57BL/6J and BALB/c; 3 to 5 markers/Ch. is suitable for selection of mice for use in production of speed congenic mice, but < 5 markers may be needed to prove the genetic background of the mice.

P019 The Effect of Hyperglycemia and Insulin Therapy in a Cecal Ligation and Puncture/Total Parenteral Nutrition Model of Sepsis

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Critically ill patients frequently exhibit both hyperglycemia and insulin resistance. A recently published clinical trial demonstrated that intensive insulin therapy, targeting glucose levels of 80 to 110 mg/dl, reduced both morbidity and mortality in critically ill patients with hyperglycemia. However, there are few established preclinical sepsis models that demonstrate the hypermetabolic state. In order to determine the effect of hyperglycemia on animals that are severely septic, studies were conducted using the cecal ligation and puncture
(CLP) model of sepsis supplemented with total parenteral nutrition (TPN). Sprague Dawley rats (n = 102) were cannulated via the femoral vein and subjected to CLP. Immediately following surgery, a stepped TPN infusion regimen was initiated that resulted in hyperglycemia (> 200 mg/dl). We subsequently determined whether TPN and the resulting hyperglycemia influenced mortality rates, glucose levels and biomarkers for up to 96 h after CLP. In addition, groups of animals were studied at 22-24 h post CLP to investigate the influence of hyperglycemia on markers of inflammation and organ damage. We found that animals that died early (≤ 30 h, n = 33) had higher blood glucose levels than animals that died later (n = 31, P < 0.003) or survived to 96 h (n = 38, P < 0.0001). Animals that were severely hyperglycemic (n = 71) by 22 h post-CLP exhibited greater mortality by 96 h than those that were mildly hyperglycemic or normoglycemic (n = 31, P < 0.005). These animals also demonstrated significantly greater organ damage and increased levels of metabolic biomarkers and cytokines. We determined that insulin (2 IU/kg/hr, started at 10-12 h) significantly lowered blood glucose levels by 24 h post-CLP (P < 0.01) and reduced mortality by 26% (P < 0.03). In conclusion, we have developed a model of sepsis/hyperglycemia that exhibits increased morbidity/mortality, and which is responsive to insulin. This model will be of value in further studies of metabolic intervention and outcome in critical illness.

P020 Threshold of Trichloroethylene Contamination in Maternal Drinking Waters Affecting Fetal Heart Development in the Rat

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Halogenated hydrocarbons such as trichloroethylene (TCE) are among the most common water supply contaminants in the United States and abroad. TCE levels of 250 ppb were found in the Tucson, AZ water basin. The maximum contaminant level (MCL) is currently 0.005 mg/L. Epidemiologic studies have found an association, but not a cause-and-effect relation, between halogenated hydrocarbon contamination and increased incidence of congenital cardiac malformations or other defective birth outcomes. Avian and rat studies demonstrated statistically significant increases in the number of congenital cardiac malformations in those treated with high doses of TCE, either via intrauterine pump or in maternal drinking water, as compared with controls. This study attempts to determine if there is a threshold dose exposure to TCE above which the developing heart is more likely to be affected. Sprague-Dawley rats were randomly placed in test groups and exposed to various concentrations of TCE (2.5 ppb, 250 ppb, 1.5 ppm, 1100 ppm) in drinking water or distilled water (control group) throughout pregnancy. A total of 98 maternal rats (distributed amongst the 5 groups) produced a total of 1146 fetal hearts. The percent abnormal hearts in the treated groups ranged from 0% to 10.48%, respectively, with the controls being 16.4%. The data from this study indicate not only that there is a statistically significant probability (P < 0.08-0.001) overall of a dose response to increasing levels of TCE exposure but also that this trend begins to manifest itself at relatively low levels of exposure (i.e., < 250 ppb). If maternal rats were exposed to greater than this level during pregnancy, an associated increased incidence of cardiac malformations in developing rat fetuses occurred.

P021 TLR4 Regulates the Early TNFα Response in a Murine Model of Bordetella bronchiseptica Pneumonia

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We have previously demonstrated that Toll-like receptor 4 deficient (TLR4−/−) mice intranasally inoculated with Bordetella bronchiseptica develop severe pneumonia. This model was used to further investigate the early inflammatory responses critical to respiratory host defense in bacterial infection. Toll-like receptor 4 (TLR4) is a mammalian innate immune receptor that detects bacterial lipopolysacharide (LPS) and plays an important, but incompletely characterized, role in limiting Gram-negative bacterial pneumonia. Microarray analysis was performed using bone marrow macrophages from wild-type and TLR4−/− mice exposed to LPS or B. bronchiseptica in order to identify genes regulated by TLR4. Tumor necrosis factor alpha (TNFα) was identified as a candidate gene whose expression was TLR4-dependent and whose product may be essential to the initial immune response. To substantiate this hypothesis, wild-type and TLR4−/− macrophages were exposed to increasing amounts of B. bronchiseptica LPS or heat-killed bacteria, and the TNFα response was measured by ELISA. The TNFα response was dose-dependent and strongly contingent on the presence of TLR4. In vivo experiments with wild-type, TLR4−/−, and TNFα−/− depleted mice also demonstrated that TLR4 regulates the early TNFα response. Groups of 3-8 mice were intranasally inoculated with 5 × 10⁴ B. bronchiseptica and sacrificed at various time points from 2 to 72 h. Lung homogenates were assayed by ELISA for TNFα, and showed the TNFα response was strongly TLR4-dependent in the first 12 h of infection. Histopathology in conjunction with colonization results also suggest that the early elicited, and TLR4-dependent, TNFα response is critical to preventing lethal pneumonia in the first 72 h in this infection model.

Conclusion: Collectively, these results indicate that during Gram-negative respiratory infection, defective early TNFα responses contribute significantly to the development of fatal pneumonia observed in TLR4-deficient mice.

P022 Gender Differences in the Blood Volume of Sprague-Dawley Rats (Rattus norvegicus)

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Hypothesis: Although blood volume is often based on an estimate of body weight regardless of sex, the hematocrit in females of many species is lower than in males. Likewise, we hypothesize that plasma and/or blood volumes are significantly different in rats between sexes, when corrected for body weight.

Methods: Sprague-Dawley rats, 67 to 77 days of age (19 female and 25 male) were surgically instrumented with indwelling femoral arterial catheters. On experiment day, the rats were weighed and placed in plastic chambers. The arterial catheter was flushed with saline. After 60 min of rest, 50 µL of saline, containing 25 µg of Evan’s blue dye, was injected into the arterial catheter followed by an additional 200 µL of saline. After a 5-min period to allow for mixing, a blood sample of 1.5 mL was obtained and placed in a vacutainer containing dry heparin. A portion of blood was also dis-
tributed to two microcapillary tubes for the determination of hematocrit by centrifugation. The blood sample was centrifuged and the plasma separated. The plasma was diluted 1:1 with distilled water and compared to standards of the dye at 0, 1.25, 2.5, and 5 µg/ml in distilled water at a wave length of 615 nm.

Results: The mean hematocrit was lower (37.6 ± 2.3 [SD] versus 39.7 ± 2.8 % PCV, \( P = 0.014 \)), plasma volume higher (5.3 ± 1.2 versus 4.4 ± 0.8 mL/kg body weight, \( P = 0.004 \)), and blood volume higher (8.5 ± 1.7 versus 7.3 ± 1.4 mL/kg body weight, \( P = 0.014 \)) in female rats than male rats. These differences do not appear to be a function of body weight, because there was no significant correlation between body weight and any of the measurements within either of the sexes.

Conclusions: Since female rats appear to have a plasma volume that is approximately 21% greater and a blood volume that is about 17% greater than male rats, assumptions of similarity between the sexes could have significant consequences for physiological experimentation.

**P023 Progress Toward the Development of a Transgenic Rat Model**

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Laboratory data clearly shows that the rat is a promising small animal model for studying HIV-1 pathogenesis. We developed the first HIV-1 transgenic rat model carrying the HIV-1 provirus with functional deletions in gag and pol regions. Expression of gp 120, tat, and nef protein have been detected in different tissues to include lymphoid tissue. We have also developed human CD4 (hCD4) transgenic rats. We have cross-bred them with the original HIV-1 TG rat, making a double TG rat with expressions of the HIV-1 transgenic rats. We have also developed human CD4 (hCD4) transgenic rats. We have cross-bred them with the original HIV-1 TG rat, making a double TG rat with expressions of the HIV-1 genes and the Human CD4 transgene. The construct of the HIV-1 transgenic rats has been well described (PNAS July 31, 2001). Founders for the hCD4 transgene were identified by Southern blot analysis. Integration-positive founders were mated with normal Fischer rats and offspring were screened by flow cytometry for expression of human proteins in peripheral blood samples. Founders were generated with different levels of expression from significant to high; the latter was used for breeding the line. Progeny were healthy and did not reveal any illness or gross pathology as compared to non-transgenic animals. The hCD4 line was crossed with the HIV-1 TG rats, and the offspring were analyzed to ensure that all genes were transferred. All phenotypes that were present in the organs of the HIV-1 TG rat with the gag-pol deleted construct were seen in the double transgenic. A key difference in the double TG rat compared to the HIV-1 TG rat was that kidney, CNS and PNS, cardiovascular, and skin diseases were more severe on the review histological slides. The disease process appears to occur earlier in life than in the HIV-1 TG rat, and the HIV-1 gene expression is abundant. The data demonstrates that the double TG rat may be a more promising model for studying the role of HIV-1 genes in the presence of the hCD4 expression.

**P024 Metastatic Basal Cell Carcinoma in a Rabbit**

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In domestic animals basal cell tumors are common, generally benign cutaneous neoplasms. Since 1992, 1103 rabbits have been submitted to the Pathology Section of the Division of Veterinary Resources. From these cases, 43 skin and subcutaneous masses were described, of which 19 (44%) were diagnosed as basal cell tumors. With one exception, these tumors were benign. A 3.5-year-old intact male, A1 and LCAT double transgenic white rabbit presented with a discrete, raised 0.5 × 1.0 × 1.0 cm mass on the face approximately 1.0 cm rostral to the medial canthus of the left eye. The mass was surgically removed under general anesthesia and diagnosed by microscopic examination as a solid-type basal cell carcinoma. Neoplastic cells were seen finger ing into the adjacent dermis, and extending to the cut border. Within 3 months the mass had re-occurred, measured 4.0 × 2.0 × 1.5 cm and was ulcerated. The tumor was again surgically excised. Based on finding neoplastic cells at the edges of the resected tissue during intra-operative examination of impressions smears, additional skin beyond the discrete tumor was excised to the maximum distance allowed by the position of the eye. Tumor cells were present in multiple vascular channels of the resected tissue, suggesting metastasis had occurred to other sites. Three weeks later, the rabbit presented in respiratory distress and died acutely. At necropsy, metastatic basal cells were present in the mandibular lymph node, lungs, liver and on the adventitial surface of the aorta. To the authors’ knowledge only five cases of basal cell carcinoma in rabbits are documented in the literature. In each of these cases, the tumors were benign and confined to the skin. This case represents the first report of metastatic basal cell carcinoma in a rabbit.

**P025 Surgical Correction of Obstructive Uropathy in Chimpanzees (Pan troglodytes)**

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Ureteroneocystostomy is the surgical transposition of the ureter via re-implantation into the bladder. The procedure is commonly performed in humans and small companion animals for renal transplantation, obstructive uropathy, ureteral trauma and congenital defects. We present two surgical cases in chimpanzee. A 33-year-old female chimpanzee with bilateral hydronephrosis and associated hydrourters of unknown cause underwent unilateral intra-vesicular ureteroneocystostomy. Three weeks post-surgery, azotemia resolved; three months post-surgery, the operated kidney returned to normal ultrasonic appearance. The second case, a 29-year-old male chimpanzee with unilateral hydronephrosis and hydrouretretery secondary to urolithiasis also underwent unilateral intra-vesicular ureteroneocystostomy. Three weeks post-surgery, the azotemia improved and ultrasound indicated less dilation of the renal pelvis. We conclude that ureteroneocystostomy can be used successfully as a palliative treatment for ureteral obstruction in chimpanzees.
P026 The Use of Fipronil and Selamectin for the Treatment and Control of Parasites on Mice

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The current treatment options for mice that are infested with parasites are limited, require repeated application and are costly over the long run. The veterinary pharmaceutical industry has developed new products that have been approved to treat parasites in companion species (Plumb, Donald C. 2002. Veterinary Drug Handbook, Fourth Edition: 371-372, 764-765.). This study examines the use of two of these products in treating parasites of mice. Fipronil was dosed at 20 mg/kg, Selamectin was dosed at 6 mg/kg. The products were diluted with 70% ethanol to yield a treatment dose of 0.1ml, which was applied along the back of the animal using an automatic pipette. Mice were group-housed in standard microisolator caging with a cage density of 4 mice per cage.

The study was conducted in two parts. Twenty-two mice (7 C57/Bl6, 15 cd/l) with confirmed diagnosis by fur plucking of Myobia musculi were randomly selected for treatment with either Selamectin (n = 8) or Fipronil (n = 8). Two cd/1 mice that had no exposure to mites were treated once and placed in direct contact with the 6 remaining positive animals.

All mice were checked weekly by fur plucking and direct examination under a dissecting microscope. After 2 weeks, no mites were found on any mice treated with Selamectin or Fipronil. Mice that were treated prophylactically were negative, and the cage mates had a decrease in the number of eggs seen on pluckings. After 4 weeks, there were no positive animals found in the any of the treated animals.

The second part of the study involved 12 cd/1 mice that were positive for both Sphacia obvelata and Aspicularis tetraptera. During the study all mice were tested weekly by fecal examination and taping. Six mice were randomly selected for one treatment with Selamectin. After 6 weeks, there was no evidence of endoparasites from the treated mice. At 8 weeks post-treatment, the remaining 6 mice positive for endoparasites were randomly introduced into the cages. After 6 weeks, only the 6 non-treated mice were positive. All mice were euthanized with CO2 and cecal contents examined microscopically. The 6 mice treated with Selamectin were negative for endoparasites while the 6 non-treated mice were positive for endoparasites.

P027 An Evaluation of Preparation Methods and Storage Conditions of Tribromoethanol

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This is the in vitro portion of a study designed to establish guidelines for preparation, storage, and use of tribromoethanol. Objectives included 1) evaluation of purity of tribromoethanol powder from three suppliers; 2) evaluation of nine common methods of preparation for a 25 mg/ml (working solution) for particulate and breakdown product formation; 3) evaluation of particulate and breakdown product formation and pH change in 1 g/ml (stock) solutions and working solutions stored under four conditions (25°C and 5°C in light and in dark); and 4) evaluation of a stock and working solution of tribromoethanol that caused lethal effects in mice. These objectives were met with the use of nuclear magnetic resonance spectroscopy, gas chromatography/mass spectrometry, particle size and UV-turbidity analysis, and pH strips. Tribromoethanol powder from three suppliers varied in purity. No significant differences in breakdown product formation, particle size, or turbidity were noted between the nine preparation methods evaluated. A low level of dibromoacetaldehyde, a potential breakdown product reported to cause toxic effects, was detectable in all newly prepared solutions. Stock solutions and the working solution stored at 5°C in the dark maintained a pH of 6.5-7.0, whereas the pH dropped for all other working solutions. Regardless of the storage condition or pH, a measurable increase in dibromoacetaldehyde was not detected in any of the solutions after 8 weeks. The stock and working solution that demonstrated lethal effects in mice had a pH of 6.5 and did not differ significantly from newly prepared, non-lethal solutions when evaluated for dibromoacetaldehyde. A decrease in pH could not be correlated to an increase in dibromoacetaldehyde or potential lethality as suggested in the literature. The toxicity associated with the lethal tribromoethanol in this study appears to be a result of a chemical reaction or breakdown product that has not been reported in the literature.

P028 Cytolethal Distending Toxin-Producing Flexispira rappini-Like Organism Isolated from Feces of a Common Marmoset (Callithrix jacchus) with Chronic Colitis

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A slowly growing microaerophilic cytotoxophilic distending toxin (CDT)-producing Flexispira rappini-like organism was isolated from the feces of a common marmoset (Callithrix jacchus) with chronic colitis. This bacterial strain possessed a pair of sheathed bipolar flagella and was positive for γ-glutamyltranspeptidase, oxidase, negative reaction for urease, nitrate reduction, alkaline phosphatase hydrolis. A 16S rRNA sequence of our isolates was identical with those of previous isolated F. rappini strains (GeneBank acc. no. AF034135) from bacteremia child with pneumonia. Bacterial sonicates and the cell culture media of our isolates induced G2/M cell cycle arrest, polymerized β-actin accumulation, cell distension and apoptotic cell death in HeLa cell lines after 72 h. The result of a CDT-specific polymerase chain reaction and its sequences also indicated that our isolates produce novel CDT. When this bacteria strain was experimentally inoculated in 20 scid mice by intraperitoneal injection and oral gavage, it induced focal erosive and subacute necrotulent colitis at 4 weeks (i.p.) and 2 weeks (o.g.) after infection. Furthermore, many spiral microorganisms were found within the crypt of the hyperplastic mucosa using modified Steiner-Steiner silver stain. The majority of the apoptotic cells in the infected mice were crypt epithelial cells and polymorphonuclear leucocytes (PMNs) cells that were localized in mucosa of the colon using TUNNEL staining. These results demonstrate the potential of this organism to be a pathogen in immunocompromised animals and marmosets.
P029 Frequent Lacrimal (Harderian) Gland and Other Tumors in Inbred White-FOOTed Mice (Peromyscus leucopus)

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In 1997 three lines of inbred Peromyscus leucopus (GS109A, GS16A1, and GS16B), were imported into the Peromyscus Genetic Stock Center. All three lines were approximately at generation 20 of brother-sister mating. Since then, records have been kept on tumors detectable by visible inspection of live animals. Two of the inbred lines, GS109A and GS16A1, presented tumors with frequencies substantially higher than that of the other inbred line or of a randombred P. leucopus stock. The average age of detection was 456 ± 75 d (n = 24) and 568 ± 168 d (n = 12) for GS109A and GS16A1, respectively. Surprisingly, the majority of the tumors detected clinically appeared to be Harderian gland, 23/24 for GS109A and 8/12 for GS16A1. In the same time period only a single tumor, a fibrosarcoma, was noted in the other inbred strain GS16B, and one Harderian gland tumor was detected in the randombred stock. Based on the number of animals born to each group, tumor frequencies were approximately 6.8%, 2.5%, 0.2%, and 0.03% for GS109A, GS16A1, GS16B, and random-bred P. leucopus stock, respectively. No apparent gender skew in the tumor distribution was evident, nor did there appear to be preference as to which eye developed the Harderian gland tumor. The ocular tumors appeared to be highly malignant with high mitotic indices, marked anaplasia, and abundant metastases, primarily to the lung. The tumors were readily transplantable to other animals of the same line. Among various species lacrimal gland tumors are relatively rare. The high frequency of such tumors observed among these particular inbred lines is likely the result of the unique combination of genes selected during the inbreeding process rather than an inherent result of inbreeding per se.

P031 A Salivary Gland Choristoma in a Pig

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Necropsy of a clinically healthy 30-kg female pig (Sus scrofa) revealed light brown, irregularly shaped soft tissue lying just cranial to the heart. The mass was composed of a 41 × 23 mm piece surrounding the proximal aorta and an adjacent 42 × 12 mm piece. The mass was assumed to be thymic tissue, but a hematoxylin and eosin stain showed the structure to be composed of glands resembling mucous acini of salivary tissue. The cells contained poorly staining granules and condensed flattened nuclei against the basement membrane. A periodic acid Schiff and a mucicarmine stain showed the tissue to be positive for carbohydrates and mucin, respectively. No mitotic figures or other evidence of neoplasia was present. This is the first known report of a salivary gland choristoma in a pig. A choristoma is defined as normal tissue found in an abnormal location. Salivary gland choristomas have also been reported in humans. Cases are rare with most heterotopic salivary glands being found in the neck and others in areas such as the middle ear and rectum.

P032 Buprenorphine Used as a CRI in Swine

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In an on-going attempt to alleviate pain within protocols for cardiac procedures, we took a drug with past success given intramuscularly and have maximized its affects via a constant rate infusion, attenuating the roller-coaster effect in analgesic properties seen with the typical 2-h intramuscular redosing time period. Having recognized the benefits of a constant rate infusion (CRI) in other animal models, a therapeutic range was developed for swine of 0.5-10 mcg/kg/hr, although 2 mcg/kg/hr is initially used and adjusted as needed. Initial testing began on acute defibrillation studies, using an anesthetic protocol consisting of a premedication of 0.07-0.12 ml/kg TKX (50 mg/ml of each of tiletamine/zolazepam/ketamine/xylazine) given IM, anesthetized further with an approximate dose of 30 mg of sodium pentothal IV, then placed on isoflurane for maintence through surgery. The CRI is started prior to any surgical attempts and continued until end of study or possibly into the recovery period. Significant results were seen immediately; a measurable difference was noticed in response to post-defibrillation, a known painful trigger, by a considerable decrease in muscle fasciculations, rigidity and tachycardia. Other vital measurements, including CO2 and peripheral blood pressures, returned to normal limits 120-150 sec faster than with IM route. The values had a smaller range of fluctuation when using the CRI compared to the IM injection, leading us to believe that we are maintaining a more constant therapeutic plane of analgesia. It was noted that there appeared to be some tolerance built up to the buprenorphine as the procedure

P030 A Naturally Occurring Fatal Case of Herpesvirus Papio 2 Pneumonia in an Infant Baboon (Papio sp.)

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A 7-day-old infant baboon (Papio anubis) was presented for anorexia of 2 days duration. This animal had been separated from its dam the morning of its birth and was being hand-raised for inclusion in a specific pathogen free (SPF) colony. Physical examination revealed a slightly decreased body temperature, lethargy and dyspnea. There was no nasal or ocular discharge. The baboon was placed on a warm water blanket, given amoxicillin/clavulinate 20 mg q12h orally and 60 ml normal saline subcutaneously. The animal continued to deteriorate despite tube feeding, subcutaneous fluid administration and antibiotic therapy and died 2 days later. Gross necropsy revealed a thin carcass and severe bilateral diffuse pulmonary consolidation. No other gross lesions were appreciated. Cultures of the lung did not show bacterial growth. Histopathology of the lung revealed severe diffuse necrotizing pneumonia. Numerous epithelial and endothelial cells contained prominent intranuclear herpetic inclusion bodies. Virus isolation and real-time PCR from lung tissue were positive for Herpesvirus papio 2 (HVP2). DNA sequencing of PCR products confirmed this identification. HVP2 is endemic in baboons and usually causes genital and oral lesions in juveniles. Over 90% of adult baboons are serologically positive for HVP2, including this infant’s dam. This case is interesting because the animal’s age at onset suggests perinatal transmission at or immediately after birth, and the disease course suggests inoculation of the virus in the respiratory tract.
Problem: *Ehrlichia canis* is an emerging tick-borne pathogen of canines causing classical canine monocytic ehrlichiosis. Dogs are susceptible to infection with several different species of *Ehrlichia*, including *E. canis*, *E. ewingii*, *E. chaffeensis*, *Anaplasma phagocytophilum*, and *A. platys*. Canine ehrlichiosis can manifest clinically with signs of fever, anorexia, and epistaxis; however, most signs are non-specific. Hematologic abnormalities include thrombocytopenia, hyperproteinemia and hypergammaglobulinemia. In the laboratory animal setting, diseases such as canine monocytic ehrlichiosis, coupled with Lyme borreliosis or rickettsiosis, can cause significant morbidity and confound research results.

Approach: Randomly selected conditioned laboratory dogs were given a physical examination, with blood taken for CBC, biochemistry analysis, tick-borne pathogen serology, and PCR. Serology included titers for *E. canis*, *B. burgdorferi*, and *R. rickettsii*. Blood was analyzed by PCR and pathology was performed at the conclusion of experiments.

Observations: Three of the 21 dogs presented with illness manifested by fever, malaise, lameness, or hemostatic abnormalities. Results revealed no particular hematological or biochemical differences between *E. canis*-positive serology dogs compared to negative dogs. Differences in hypergammaglobulinemia and albumin/globulin ratio were detected among groups serologically positive for one pathogen compared to multiple pathogens (*P* = 0.0047 and *P* = 0.0081, respectively). *E. canis* PCR was negative for all dogs. Universal PCR for detection of *Ehrlichia* and *Anaplasma* 16S rRNA genes yielded 47% positive samples. Histopathologic examinations revealed lesions of glomerulonephritis and hepatitis.

Conclusions: From this cohort of dogs, serologic and molecular results indicate prior exposure and persistent infection in the absence of hematologic or biochemical abnormalities and overt clinical disease. Protein abnormalities such as hypergammaglobulinemia could reflect chronic infection and prompt further diagnostic work-up. Infection with these pathogens could also confound histopathologic examinations and physiologic responses resulting in questionable interpretation of research models.

**P034 Efficacy of Transdermal Fentanyl Patch Use in Sheep**

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Our institution is faced with the issue of providing around-the-clock post-operative pain management in sheep that have undergone surgical procedures such as a thoracotomy or laparotomy. This dilemma is not unique at the University of Pennsylvania; most laboratory animal facilities involved in research protocols must, at some point, address the challenge of providing such pain relief. The predominant factors contributing to this challenge are the relatively short duration of commonly used analgesics, personnel shortages, and budget constraints.

In sheep, to cover a 24-h period of analgesia, the best case scenario would require a “after-hours” administration of an 8-12 h analgesic (e.g., flunixin meglumine or buprenorphine), thus requiring overtime for the technician administering the medication. By utilizing an analgesic that does not require repeated short-term dosing, such as transdermal fentanyl patches, the need for extended personnel time and any associated cost may be minimized.

This study examined the proper placement of fentanyl patches and confirmed the maintenance of analgesic blood plasma levels (based on the human literature) in Dorset-cross sheep. As compared with dogs and pigs, one unique complication in using fentanyl patches in sheep is the oily nature of the sheep’s skin. The oil inhibits the adherence of the patch to the skin’s surface, therefore preventing proper absorption of the drug. In this experiment, three different fentanyl patch application techniques were investigated. The pharmacokinetics of fentanyl was used to confirm proper adherence, absorption and analgesic blood plasma levels. This series of experiments provides the laboratory animal community with a more convenient option for providing analgesia in post-op sheep by confirming the efficacy of Duragesic® fentanyl patches.

**P035 Epidemiological Findings of the Cardiovirus GDVII-Like Virus in Rats**

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Little is known about the virus in rats that causes seroconversion to GDVII virus antigen. The presumptive genetic sequence has been published and reports have been contradictory regarding pathological lesions caused by the virus, but no epidemiological information has been published. This presentation describes information related to the infectivity and transmission of the virus. The data were collected in the course of a “burn-out” attempt of genetically unique, severely hypertensive congenic rat strains. Routine colony surveillance of the containment barrier has demonstrated seroconversion of conventionally housed, dirty bedding-exposed surveillance rats to GDVII-like virus and the parvovirus RMV. At weaning, representative pups from 6 rat strains were moved to a containment/exclusion barrier area, individually housed in autoclaved, ventilated cages, and managed using sterile technique. Serology at 13-19 weeks of age of the 57 surviving rats from this first generation, was negative for antibody to GDVII and parvovirus. Brother-sister pairings were created but all other isolation procedures were continued. The breeding resulted in 111 pups in 15 litters from 5 strains. Serology of this second generation at 11-14 weeks identified 12 GDVII-seropositive rats from 6 litters. The positive ELISA results were confirmed by IFA by the testing laboratory and a confirming laboratory. The saved serum from the first generation was retested by IFA by 2 laboratories and confirmed negative. The serological evidence for the parvovirus was not found, and neither virus was seen in unrelated, conventionally housed colonies at the facility. These results indicate that the GDVII-like virus can be transmitted by indirect contact and can be transmitted by direct contact by seronegative rats. Shedding apparently occurs in seronegative rats for as much as 13.5 weeks after the last known exposure.
P036 Gastric Foreign Body and Resulting Severe Weight Loss Associated with Environmental Enrichment Devices in a Rhesus Macaque

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A 9.5-year-old female rhesus macaque (*Macaca mulatta*) housed from birth in a B-virus/retrovirus-free breeding colony presented with diarrhea, reduced appetite, and severe weight loss. This animal had a history of trauma by conspecifics due to her subordinate position in the colony group, and had thus been housed with only one young male for the preceding 6 months. She had been previously hospitalized in May 2000 for weight loss, and at that time a large phytobezoar (hay) was removed from the stomach. On presentation in February 2004, feces were soft, but were within normal limits the following day and remained normal throughout hospitalization. Weight was 4.2 kg, down from 6.4 kg noted on routine physical examination one month prior. Hematology and serum chemistry were unremarkable except for low serum creatinine (0.6 mg/dL), and low total protein (5.8 g/dL, both globulin and albumin were low). Low serum creatinine in humans is consistent with inadequate food intake. No improvement was noted after 2 weeks of single housing with monitored food intake. Though no masses were externally palpable and contrast radiographs were inconclusive, blue plastic particles and hair-like particles were observed on gastroscopy. A small mass formed of hay interspersed with 1 mm plastic particles from an enrichment device was removed surgically. Two months later, the animal remained healthy and weighed 7.1 kg, and was successfully acting as an “aunt” to a troop of weanlings. Hay had been used in the past as a foraging substrate, though not for one year prior to this case, illustrating the importance of selecting suitable enrichment strategies for nonhuman primates.

P037 Honey as a Topical Dressing to Treat a Large, Devitalized Wound in a Stumptail Macaque

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There are many reasons why wounds are managed as open wounds and not by primary closure. Indications include gross contamination, infection and skin loss resulting in inadequate adjacent tissue available for wound closure. The most common method to manage an open wound is with wet-to-dry dressings. Wet-to-dry dressings provide mechanical debridement and promote the movement of viscous exudates away from the wound. Wet-to-dry bandages should be changed relatively infrequently. Honey decreases direct effects on tissue and antibacterial properties. Additionally, dressings with honey can be changed relatively infrequently. Honey accelerates healing because of direct effects on tissue and antibacterial properties. Additionally, dressings with honey can be changed relatively infrequently. Honey decreases inflammatory edema, hastens devitalized tissue sloughing, attracts macrophages which cleanse the wound, provides a local cellular energy source and protectively covers the wound. A high osmolarity, acidity and hydrogen peroxide content confer honey with antibacterial properties. Here we describe the use of honey to manage a bite wound in a stump tail macaque. The wound initially measured approximately 3 × ½ in. and was more than 1 in. deep. After debridement, the wound was bandaged with a layer of sterile gauze soaked in honey, a layer of rolled cotton, and a layer of self adhesive tape. Wound dressings were changed two to three times weekly. The wound healed rapidly. By the end of two weeks, there was no exudate or necrotic tissue and the wound measured less than ¼ in. deep, ¼ in. long and ¼ in. wide. This report suggests that honey may be helpful in the management of open wounds in nonhuman primates and may minimize the frequency of handling.

P038 Insulinoma in a Rhesus Macaque (*Macaca mulatta*)

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A 34-year-old sexually intact female rhesus macaque (*Macaca mulatta*) presented with acute onset of lethargy and weakness. The animal had been obtained from another academic institution 19 years prior and individually housed indoors for use in a neurobiological study involving cognitive function and catecholamine receptors in the prefrontal cortex. Routine yearly serologic sampling performed 10 weeks prior revealed seroconversion to SRV. Physical examination findings included hypothermia (36°C) and mild dehydration. Blood glucose was below the detectable range (38 mg/dl) of a hand-held glucometer. Empirical treatment for hypoglycemia, sepsis, dehydration, and hypothermia resulted in a dramatic improvement. Pretreatment blood samples were submitted to a commercial veterinary diagnostic laboratory for analysis. Serum glucose/insulin ratio confirmed hypoglycemia and relative hyperinsulinemia. Due to a poor prognosis, the animal was euthanized and submitted for pathological examination. Necropsy revealed a 2-cm round, circumscribed pancreatic mass. Microscopically the mass was composed of neoplastic cells arranged in disorganized clusters suspended in highly vascular fatty connective tissue stroma. Polymorphic granules with crystalline cores characteristic of beta cells were visible on electron microscopy. Immunohistochemical staining was moderately positive for the neuroendocrine cell marker chromogranin A, weakly positive for insulin, and negative for somatostatin and glucagon. Islet cell adenomas have previously been reported in nonhuman primates. They are typically nonfunctional neoplasms, producing no clinical signs, and are often considered incidental findings at necropsy. This report is unique in that it describes a pancreatic neuroendocrine tumor in a rhesus macaque with clinical, biochemical, histological, and ultrastructural findings consistent with an insulinoma. Insulin secreting tumors should be included as a differential diagnosis in instances of hypoglycemia in aged rhesus macaques.

P039 Left-Sided Heart Failure in Squirrel Monkeys (*Saimiri sciureus*)

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This is a retrospective analysis of necropsy findings of 6 colony-bred squirrel monkeys (*Saimiri sciureus*) that died between 2002 and 2004.
with a diagnosis of heart failure. As the colony of squirrel monkeys at our institution has aged, morbidity and mortality due to this syndrome has increased. Limited published data are available on heart failure in squirrel monkeys because this species has not typically been maintained long term. The cases include 4 multiparous females with an average age of 18 years and 2 intact males with an average age of 20.5 years. As is the case for other small animals experiencing heart failure, affected monkeys could be expected to present with a spectrum of signs such as exercise intolerance, dyspnea, tachypnea, cyanosis, cough, syncope, weak pulses, cool extremities, harsh lung sounds or crackles, arrhythmias, and sudden death. Three of the six monkeys presented with sudden death, while the others had varying clinical presentations prior to death or euthanasia. Clinical signs seen included tachypnea, hypothermia, and hind limb paresis. Ancillary tests such as CBC, serum chemistry, radiography, and chest auscultation were performed where possible. Antemortem differential diagnoses for these animals varied, but all had heart failure as a prime differential based on clinical signs and the previous diagnosis of this problem in aged squirrel monkeys. Postmortem findings from the six animals included multifocal myocardial fibrosis, atherosclerosis of the aorta, pulmonary edema, and free-fluid within the thoracic cavity. Three monkeys had subjectively enlarged left ventricular free wall measurements. One had atherosclerosis of the coronary arteries in addition to aortic atherosclerotic lesions. Another monkey had an acute aortic thromboembolism (saddle thromboembolism). Pulmonary edema, free fluid within the thoracic cavity, left ventricular hypertrophy, and aortic thromboembolism are all findings consistent with left-sided heart failure. Both dilative cardiomyopathy (as previously reported from this colony) and left-sided hypertrophic cardiomyopathy have now been identified. We are exploring a possible mode of inheritance within our colony, and are adopting a health surveillance program for aged squirrel monkeys in hopes of identifying those animals at greatest risk of developing heart failure. Ongoing investigations include efforts to understand the underlying etiology and to find potential diagnostic markers for future identification of monkeys at risk of developing heart failure in the breeding colony.

P040 Perineal Hernias in FVB/N Mice

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An increased incidence of perineal hernias was noted in several transgenic lines as well as their wild-type counterparts; all were on an FVB/N genetic background. Mice ranged in age from 6 weeks to 8 months. The mice were housed in several different rooms, all of which had no evidence of infectious disease on previous serology, histology and parasitological testing. At least 130 animals were identified, 4 of which were evaluated with the objective of further characterizing this syndrome. All four animals exhibited significant perineal swelling and gross necropsy of one male mouse revealed a large perineal sac containing most of the abdominal organs. The distal portion of the duodenum was incarcerated within this sac and was almost completely obstructed. In the remaining three animals, perineal swelling resolved with normal organ positioning noted at necropsy. Histologic examination of multiple organs including the pelvic musculature and bones revealed no lesions. A pooled fecal sample was positive by PCR analysis for both Helicobacter hepatitis and H. rodentium; however, this was considered an incidental finding as no histologic evidence of lower bowel inflammation was found. No other evidence of infectious disease was found on serology, parasitology, and microbiology examinations. Two of the affected FVB mice were matched to control animals from another facility. Computerized tomography (CT) scans clearly showed pelvic herniation and slight differences in the shape and diameter of pelvic canals when comparing affected to control animals. Culling of affected breeding mice significantly reduced the incidence of perineal herniation in the colony, suggestive of a genetic component to this syndrome.

P041 Spontaneous Squamous Cell Carcinoma in a Sooty Mangabey (Cercocebus torquatus atys)

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Spontaneous malignant tumors in nonhuman primates are relatively rare. The most frequently reported neoplasms in nonhuman primates are carcinomas, with the digestive system being the most commonly affected organ system. Squamous cell carcinomas (SCC) usually arise from the skin and stratified squamous epithelial cells of all body openings, and have been recognized in all domestic animals. In nonhuman primates, SCC has been reported in squirrel monkeys, capuchin monkeys, tamarins, marmosets, orangutans, baboons and different species of macaques, with most of the reports originating from the oral cavity or in areas of mucocutaneous junctions. Here we report a cutaneous squamous cell carcinoma diagnosed in an 11-year-old, 8.3-kg castrated male sooty mangabey (Cercocebus torquatus atys). During physical examination of this individual, a solid mass arising from the skin of the lateral cervical area was noticed. Grossly, the mass was firm, elongated, ulcerated at the tip and measured 2.7 × 2.0 × 2.3 cm. The mass was surgically excised, placed in 10% neutral buffered formalin and submitted for histopathologic evaluation. On histopathology, this tumor was composed of irregular masses and cords of neoplastic squamous epithelial cells, often undergoing keratinization, that invade the dermis and the subcutis. Additional tests, including hematologic evaluations and radiographic views of the abdominal, thoracic and cervical areas were normal. To the authors’ knowledge, this is the first reported case of a spontaneous cutaneous squamous cell carcinoma in a sooty mangabey.

P042 Staphylococcus aureas as the Causative Agent for Bronchopneumonia in a Captive Baboon

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A 1-year-old gang-housed (indoors/outdoor facility) female baboon (Papio anubis) was observed to exhibit generalized signs of depression/listlessness and specific signs of coughing/dyspnea. The animal was immediately lightly sedated and presented for medical evaluation consisting of physical examination, CBC/chemistries, fecal/urinalysis and radiography. Significant results of the evaluation included the following: (1) moist lung sounds, (2) productive cough, (3) mild muco-purulent nasal discharge, (4) slightly elevated leukogram, (5) negative fecal for ova/parasites, and (5) generalized increased lung radiopacity with multiple diffuse discrete areas of radiopacity suggestive of microabsceses. Based on these findings a presumptive diagnosis of bronchopneumonia was made. Accordingly, the animal was started on a preliminary course of enrofloxacin 4.4 mg/Kg TID. Additionally, blood/throat cultures and a trans-
tracheal washing (TTW) were obtained for culture/sensitivity. The blood and throat cultures were negative; however, the TTW grew a pure culture of *Staphylococcus aureus* (sensitive to erythromycin/ clindamycin). The animal was immediately started on erythromycin (10 mg/Kg TID) and showed dramatic clinical improvement within 3 days. The baboon was continued on antibiotic for an additional 7 days, at which time all signs were resolved. Following an additional 5 days cage rest, she was returned to her home troop. To our knowledge, bronchopneumonia due to *Staphylococcus aureus* is generally an infrequently/rarely observed condition among nonhuman primates and specifically has not been fully documented in the baboon.

P043 Successful Treatment of Decubitus Ulcer on the Plantar Aspect of Foot of an Adult Male Cynomolgus Macaque (*Macaca Fascicularis*)

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Traumatic injuries are common medical problems of macaques. Wounds over pressure points can result in decubitus ulcers. Treatment requires the patient be kept off the ulcer. Wound dressings that stimulate granulation tissue are also used to encourage healing.

This case report describes the successful treatment of a decubitus ulcer due to trauma in a macaque.

An 8-year-old cynomolgus macaque sustained numerous bite wounds after fighting with another male. Of greatest concern was a laceration on the left lower leg over the Achilles tendon. The animal became increasingly lame despite treatment. Although the tendon appeared intact initially, the left heel had dropped and the animal was bearing weight on the plantar surface of the foot in an abnormal position, indicating that the Achilles tendon was damaged. A decubitus ulcer developed just dorsal to the tuber calcanei. The ulcer was debrided, the Achilles tendon was repaired, the animal’s foot was casted, and the lesion healed. However, after the cast was removed and the animal started to bear weight on the foot, the ulcer recurred. After consultation with veterinarian who specializes in wound healing, we tried the following approach. The animal’s foot was encased in a fiberglass cast with a window over the ulcer. Two products known to enhance early formation of granulation tissue and collagen deposition (acemannan and maltodextrin) were applied to the ulcer. Since these granulation tissue stimulants lose their efficacy after one week of treatment, we alternated between the two, treating daily for a week with one product, then the next week with the other. The process was repeated for two months until the wound was healed. Finally, a paw pad toughener was applied. The results were excellent and the cast was removed.

P044 The Role of Commensal Microorganisms and Genetic Background in Immunodeficient Mice

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Many microbes that were previously considered commensal organisms have been shown to be primary pathogens of immunodeficient mice. To prevent these organisms from infecting immunodeficient mice, strict aseptic technique is required. Despite these practices, an outbreak of staphylococcosis, pneumocystosis, and pasteurellosis in T-cell receptor mutant mice occurred in our facility. These mice were derived from a BALB/c x C57BL/6 cross and carry a T-cell receptor mutation that results in autoimmune gastritis and a concurrent decrease in the total functional T-cell population. Several months after these mice were transferred to our facility they began displaying conjunctivitis, focal and systemic abscesses, and dyspnea. Upon necropsy, *Staphylococcus aureus* was found in all cases of conjunctivitis and abscessation, with occasional isolation of *Pasteurella pneumotropica*. The majority of the conjunctivitis was only seen in the right eye, a phenotype that may have arisen from a congenital C57BL/6 right eye microphthalmia. Several of the mice had acute alveolitis caused by *Pneumocystis carinii*. There was no evidence of gender or age predisposition among the affected mice; however, mice with dyspnea or mice that had been crossed to *rag2* knockout mice did not respond to treatment with oral or topical antibiotics. In an effort to identify the source of the causative agent cultures were taken of caretaker gloves, sterile caging prior to animal housing, and the cages housing the mice. Bacteria were cultured from caretaker gloves and cages housing mice but were not found in the sterile caging prior to animal housing, suggesting exposure during handling or from cagemates. This report reinforces that even when microisolator technique is performed in a HEPA-filtered change station, commensal and environmental organisms often spread to immunodeficient mice, resulting in disease; additionally, it demonstrates that disease manifestation may be influenced by the genetic background of the mouse.

P045 The Use of Buprenorphine Following Blatocyst Transfer

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Embryo transfer in rodents is a common surgical procedure performed by those working with genetically modified mice. The procedure involves two small (< 5mm) incisions made over each ovary. Embryos are injected through the incision site, into the uterus or oviduct of a foster female. Incisions are then closed, typically with surgical clips. While it is a simple process, it is a surgical procedure, and the potential for associated pain or distress is a concern. The controversy arises, however, as to whether analgesia should be administered to these animals. The main concern is that implantation of the embryo will be affected by the analgesic. Certainly manipulation and handling of these mice is curtailed following the surgery to minimize the loss of embryos, but the question remains whether an analgesic would have any affect on implantation. In order to examine this further, we selected the analgesic buprenorphine, a mixed partial opioid mu agonist and kappa antagonist, to be given at the end of surgery. We then monitored if the rate of implantation was affected by the administration of buprenorphine as well as whether it altered post-surgical behavior. Buprenorphine-treated groups vs. controls (n = 5) were used. Initial studies indicate that animals recover well under this regimen. Implantation rates of the buprenorphine treated groups were equal or better than that of the non-treated animals (mean implantation rate of 5.77 versus 3.25, respectively). These results indicate embryo implantation is not affected by a one-time administration of buprenorphine following surgery.
A 2-year-old and a 7-year-old rhesus macaque developed toxic epidermal necrolysis following administration of rituximab, a chimeric, monoclonal antibody against the CD20 antigen found on B lymphocytes used to treat certain B cell neoplasias. The macaques were part of a gene therapy study that involved administration of an adenoviral vector (AVV) and plasmid for human factor IX. Both of these animals developed antibody against the AVV, which resulted in low expression of the gene. Rituximab was administered to deplete the population of B cells producing antibody against the vector in hopes of enhancing gene expression. Both animals were administered rituximab according to recommended guidelines following pretreatment with corticosteroids and antihistamines to decrease the possibility of hypersensitivity. Two days post-treatment, the 7-year-old animal developed indurated and erythemic skin lesions which quickly progressed to ulceration. Despite aggressive treatment with analgesics, antibiotics, and corticosteroids, the lesions progressed, became necrotic, and required the animal to be euthanized 5 days later. Histopathologic findings were consistent with diagnosis of toxic epidermal necrolysis. The 2-year-old macaque had no reaction to the initial rituximab and received a second infusion 2 weeks later. Two days following drug administration, skin lesions developed and aggressive analgesic, antibiotic, and steroid treatment were initiated. The lesions healed with treatment in approximately two weeks. A third dose of rituximab was given approximately 2 months following the second rituximab treatment. Skin lesions developed again and were treated. The lesions were biopsied and histopathologic findings were consistent with toxic epidermal necrolysis. With antibiotic and steroid treatment, the animal made a full recovery. Toxic epidermal necrolysis is a severe, life-threatening condition which presented was unusual in that the macaque had normal appetite and mentation, and the edema was confined to the scrotum and prepuce in spite of marked clinical and histopathological abnormalities. Chronic renal failure must be on the differential list in cage-confined macaques with scrotal and preputial edema as the inguinal region is the dependent site for fluid accumulation.
limited to the ventrum, specifically the axilla, rostral mandible, cervical and inguinal regions. Differential diagnoses included infection, dietary deficiency, metabolic abnormality, endocrinopathy and immunological injury such as hypersensitivity.

Diagnostic tests included complete hemogram, blood chemistry, skin scraping for ectoparasite detection, hair plucks for dermatophyte culture, and a serum-based allergy panel. Results for all tests were within normal limits. Skin biopsies revealed lesions consistent with active allergic dermatitis and a diagnosis of atopic dermatitis (AD) was made. Therapy with oral cyclosporine (5 mg/kg/day) rapidly eliminated clinical evidence of dermatitis. Histologically, lesions resolved after 1 year of treatment. Clinical signs reappeared, however, when the dose of cyclosporine was reduced to 2.5 mg/kg/day and resolved once the dose was increased back to 5 mg/kg/day. AD is an inflammatory skin condition for which there are no pathognomonic clinical or diagnostic features other than there is one successful therapy. Basic criteria such as pruritus, lichenification, chronic relapsing course and history of personal or familial allergies strongly support the diagnosis. Many pharmacological agents have been tried to treat AD. One of the more successful therapeutic agents is a calcineurin inhibitor, cyclosporine. It represents a safer class of immunomodulatory drugs than corticosteroids and provides targeted alteration of lymphocyte function. It has been used to control or eliminate AD in humans and dogs, although chronic use requires regular monitoring of renal and hepatic function. To our knowledge this case represents the first reported successful treatment of AD utilizing cyclosporine in a nonhuman primate.

P050 Zinc-Induced Survival of Leydig Cells in Fischer Rats (Rattus norvegicus) Treated with CdCl₂

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Zinc is known to prevent cadmium-induced carcinogenesis and Leydig cell destruction in rat testes; however, its mechanism of action is not known, although it has been suggested that pituitary feedback increases the production of luteinizing hormone (LH) in response to low-circulating androgen. We therefore examined the biological role of zinc in reducing cadmium toxicity in Leydig cells of Fischer rats. Two groups of eleven 6-month-old rats were injected with 20 µmol CdCl₂/kg weekly for 5 weeks; one of these groups also received 1 µmol/kg zinc acetate weekly for the same 5 weeks. A third group received 1 µmol/kg zinc acetate weekly for 4 weeks, and a fourth group was injected with saline solution for 5 weeks. The number of surviving Leydig cells was significantly lower (P ≤ 0.05) in the cadmium group (7.34%) than in the cadmium-zinc group (20.85%) or control group (91.2%). Moreover, the concentrations of serum testosterone and LH were significantly higher in the cadmium group than in any of the other groups. These findings suggest that this may be a good model system for testing the effects of cadmium and zinc on the production of testosterone, LH, and other androgens.

P051 Variation in Recovery of Inbred Mice after Cranial and Abdominal Surgery

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Inbred strains of mice vary in many physiological and behavioral characteristics. To study circadian patterns of sleep and temperature, we performed surgery on 241 adult male mice of nine strains. Mice were anesthetized with ketamine/xylazine and received supplemental anesthetic as needed. Aseptic technique was used. Four EEG-recording electrodes were positioned under the skull; EMG electrodes were placed subcutaneously overlying the nuchal muscles. Electrodes were inserted into a pedestal that was secured to the skull with dental acrylic. Mice were then implanted with intraperitoneal transmitters to telemetrically quantify locomotor activity and core temperature. Sterile saline was administered intraperitoneally on the day of surgery and again the next morning to lubricate the transmitter and to support hydration. Mice received 1% ibuprofen in the drinking water beginning the day before surgery and continuing for 7 days thereafter. After surgery, mice were housed individually under a 12:12 h light:dark cycle at 22 ± 1°C. Mice were observed at least daily after surgery. Animals that developed palpable hypothermia, inability to ambulate, or behavioral unresponsiveness were euthanized. Marked strain variation was noted in the percentage of mice that survived. Recovery was closely correlated to body weight at the time of surgery. A/J mice, which weighed an average of 24 g, had only a 45% survival rate. In contrast, 80 to 95% of mice that weighed over 26 g survived. Some mice died within 48 h after surgery without full recovery from anesthesia. In others, a moribund condition or death occurred a few days after surgery. In these cases, necropsy often revealed intestinal necrosis and apparent obstruction. These findings indicate that variation in weight, which may be associated with variation in age, is a major consideration in promoting recovery of mice from cranial and/or abdominal surgery.

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P052 Phenotypical Features in a Mouse Model of Sanfilippo Syndrome Type B

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Sanfilippo syndrome type B, or mucopolysaccharidosis type III B (MPS IIIB), is a lysosomal storage disorder which is inherited in an autosomal recessive fashion. It is characterized by systemic heparan sulfate accumulation in lysosomes due to deficiency of the enzyme α-N-acetylgalcosaminidase (Naglu). There are devastating clinical abnormalities with severe central nervous system involvement and somatic disease leading to premature death. A mouse model of Sanfilippo syndrome type B was created by the targeted disruption of the gene encoding Naglu, providing a powerful tool for understanding pathogenesis and developing novel therapeutic strategies. However, the utilized JAX® GEMM® Strain B6.129S6-Naglu<sup>−/−</sup> mouse, although showing biochemical similarities to humans with Sanfilippo, exhibits aging and behavioral differences. We observed idiosyncrasies such as skeletal dysmorphism, hydrocephalus, ocular abnormalities, organomegaly, growth retardation, and anomalies of the integument in our breeding colony for the Naglu mutant mouse and determined several of them were at least partially related to the background strain C57BL/6. These background strain abnormalities therefore potentially mimic or overlap symptoms of the induced syndrome in our mice.

An observed pathology with a high incidence in the Naglu mouse strain is a distended urinary bladder elicited by urinary retention
that eventually leads to hydrenephrosis and subsequent death. In the ongoing study, it is important to evaluate the pathogenesis of urinary tract abnormalities, including kidneys, ureter, urinary bladder, and urethra, in the mutant mouse strain in relationship to the disease in humans.

P053 Acute Mortality of *Xenopus laevis* Tadpoles

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A mortality of 50 tadpoles occurred in January 2004 in a laboratory at the University of Washington. The tadpoles were being used to study a cell membrane transporter protein. The oocytes were injected with a GFP tagged transgene, then fertilized in vitro and grown to tadpoles. We used 0.1 X Modified Ringer’s (MR) (100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, and 4 mM NaHCO₃) for raising the amphibian oocytes and embryos in vitro. The tadpoles were housed statically in covered, food-grade plastic containers holding 2-3L of water. The water was aerated by an electric pump and air stones. Containers received a 90% water change every second day. Tadpoles were fed twice daily with a commercial fish food. The tadpoles died 15 days after transfer from 0.1 X MR to distilled water. Differential diagnosis included: poor water quality, improper nutrition, toxins, or infectious agents. The investigator had transferred the tadpoles to distilled water rather than tap water in an effort to provide “cleaner” water. A tentative diagnosis was made that the acute mortality in the tadpoles was due to osmoregulatory failure as a result of minimal to no salt or minerals in the water. The water source was changed to potable, city tap water dechlorinated with a commercial product. This change reduced, but did not eliminate, the mortalities. The water quality was tested using a commercial water quality testing kit. The hardness was 34.2 mg/L CaCO₃ (optimal >150 mg/L), alkalinity 20.5 mg/L CaCO₃ (optimal > 50 mg/L) and conductivity was 278 µS (optimal 500-1500 µS). Ammonia, nitrite and pH were within normal limits. We are currently working with the group to test various concentrations of MR to improve the water quality in order to prevent further tadpole mortality. This case illustrates the importance of adequate water quality for optimal health of amphibian tadpoles.

P054 Epidermal Lichenification in an African Clawed Frog (*Xenopus laevis*)

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A female adult South African Clawed Frog (*Xenopus laevis*) used for oocyte harvest was presented with diffusely thickened skin that had a cobblestone appearance (lichenification). The frog was housed with 40 others in a flow-through tank system. The frogs were obtained from a commercial vendor and were quarantined for 4 weeks prior to entering the colony. The frog was in a moderate body condition; the skin was flaky and lacked normal luster. Multifocally, the ventrum was mild to moderately erythematous. The differential diagnoses included *Capillaria xenopi*, *bacterial dermatosepticemia* (Red Leg Syndrome), *Basiidiobolus ranarum*, *Batrachochytrium dendrobatis* (Chytridomycoses), *Chromomycosis*, and *Mycoplasma spp*. Gross necropsy findings were confined to a diffuse, severe and chronic dermatopathy with algal growth and no slime layer. Skin scrape contained many filamentous bacteria, non-septated fungal hyphae, adult nematodes, squamous epithelial cells and inflammatory cells. *Alcaligenes sp.*, *Aeromonas hydrophila* and *Aeromonas casta* were cultured from the coelomic cavity. *Alcaligenes sp.* was cultured from the heart blood. Epidermal thickening (acanthosis), epidermal erosions, epidermal and dermal intercellular edema with inflammatory cells invading the dermis were noted on histopathological exam. Multifocally there were cross-sections of nematodes and larvated eggs. The final diagnosis was verminous dermatitis caused by *Capillaria xenopi* (*Pseudocapillaroides xenopi*) and bacterial septicemia caused by *Alcaligenes sp.* A low level of *Capillaria xenopi* is common in commercial colonies and frogs may be asymptomatic until significantly stressed. Nematodes burrowing through the skin serve as portals of entry for bacteria. Skin damage and subsequent inflammatory changes disrupt normal osmoregulation and can result in anorexia, wasting and death. Continued surveillance for parasites in *Xenopus laevis* at necropsy will determine the frequency of infection and severity of parasite load in our colony. We will base our decision whether or not to treat the colony on this information.

P055 Rederivation of a *Helicobacter*-Infected Diabetes-Prone Biobreeding Rat Colony

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We previously reported the occurrence of intermittent, chronic diarrhea over a 3-year period in a lymphopenic diabetes-prone biobreeding (DPBB) rat colony housed in an SPF rodent facility at the University of Washington (Contemp. Topics 42:64, 2003). All known rodent pathogens were excluded as etiologic agents, and fecal samples from the affected rats were PCR-positive for a novel *Helicobacter spp.*, and *H. rodentium*. In order to determine the role of *Helicobacter* in causing gastrointestinal disease, this colony was rederived as *Helicobacter*-free by embryo transfer. Techniques for rederivation by embryo transfer in rats are not as well characterized as in mice and natural matings rather than superovulation are generally used for blastocyst transfer. We were successful in rederiving this DPBB rat colony as *Helicobacter*-free using superovulation and two-cell stage embryo transfer using *Helicobacter*-negative 7- to 9-week-old Wistar females and vasectomized males. Offspring obtained were fecal PCR negative for *Helicobacter*. Rederivation of this colony was performed in a rodent transgenic facility and SPF rodent room that also housed *Helicobacter*-infected animals using SOPs to maintain animals as *Helicobacter*-negative. *Helicobacter*-negative animals were handled in the same changing station as *Helicobacter*-infected animals. Presently, there are small numbers of *Helicobacter*-negative BB rats in this colony that are available for histopathologic evaluation. However, thus far none of the DPBB rats have shown clinical signs of gastrointestinal disease. Interestingly, histopathologic evaluation of the gastrointestinal tract of two *Helicobacter*-negative BB juvenile rats has shown a 60-80% reduction of GI disease, suggesting other bowel microflora are contributing to the enterocolitis. Our findings suggest that a) superovulation combined with two-cell stage embryo transfer into the oviduct is an effective means of strain rederivation in rats, and b) rederivation of animals as *Helicobacter*-free can be done while animals are housed in the same room as *Helicobacter*-infected animals.

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A surgical model of Chronic Renal Disease (CRD) can be created in the mouse with a 5/6 nephrectomy. Advantages of a mouse model over a large animal model are numerous. It allows the model to be combined with genetic engineering techniques available in the mouse, and allows for lower animal and husbandry costs with close regulation of health status. Our surgical model for 5/6 nephrectomy has been developed by modification of published techniques. Published models have multiple approaches to the surgical modification of the remaining kidney, and there is a lack of consensus about performing both modifications in a single surgical session (one stage) or separating the surgical steps by a healing period. Our goal was to optimize the technique in a controlled setting. Briefly, non-absorbable suture is used to ligate the inferior left branch of the renal artery. A portion of the kidney’s upper pole was subsequently removed by electrocautery, leaving approximately one-third of the kidney functional. The right kidney was removed with standard nephrectomy techniques. Both modifications were performed through a single ventral incision. One group of Swiss Webster male mice (n = 20) was modified using the one-stage 5/6 nephrectomy, while another group of Swiss Webster males (n = 20) was modified using the two-stage approach. Both groups were closely monitored over a 14-day period for direct comparison of the two approaches. The survival rate after 14 days was 69% for the one-stage group and 47% for the two-stage group. We will present additional clinical pathologic data from this surgical model and will further characterize the response from different stocks and strains of mice to this surgical model creation. This will provide information to the biomedical research community as to which strains of mice are more conducive to this surgical approach as well as help predict the effect of background strain on potential genetically engineered lines.

The goal of this project was to create a method to place a length of 32-gauge tubing through the aortic valve and subsequently cause aortic valve damage for endocarditis studies. The left carotid artery is aligned with the ascending aorta and can be used to direct a catheter into the aortic valve. In order to accomplish this, a length of 32-gauge tubing was modified in the following manner to create the stylet. We measured and cut 2 cm of tubing. The catheter was heat sealed, creating the distal end; the tubing was then marked 1.3 cm from the distal end. A 2.0-cm length of wire material was inserted into the proximal or open end. The mice were anesthetized with isoflurane (5% for induction, 1.5-3% for maintenance). The left carotid artery was then isolated and ligated cranially with suture (6-0 silk). A small hole was made in the carotid artery with a 27-gauge needle; the tubing tip was inserted into the carotid artery and fed retrograde through the aortic valve up to the 1.3-cm mark. The tubing was then secured in place by two 6-0 silk sutures placed proximal and distal to the insertion point. Placement of the tubing in the aortic valve was confirmed by viewing the magnified radiographic images of the anesthetized mice produced by a Faxitron MX20. Once placement was confirmed, the wire was removed and the incision was closed in a simple interrupted pattern with 5-0 monofilament. The catheters were left in place for 48 h. The mice were euthanized; the hearts were flushed with saline and immersion-fixed in 10% BNF. Transverse serial sections of the heart were stained with H&E and examined microscopically. The catheter-induced changes included focal areas of erosion of lining endothelium covered by layers of fibrin along aortic valve leaflets and endocardial surface of the left interventricular septum.

The fluorescent nucleic acid polymerase chain reaction assay combines PCR with an internal fluorogenic hybridization probe, which ultimately provides quantitative results, enhanced sensitivity, and elimination of post-PCR processing. Fluorogenic nucleic acid polymerase chain reaction (fnPCR) assays were therefore developed to detect all known Mycoplasma spp. and to specifically detect M. pulmonis. Primer and probe sequences for the two assays were targeted to 16S rRNA sequences conserved among all known Mycoplasma spp. or specific to M. pulmonis, respectively. Each assay detected the equivalent of < 10 copies of template DNA. When evaluated against a panel of 30 species of bacteria, the Mycoplasma spp. assay detected all of the Mycoplasma spp. evaluated (M. pulmonis, M. arthritidis, M. neurolyticum, M. felis, M. bovis), while the M. pulmonis assay detected only the mouse and rat M. pulmonis isolates. When 10-fold serial dilutions of cultured M. pulmonis were evaluated by multiple detection methods, the M. pulmonis fnPCR assay was equally as sensitive as Dutch agar culture in detecting viable M. pulmonis organisms, while the mouse antibody production test displayed positive serologic results at dilutions beyond those in which viable organisms could be detected. Finally, the M. pulmonis fnPCR assay was able to detect M. pulmonis DNA in nasopharyngeal wash fluid, trachea, and lung collected from naturally infected mice that were seronegative for M. pulmonis, but did not detect M. pulmonis in similar samples collected from mice that were seropositive for M. pulmonis. In conclusion, the M. pulmonis fnPCR assay provides a high-throughput, PCR-based method to detect M. pulmonis in infected rodents and contaminated biological materials, while the Mycoplasma spp. fnPCR assay should prove useful for the detection of Mycoplasma spp. in contaminated biological materials, particularly contaminated cell culture lines.

The cotton rat (Sigmodon hispidus) is an excellent animal model for infectious diseases, notably for measles virus and respiratory syn-
Platelets play a crucial role in maintenance of normal hemostasis; perturbations in this system can lead to pathological thrombus formation and vascular occlusion, including myocardial infarction, stroke, and unstable angina. In these studies, novel P2Y_12 receptor antagonists were screened using dual catheterized rat model to continuously infuse compounds and collect serial blood samples for use in testing ex vivo platelet aggregation. Catheters were implanted in the femoral vein and femoral artery of rats then exteriorized dorsally between the scapulae. Animals were tethered and the catheters were connected to dual channel swivels to facilitate infusion into the femoral vein catheter and blood sampling from the femoral artery catheter. Arterial catheters were maintained by attachment of an injection cap to a catheter extension from the swivel. Twice-daily flushing with saline and locking with a heparinized saline solution maintained patency of the arterial catheters. Platelet aggregation in whole blood was then measured using a platelet aggregometer and challenging with a known concentration of adenosine-5'-diphosphate (ADP) at a level slightly above the effective concentration at 50% (EC_{50}), usually 3 μM. Arterial blood was used to assess platelet aggregation because previous in vitro work in the rat model used blood collected from the abdominal aorta; however, this model also provides some distinct advantages over catheterization using other vessel combinations. Use of the dual femoral catheter model allowed blood sampling that was not in close proximity to the infusion site. Other advantages were that the animal could be effectively tethered for a longer period of time while facilitating arterial catheter patency for as long as 3 weeks using twice-daily flushing and locking with a heparinized saline solution.
P063 Study of the Applicability and Efficacy of Polypropylene Mesh (Sepramesh) on Abdominal Wall Defect in Mice

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Incisional hernia is the most common abdominal-wall defect usually formed by trauma infection or tumors. Due to insufficient or inadequate autogenous tissue, repair of such defects and prevention of subsequent adhesions are a clinical concern when replacement is accomplished by patching the defect with prosthetic materials. The present experimental study on mice evaluates the efficacy of polypropylene mesh (Sepramesh) inserted in a sandwich form between the injured peritoneum and abdominal muscles. There were evaluated alone and in combination with Seprafilm, a bioresorbable membrane that adheres and promotes the normal healing process.

In a pilot study, 27 6-month-old male albino mice were randomly divided into three groups: sepramesh, sepramesh/seprafilm and seprafilm as control group. Mid-line laparotomy was performed, skin flaps were raised and a 1 × 1.5 cm rectangular and full thickness defect consisting of fascia, muscles and peritoneum was created in the abdominal wall as an established model for hernia. In sepramesh group, a 1.5 × 2-cm piece of sepramesh was cut, socked in 0.9% normal saline solution, and fixed intraperitoneally to the cut margins of peritoneum using 4-0 nylon taper-cut needle in simple interrupted fashion with sutures placed 0.25 cm apart. In the seprafilm/sepramesh group, the abdominal wall defect was repaired first by laying seprafilm intraperitoneally over the underlying viscera, then leaving sepramesh between muscles and peritoneum using the sandwich technique. Mice in the seprafilm (control group) received seprafilm in proper size deep to the abdominal wall defect. Then the skin was closed, as in the other groups with 3-0 silk in an interlock pattern. The abdominal incision, peritoneal cavity and all abdominal organs were evaluated for adhesion scoring and histological wound-healing process. All the animals were sacrificed at 28 days following mesh repair. The abdominal incision, peritoneal cavity and all abdominal organs were evaluated for adhesions and any other abnormalities. The presence of adhesions between bowel, seprafilm and sepramesh was assessed and the estimation was calculated by sectioning the defect surface into three fields. The assessment was made according to their tenacity and extent with a scale of 0-3 and 0-4 respectively. The median scores of extent and tenacity of the adhesions were reduced significantly in the sepramesh in sepramesh/seprafilm treated mice (P < 0.05). All seprafilm group animals developed some degree of adhesion between abraded small bowel and peritoneum. In the sepramesh groups, epithelialization of the inner surface of the mesh was completed without evidence of adhesion formation. There was good collagen accumulation, which is an implication of good and proper wound healing with resultant acceptance of sepramesh repair of the ventral in mice.

P064 A Comparative Analysis of Microenvironmental Conditions and Breeding Performances in Voles (Microtus spp.) Using Different Caging Systems

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Prairie voles have very high circulating levels of corticosterone, as much as ten times the level of rats. Because of this naturally high level of corticosterone, increased environmental stress induced by handling during frequent cage changes decreases pair bonding and influences their social organization. This could have a dramatic impact on their use for behavioral studies. Because of the high risk of deterioration of the microenvironmental parameters, open-top cages and individual ventilated cage (IVC) systems would be the only two viable possibilities to achieve proper housing with reduced frequency (once every other week) of cage changes. However, with the very particular requirements to maintain breeding colonies of transgenic or knockout mice in the same housing facilities as voles, cross-contamination is too high of a risk to use open-top cages. As for the forced-air IVC, the noise, the high rate of air change and the vibration produced by those cages could negatively impact the behaviour of vole species. To overcome these problems, we evaluated a new type of IVC system and compared it to our current static filter-top housing system for voles. The new IVC consists of a closed-system cage on an exhaust-ventilated rack that uses filtered ventilation by exhaust HVAC-assist (heating, ventilation, air-conditioning system) and direct exhaust venting. It provides adequate air changes without drafts, metabolic contaminant buildup, and need of mechanical ventilation. Breeders and weanling voles were housed in those two types of housing system. Our comparative data show that the level of ammonia measured after 7 and 14 days in the new IVC system is much lower than in static filter top cages (1-20 ppm compared to 20-110 ppm, respectively). Also, breeding performances in new IVC were equivalent to breeding performances in static filter-top cages used for several years with that breeding colony. In conclusion, the closed-system exhaust-ventilated cage rack offers improved housing capabilities for voles. It provides an extended cage-changing period, better animal care, and a high degree of confidence for experimental research.

P065 A Novel Surgical Model for Use in a Plethysmography Validation Study in the Rat

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A study was designed to validate the use of plethysmography equipment to measure pulmonary function in the rat. The study required combining plethysmography with arterial blood gas analysis to provide a more complete evaluation of pulmonary function without increasing the number of animals on study. A rat surgical model was required which allowed timed arterial blood collection on animals restrained in head-out plethysmographs. Restraint-acclimated rats were implanted with arterial catheters and vascular access ports 7 days prior to pulmonary assessment. The catheters were surgically placed in the femoral artery and the ports were located on the left flank of the animal to facilitate access during plethysmograph restraint. The animals were dosed with saline or positive control article (sodium pentobarbital, 10, 25, or 45 mg/kg) intraperitoneally, sterile extension tubing was passed through a sampling port on the plethysmograph, and the vascular access ports were accessed via huber needles. The animals were secured in the plethysmographs and pulmonary assessment commenced. Pulmonary function data consisting of respiratory frequency (F), tidal volume (TV), and minute volume (MV) were collected at baseline and at 0-6 h and 24 h post-dosing. Systemic arterial PO2 (PaO2) and CO2 (PaCO2) levels were determined in blood samples collected at pretest, and at 0.25, 1, 2, and 24 h post-dosing. Expected depression of F, TV and MV
were observed in the 25 and 45 mg/kg sodium pentobarbital groups and the PaCO2 increased inversely proportional to the decreased minute volume. Ninety-four percent of attempted blood samples were collected at the scheduled time and without incident; collection failures were due to the animal’s position in the plethysmograph. In conclusion, this surgical model provided an excellent method of combining plethysmography and arterial blood gas analysis within the same animals to provide more complete pulmonary function data in the rat.

P067 An Evaluation of Chronic Venous Catheter Patency

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Jugular vein catheterized rats are extensively used in pharmacokinetic studies. Once implanted, the duration of catheter patency is a concern for most investigators. In this study, four groups of 10 male CD rats (Crl:CD(SD)IGS®BR) were evaluated for catheter patency over a 28 day period. The targeted tip location of these silicone catheters was 1 mm passing the junction of superior vena cava and right atrium. After catheter implantation, catheters were not manipulated between days 0-6. Beginning on day 7, the catheters of rats in group one were flushed with saline every three days for three weeks. Groups 2-4 were not manipulated in any way until evaluated for patency at 14, 21, and 28 days respectively. Patency was classified as 1) fully patent - successful withdrawal on first attempt, 2) patent on flush - successful withdrawal after infusion of saline, 3) partially patent - unsuccessful withdrawal but patent for infusion, and 4) non-patent - unsuccessful withdrawal and infusion. All catheters in groups one and two maintained full patency for up to two weeks. Eighty percent of group one catheters were only partially patent by day 28. One hundred percent of group two catheters were fully patent at day 14. One hundred percent of group three catheters were either fully patent or patent on flush at day 21. One hundred percent of group four catheters were either fully patent or patent on flush at day 28. No catheters were found to be non-patent over the 28 day period. The results of this study indicate that jugular vein catheters can maintain patency for up to four weeks with no prior flushing or other manipulation of the catheter. Re-fraining from catheter flushing may result in better initial catheter patency at the start of a study.

P068 African Green Nonhuman Primate Enrichment

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To enrich the living conditions of our African Green nonhuman primates we implemented a four prong program consisting of pair housing, exercise caging, food treats, and manipulata. The most challenging and rewarding component of our environmental enrichment program is pair housing. Select pairs are housed with plexi-panels between them for at least one week, then we replace the plexi-panels with a grated panel for one week. At the end of this adaptive period, pairs are allowed access to each other by removal of the grated panel separating them for a supervised period of one hour. The daily amount of time monkeys access each other is increased gradually until transition to pair-housing is complete. For exercise caging, nonhuman primate pairs are selected for 2 week rotations in specially made oversize cages containing numerous custom designed enrichment devices. Daily food treats using a variety of fruits and vegetables augment our enrichment program. Chew toys, challenger balls, mirrors, fleece boards, puzzle feeders, dumbbells, finger tubes, kong toys, nyla-balls, nyla-bones, steel rings, jingle balls, and tug toys provide a variety of tactile stimulation. As a whole this program effectively mitigates boredom preventing the development of negative behaviors in our African Green nonhuman primates.

P069 Biocompatibility and Safety Evaluation of InnoPol®, a Biodegradable Polymer Scaffold

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Biodegradable materials have various important applications in the biomedical field. Porous scaffolds with an open pore structure are often desirable in many tissue engineering applications in order to maximize cell seeding, attachment, growth, extracellular matrix production, vascularization, and tissue ingrowth. Porous poly (L-lactic-co-glycolic acid, or PLGA) foams have been utilized for the regeneration of various tissues and organs such as cartilage, bone, heart, valves, nerves, and muscles. Before a biomaterial can be applied in the clinic, it has to be certified as to be non-cytotoxic and biocompatible.

In the present studies, PLGA 65/35 scaffold manufactured by special gas foaming methods in Korea, InnoPol®, has been subjected to assessment of the physical-chemical characterization of scaffold and its extract. In addition, toxicity studies were performed to evaluate the InnoPol® as a medical device. Scanning electron microscopic (SEM) studies revealed that InnoPol® had a relatively homologous pore structure, with an average pore size of 73.98 µm and porosity of 89.69%. Differential scanning calorimetry thermograms showed that InnoPol® has a glass transition at 56.1°C. The toxicity studies showed that
InnoPol® had no side effect such as cytotoxicity, hemolysis in vitro, genotoxicity, acute systemic toxicity, pyrogenicity, intracutaneous skin irritation reactivity, encapsulation in implantation or sensitization.

In order to evaluate biocompatibility and subacute systemic toxicity for six months, C57BL/6 mice and SD rats were implanted s.c. with InnoPol® discs (10 x 3 mm and 10 x 1 mm, respectively) and sacrificed 8, 12, and 24 weeks after implantation. No test material-related effects were observed on mortality, clinical signs, body weight changes, food and water consumption, ophthalmologic signs, urinalysis, hematology, serum biochemistry parameters and organ weights of animals treated with InnoPol®. In addition, there were no significant lesions related to implantation of InnoPol® in major organs of mice and rats. Although some lesions were observed in InnoPol®-implanted animals under microscopic examinations, except for skin, lesions found in the control group were also observed in the treatment group. Also, metabolic or progressive symptoms, including infectious diseases, were not observed. To investigate the degradation and tissue compatibility characteristics of InnoPol® skin tissue samples of the implanted sites were removed at post-operative weeks 4, 8, 12, and 24, and fixed in formalin solution, stained with hematoxylin & eosin or Masson's trichrome stain, and examined histopathologically. InnoPol® produced mild inflammatory reactions and completely degraded at the end of 24 weeks, leaving implant tissues that were similar to surgical wounds without implants in mice and rats.

These results suggest that InnoPol® may have good mechanical properties and tissue compatibility with neither side effects nor systemic toxicities except local inflammatory reaction at the site of implantation. Therefore, it is suggested that InnoPol® could be a relatively safe medical device for implantation and that this study will be a good model for assessment using laboratory animals on biocompatibility. Also, these consequences provide useful information for assessment on biocompatibility and fundamental biomaterial research.

P070 Development of Genotyping Assays for Obese and ApoE Mutant Mice Using Pyrosequencing
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Genetic models have become a crucial tool for modern biological research. With the increasing use of these models, the development of efficient genotyping assays becomes more important. This is particularly critical when large numbers of animals must be genotyped and rapid results are essential. We envision large numbers of mouse lines with single nucleotide polymorphisms (SNPs) to come out of N-Ethyl-N-nitrosourea (ENU) mutagenesis programs in the future. These would have to be genotyped by restriction fragment length polymorphism (RFLP) or by sequencing. In RFLP assays, a PCR is followed by enzyme digestion, and the reaction products are analyzed by agarose gel. Agarose gels are difficult to automate, and this step limits the efficiency of the genotyping process. Most current sequencing techniques, on the other hand, are quite costly. Therefore, we adapted an alternative sequencing technology to our genotyping needs. New genotyping assays for leptin and ApoE mice (B6.129P2-Apoetm1(APOE4)Mae, B6.129P2-Apoetm1(APOE2)Mae, B6.129P2-Apoetm1(APOE3)Mae, B6.129P2-Apoetm1(APOE3)Mae) or two, (Apoetm1(APOE4)Mae, B6.129P2-Apoetm1(APOE3)Mae, B6.129P2-Apoetm1(APOE3)Mae) were developed at Taconic Biotechnology. The assays are based on one (leptin) or two (Apoet) PCR reactions, and a few bases of PCR product are sequenced using the novel technology, Pyrosequencing®. PCR products are purified, and sequencing primers are extended by sequential addition of nucleotides. Every extension of the primer causes a light flash, which is detected by the Pyrosequencing equipment. Alternative primer pairs and sequencing primers were tested for each gene, PCR cycling conditions were optimized, and the assays were validated on more than 700 samples. The resulting assays will reduce cost and turnaround time for routine genotyping.

P071 Downdraft Platform for Mouse Anesthesia
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Mice are traditionally anesthetized by administration of gas anesthesia through a chamber, nose cone, or endotracheal tube with or without a ventilator. However, when carotid or jugular cannulation surgeries are performed, the anesthetic equipment can obstruct the surgical site and be a hindrance to the surgeon. We have constructed a novel downdraft anesthetic platform designed to alleviate some of the problems associated with commercial anesthetic delivery systems. The anesthetic delivery and scavenging source are positioned under the mouse’s nose, allowing an unobstructed surgical field for the surgeon to manipulate. Advantages of the novel downdraft anesthetic platform is that it is angled to allow for easier access to the operative site, it can be easily positioned under an operating microscope, is lightweight, easy to sanitize and it doesn’t require the mouse to be intubated. A disadvantage of this system is in its design. The anesthetic delivery port is surrounded by the scavenging vacuum and will sometimes interfere with the amount of anesthetic delivered if the vacuum is excessive. The proper balance of anesthetic gas and vacuum is critical in maintaining an appropriate surgical plane. The system is not designed to allow intubation and subsequent ventilation, which limits the types of procedures that can be performed. Lastly, the platform is not commercially available and needs to be constructed in-house. This alternative design has many advantages over commercially available anesthetic delivery systems. Specifically, mouse jugular and carotid cannulations are performed with greater ease and success utilizing this platform.

P072 Effect of Green Tea Polyphenols on Viability and Fertility of Mouse Epididymal Spermatozoa after Cold Preservation
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Green tea contains various antioxidative polyphenols, which exert potent inhibitory effects on LDL oxidation in vitro and ex vivo in humans. Most recently, successful short-term storage of rat fibroblasts and adontoblasts at 4°C have been performed by using polyphenol, such as (-)-epigallocatechin gallate (EGCG). In this study, to examine whether polyphenols are effective for short-term storage of mouse spermatozoa, we attempted 4°C-storage for 1-10 days with stock solutions containing polyphenols, such as EGCG and (-)-epicatechin (EC).

The viability and fertility of isolated mouse epididymal spermatozoa kept with polyphenols for 1-10 days at 4°C were determined. Spermatozoa kept for 3 days at 4°C after treatment with various concentrations of EGCG or EC were still active. When spermatozoa treated with EGCG was left for 10 days at 4°C and assessed for their motility, motility rates of spermatozoa treated with 100 µM and 50 µM EGCG were 8% and 29%, respectively, while motile spermatozoa kept with 200 µM and 0 µM EGCG were not observed. In EC-treated groups, actively motile spermatozoa were observed in all groups except for...
with the limited spatial resolution of micro-PET scanners significant for determining regional metabolic activity in the brain. However, Pfizer Global Research and Development, Ann Arbor, MI


Pfizer Global Research and Development, Ann Arbor, MI

18F-FDG positron emission tomography (PET) scanning is useful for determining regional metabolic activity in the brain. However, in rats, intense uptake of FDG by the harderian glands combined with the limited spatial resolution of micro-PET scanners significantly reduce the ability to assess neuronal activity of frontal brain structures by this technique. Surgical removal of the harderian glands before FDG PET imaging would, theoretically, eliminate the confounding uptake of the radioactive tracer and thereby permit visualization of glucose metabolism in frontal brain structures. A pilot study involving removal of the harderian gland on one side only, leaving the contralateral gland intact for comparison, was conducted. To remove the harderian gland, the rat was anesthetized and the nictitating membrane grasped with forceps and retracted out of the conjunctival sac. A small incision was made below the nictitating membrane cartilage and the harderian glands dissected free from the conjunctival tissue. One week after surgery the rat was injected IV with FDG and the brain was scanned at 30 min post-FDG. The resulting PET images demonstrated that the frontal cortex on the surgical side was clearly visible with only background FDG accumulation in the surgical bed observed. The frontal cortex on the side with the intact harderian gland was mostly obscured by the intense accumulation of FDG in the harderian gland. This simple surgical procedure may become a valuable tool for the visualization of the frontal cortex of the rat brain by FDG micro-PET.

P075 Materials and Techniques for Long- and Short-Term Infusions in Various Species

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Due to issues associated with bioavailability and metabolism, or safety evaluations where remote dosing is critical, test compounds may be required to be administered as an infusion. Depending upon the test compound and the duration of the study, an infusion may range from 5 min to several months (short-term versus long-term infusions). The methods described will provide solutions and materials of proven, economical techniques for both long- and short-term infusions. These will include a system for long-term intravenous infusions in the rat, a system for short-term infusion in the rat, using a unique rat restraint created from an inhalation nose-only restraint cone, a short-term canine infusions using a restraint sling in order to infuse multiple dogs at once, a primate and canine short-term infusion using a programmable ambulatory pump and jacket system, and a long-term primate and canine infusion system using a tethered model, for ease of dose verification and maintenance. Photos of all techniques and supplies will be shown, as well as technical methods that can be implemented to prevent possible misdosing and help the infusions run smoothly.

P076 Mice on the Move!

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The number of worldwide live animals shipments has increased significantly in recent years due to the huge international demand for transgenic animal models. The requirements for monitoring environmental conditions during transport lags far behind that required within animal facilities. Animals shipped internationally may be subject to vast temperature changes during transit; these changes go unnoticed unless a monitoring system is put in place. The use of a small temperature datalogger is a cheap and effective way of giving retrospective data for local and international shipments. Data retrieved using this method has shown that ‘in crate’ temperatures have ranged from 7.9-32°C during worldwide transit and airport holding, demonstrating the need
for awareness in this area. This information has been used to assess ways in which shipment conditions can be improved or modified to improve animal welfare. Examples include modifying collaborative agreements to include a requirement to minimize time in transit at both ends of a shipment, including out-of-hours collections from airports. Data has also been used to lobby the UK legislative authorities to progress changes to the laws pertaining to the import of germplasm, simplifying the process and thus avoiding the welfare concerns of shipping live animals.

**P077 Mobile Rodent Anesthetic System**

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Abbott Laboratories, Abbott Park, IL

The development of a mobile anesthetic system utilizing inhalation anesthesia came from the demand for alternatives for providing safe anesthesia for prolonged periods, the difficulty of using injectable controlled substances, and the need for use of the system by multiple investigators.

In order to increase the use of inhalation anesthesia, a stainless steel cart to transport a complete inhalation anesthetic system was designed. The goal was to create a system that is easy to use and is 100% mobile so that the system can be used by multiple investigators. The system included the modified cart, which is capable of safely and securely transporting two “E” oxygen cylinders. The remaining space on the cart was used for placement of a sevoflurane or isoflurane vaporizer, flow meter, an induction chamber and nose cone manifold. The system is set up to scavenge all unused anesthetic through either the room HVAC or with the use of a charcoal collection canister. This increases the flexibility of the system by making it entirely self-sufficient and mobile.

Currently the Comparative Medicine department has two mobile systems available for use, in which researchers sign the systems out by reserving them on a centrally located calendar. This eliminates the need for researchers to purchase their own equipment, the main deterrent in using this type of system for investigators.

**P078 Modification of the Digestion/Flotation Procedure for Detecting Murine Fur Mites**

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The diagnosis of murine fur mite infestation is routinely performed by microscopic examination of scrapings from the pelt or tape applied to the hair and by macroscopic examination at necropsy. These procedures have low sensitivity and may not detect fur mites accurately. The digestion/flotation procedure is more sensitive for detecting low-level infestation often missed by other methods. The purpose of this study was to develop a simplified and sensitive detection method using a modification of the digestion/flotation technique. In this procedure, the pelt is dissolved by boiling in potassium hydroxide (KOH). This solution is centrifuged and the KOH supernatant is decanted, leaving a pellet that includes undigested mite eggs, nymphs and adults. The pellet is re-suspended in a flotation solution of dextrose and water, then allowed to float for 1 h. The eggs, nymphs and adults float to the top, where they are recovered on a glass slide for microscopic examination. In addition to being time-consuming, this technique has several unique problems, such as the collection of fat globules, air bubbles and other material that inhibit visualization on the slide. Also, dextrose can leave a problematic residue on laboratory equipment. We have modified the digestion/flotation procedure by replacing the flotation step with one that uses a detergent solution to solubilize the sediment, allowing filtration of the suspended pellet. Using a filter collection system, the solution is passed through a 20-micron polycarbonate filter. The filter is washed with water, placed on a glass slide, cover slipped and examined for eggs, nymphs and adults. This technique provides a simplified method for the examination of the entire pelt by eliminating the cumbersome flotation step without compromising the sensitivity of the assay.

**P079 Modified Primate Racks for Use by Ferrets**

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Specific ferret housing is not commercially available; therefore, Abbott uses a modified primate rack. Each cage had been fitted with a cloth hammock which had to be removed, laundered, and then replaced in each cage. Ferrets were fed in bowls that are placed inside a shoebox cage. The ferrets frequently knocked the shoebox cages over, spilling all of their chow through the slatted flooring. The squeeze mechanism also creates an entrapment issue for the inquisitive juvenile ferrets.

The cloth hammocks were frequently chewed; animals then tunneled between the layers of material, making observations difficult. The hammocks also posed strangulation and smothering risks. Therefore, a PVC hammock was constructed that is safer, more efficient, durable, economical, and allow better visibility of the animals. The hammock was made from 6 in. pipe with stainless steel chain and hooks fitted on the hammocks for hanging in each cage. These hammocks do not need to be removed for cage wash and thus remain in the cages indefinitely. More than 200 hammocks have been fabricated.

Ferrets are playful animals and commonly flip over the food dish, leading to food wastage. To prevent flipping, the shoebox cages were seated into a bracket in each cage. The end result showed a 40% decrease in food usage.

When juvenile ferrets arrive, some crawled behind the squeeze mechanism and became lodged, creating a potentially life-threatening situation. Our welding specialist removed the squeeze mechanism from each cage, thus removing the possibility of an animal becoming trapped.

Our current system has been a work in progress to allow for the safe and effective housing for ferrets.

**P080 Novel Restraint Device for Nasogastric Dosing of Nonhuman Primates**

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In response to an increasing demand for nonhuman primate (Macaca fascicularis) studies using more robust experimental designs (e.g., > n), we developed an alternative restraint method for nasogastric intubation that improves dosing efficiency. We decided to custom fabricate and use a half-tube restraint device to evaluate its efficiency for nasogastric administration. The half-tube device is made from lightweight PVC material and the material costs are low (~$60). The efficiency for the custom device was determined by comparing the average dose times per animal using the restraint box and half-tube device. Use of the half-tube provided a higher comfort level for the animal based on a decrease in struggling. Additionally, there were
fewer steps involved in positioning the animal. Collectively, this approach resulted in an increased efficiency. The average dose time per animal by using the restraint box was 3 min, whereas the average time by using the half-tube device was 1 min per animal. Thus, based on the increased comfort level for the animal and the increased dosing efficiency, the half-tube device became the preferred restraint method for nasogastric dosing in nonhuman primates at our facility.

P081 Proper Acute Staining Method of Viable Myocardium

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Background: Multiple energy sources for the surgical treatment of AF are currently being evaluated worldwide. Microwave energy is one of these sources. In order to determine the transmurality of lesions created by this source on the myocardium, we employed triphenyl-tetrazolium-chloride (TTC). TTC is a vital stain commonly used to determine grossly viable and nonviable tissue. We hypothesized that staining would be improved by injecting TTC intravenously (IV) followed by incubation versus TTC incubation alone.

Methods: Porcine hearts (n = 8) were excised after the procedure was performed. The hearts were divided into two groups with group I (n = 55) consisting of intact hearts incubated in 1% TTC for 1 h at 37°C with constant mixing and group II (n = 52) comprised of 2% TTC solution injected IV 20 min prior to euthanasia followed by the same incubation protocol as in group I. Right ventricular tissue was used for both groups and divided into 0.5-mm sections. Each section was photographed and the tissue was subsequently graded blindly for completeness of staining (1 = 95-100%, 2 = 50-95%, 3 = > 50%). Statistical analysis was performed with a non-parametric Mann-Whitney U test.

Results: Group I had a median grade of 2.0 [1-3]. Group II had a median grade of 1.0 [1-2]. The difference between these two groups was found to be significant (P < 0.001).

Conclusion: The combination of IV injection with incubation was superior to incubation alone. From these findings we recommend that all staining of myocardial tissue with TTC should be injected IV prior to euthanasia to optimize the efficacy of this stain to differentiate between viable and nonviable tissue.

P082 Rabbit Mini-Pump Implantation Using a Topical Anesthetic

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This rabbit surgery technique was developed by the veterinary staff to reduce anesthetic risks associated with general anesthesia. The rabbit is placed into a feline restraint bag; a 10 x 8-cm area over the left shoulder/thorax area is shaved, and a topical anesthetic is spread generously over the exposed skin. The rabbit is then removed from the bag and returned to its cage for 30-40 min to allow adequate contact time for the local anesthetic to be effective.

The rabbit is again placed in the feline restraint bag. The shaved area is disinfected with a surgical scrub and alcohol, and 1 ml of an injectable local anesthetic is given s.c. along the anticipated incision site and the area where the mini-pump will be placed. After the area is again disinfected, a 3-cm incision is made into the anesthetized skin, a small subcutaneous pocket is created, and the mini-pump inserted. The subcutaneous tissue is closed with 3-0 absorbable suture using the simple interrupted technique. The dermal layer is closed using 3-0 absorbable suture with the continuous subcuticular technique. After closing, the area is cleaned with hydrogen peroxide. The rabbit is removed from the restraint bag and returned to its cage. Post-surgically, the incision site is monitored for the next three days. Any signs of swelling or infection are immediately reported to the veterinarian.

Before this technique was developed, technicians spent extra time anesthetizing the rabbit and monitoring the recovery of the animal. Avoiding general anesthesia prevents life-threatening conditions such as hypothermia and bradycardia from occurring during surgery. Use of topical and local anesthesia prevents the rabbit from feeling pain caused by the incision and placement of the mini-pump. The rabbit does not vocalize, move or increase its respiration rate during the surgical procedure. In the past year 30 mini-pump implant surgeries have been performed, and only 2 have resulted in postsurgical complications. This technique has proven to be a valuable alternative to general anesthesia.

P083 Redesigned Minimal Restraint Chair for Nonhuman Primates

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In 2002, we presented our nonhuman primate minimal restraint chair, originally designed in 1989. The chair was based on normal sitting posture and behavior of cynomolgus monkeys. It consisted of a metal pole frame on casters with a nylon platform perch and a clear top plate with a slotted groove for the collar; the top plate could be raised and lowered from a front hinge to allow the animal a more natural posture, and enrichment was provided via a hand access port, foot rests, and nylon bars. The design did have some drawbacks: stress points on the Lexan™ top at the screws caused fractures and required frequent replacement of the entire top; clouding of the Lexan top and side-plates caused viewing limitations; screw threads stripped; animals could grab the technician when the access port in the top was opened or closed; enrichment devices were attached by cable ties; and small parts (screws, bolts, nuts) constantly came loose or were lost. Maintenance was a full-time job. The new design minimizes all small parts. The top is now surrounded on three sides with an aluminum channel that holds three removable Lexan plates with no screws. An additional bar across the back of the top plate reduces the risk of escape to nearly zero. A Lexan plate can now be slid down behind the animal as a kick plate. The side plates are now metal grids that allow unlimited use of enrichment objects, eliminates the foot rest, and allows clear viewing. A water bottle holder and screw holes along the outer rim of the channels also permit direct attachment of foraging devices. No parts are left that can be stripped, distorted or damaged by high heat during cleaning, loosen with use, or get lost. The new design is extremely user-friendly for both animal and technician.

P084 RT-PCR Detection and Nucleic Acid Sequence Confirmation of Reovirus Infection in Mice with Discordant IIFA and ELISA Serology Test Results

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Reoviruses (family Reoviridae) are double-stranded RNA viruses that have been detected in nearly all mammals. In mice, reovirus infec-
tions are typically subclinical, though they have been associated with minor respiratory and gastrointestinal illnesses. Many diagnostic laboratories use a commercial enzyme-linked immunosorbent assay (ELISA) to screen for reovirus antibodies, and an indirect immunofluorescence assay (IIFA) as a secondary test for confirmation. The Loyola University Medical Center (LUMC) Comparative Medicine Facility Diagnostic Laboratory (CMFDL) uses IIFA in-house for primary screening of rodent serum for anti-virus antibodies. Send-out tests of matched serum samples are used for outside confirmation of CMFDL test results; a leading commercial serology testing laboratory provides this service utilizing both ELISA and IIFA tests. From 1999 to 2002, 202 of 984 serum samples taken from sentinel mice were IIFA seropositive for reovirus. The sentinels were reovirus-seronegative prior to use, but seroconverted after exposure to soiled bedding from reovirus-seropositive research mice that had been obtained from various commercial and academic sources. IIFA tests demonstrated the same cytoplasmic staining pattern as visualized with the Reovirus strain Dearing control, though with reduced intensity. Matched serum samples sent to the commercial testing laboratory resulted in negative ELISA and positive IIFA tests, and were reported as “false positives.” We asked if these results were always “false positives,” and were able to explore this question with the recent acquisition of 12 reovirus seropositive transgenic mice. Six reovirus seronegative sentinel mice were exposed to soiled bedding taken from the transgenic mice, and stools were collected monthly for 3 months. In-house IIFA tests indicated that all 6 sentinels seroconverted to reovirus by 3 months. Attempts to isolate virus from the stool specimens in tissue culture using trypsin-activation of the stools and other standard reovirus methods failed. However, reoviruses are often difficult to isolate in tissue culture, and negative virus isolation did not rule out the presence of reovirus in the stools. Reverse-transcriptase (RT)-PCR and nucleic acid sequence analyses established the presence of reovirus RNA in stool samples taken at 2 months. Stool samples taken from reovirus seronegative animals were RT-PCR negative. Nucleic acid sequence analyses confirmed a reovirus identity. The virus detected in the sentinel animals had unique gene sequences which had a higher homology to recently described reovirus strains than to commonly studied reovirus strains Jones, Lang, and Dearing, the three of which were isolated in the 1950s. This study indicates that true reovirus infections of mice can result in IIFA-positive, ELISA-negative test results using current commonly used reagents or tests.

P085 The Use of Isoflurane and Hypothermia as an Anesthetic Protocol for Surgical Subcutaneous Implants in Neonatal Rats

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Anesthetizing neonatal rodents has been associated with issues such as poor or unpredictable anesthetic depth and excessive mortality. This evaluation was done to develop a simple method that could provide safe and effective anesthesia for neonatal rats. Initially, a published method using ketamine (100 mg/kg of body weight, i.p.), and xylazine (10 mg/kg, i.p.) was used and resulted in prolonged time to recovery and a high rate of mortality (> 50%); this was deemed unacceptable. The goal was to establish a protocol to ensure that neonatal rat pups could be successfully anesthetized and recovered for minor survival surgical procedures. The 12- to 14-day-old rat pups were anesthetized by face mask with isoflurane (1-2% via Bain circuit) and induction of hypothermia (by placement of animal on a draped icepack). Isoflurane without hypothermia was not evaluated upon recommendation by the technical staff at Charles River Inc. Animals were observed for loss of righting reflex, depth of anesthesia (indicated by lack of pedal reflex and no response to skin pinching), respiratory rate and recovery of righting reflex. The pups were shaved and prepared for aseptic survival surgery, and multiple subcutaneous implants were performed along the dorsal surface of the animal. Time from induction of anesthesia and surgery to recovery was approximately 45 min. Pups were readily accepted when returned to the mother. This method proved easily administered, controllable and effective in inducing short-term surgical anesthesia with low mortality (< 0.02%) and no long-term effects. Our evaluation did not include animals anesthetized with isoflurane alone upon recommendation by the technical staff at Charles River Inc.

P086 Use of Assisted Reproductive Technologies in Optimizing Transgenic Colony Management

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From the animal room to the lab, experienced transgenic technicians work together to rejuvenate poorly breeding lines of mice. In our experience, approximately 5% of transgenic founder lines do not breed well. Although this percentage seems small, it could be a significant loss. Just one non-productive founder can result in the loss of the highest expresser for that line. In those instances, we need to assist colony production with Assisted Reproductive Technologies (ARTs). Determining which technique to employ depends on many factors, such as gender, age and physical limitations of the strain. These technologies include ovary transplantation, modified vasectomy combined with in vitro fertilization, assisted in vitro fertilization using acid tyroses, intracytoplasmic sperm injection (ICSI), and partial zona-pellucida incision by piezo-micromanipulator (ZIP). We have salvaged numerous founder lines by employing various ARTs. The resulting transgenic offspring are utilized for line expansion and future study support. One of the key factors of successful line recovery is early recognition. The mouse room technician must recognize potentially non-productive founders. Thereafter, the lab technician consults and both work together to determine which technique to employ. This team effort increases transgenic breeding efficiency and improves study support. In conclusion, Assisted Reproductive Technologies can be used successfully to salvage lines that would normally be lost.

P087 Use of HemaBlock as a Clotting Agent in the Reduction of Direct Pressure Related Pain and Distress in Rabbits

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Rabbits are bled from the medial artery using a 19-gauge infusion set. Once the blood is drawn, the infusion set is removed and direct pressure is applied to the puncture using gauze and a plastic clothespin. The clothespin is left on the wound for approximately 20 to 30 min, at which point sufficient clotting has taken place and the bleeding has ceased. This method, however, has a negative health impact on the rabbit. The pain and distress from the pressure asserted by the clothespin is visually apparent. Additionally, if the clothespin is not removed promptly, tissue damage can result from the lack of blood flow below the point of pressure. A study was performed to determine if HemaBlock, a commercial clotting agent, would eliminate the need for the continued direct pressure used in the current process. With this
trial method, light pressure was applied to the puncture by hand and HemaBlock was applied directly to the wound. Once the wound site was covered with HemaBlock, light fingertip pressure was applied directly on the puncture for approximately 1 min. An average of 75% of the rabbits were able to achieve full clotting within 1 min using HemaBlock. Any rabbits that continued to bleed from the puncture required the use of a clothespin. However, the overall time required to produce a clot with the clothespin after the HemaBlock application was 5 min, a 15-25 min reduction in clotting time. As a result of the use of HemaBlock, there has been a direct reduction in the observed pain and distress to which the rabbits are subjected. Further, the concern of tissue damage caused from a prolonged blockage of the artery has been eliminated.

P088 New Forceps Design Offers Ergonomic and Animal Welfare Benefits

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At our institution the animal care technicians move the mice primarily with 10-in. stainless steel forceps. These forceps vary in weight and resistance and have been associated with some repetitive motion injuries. In an attempt to prevent or reduce these injuries and maximize comfort for both technician and mouse, new forceps designs have been considered. Searching through vendor catalogs and the Internet did not yield a forceps design that integrated all the specific attributes desired; thus, modifying the existing forceps became the goal. This lead to a new forceps design which is quieter, lighter, requires less force to manipulate, and introduces a mouse-friendly tip design which displaces the pressure applied by the forceps, lessening the possibility of injury and reducing stress to the animal. Unique adjustable finger guides allow the technician to change the forceps position in the hand as desired. The prototype as designed with improvements still weighs about 30% less than the original model. Initial response by animal care technicians has been favorable. Additional analysis by a test group of technicians has been favorable. Additional analysis by a test group of animal care technicians will be conducted before considering these as standard husbandry equipment.

P089 A Unique Procedure That Provides Perfused Tissue for In-Vitro Analysis of the Effects of Drugs on Lung Tissue

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The production and release of various cytokines such as IL-12 and IL-10 are thought to be important in modifying and controlling symptoms associated with diseases such as asthma and allergic rhinitis. In the lungs, these cytokines are thought to come from alveolar macrophages and cells in the pulmonary tissue. However, they can also come from mononuclear cells in the blood. The objective of this research was to establish a simple perfusion technique which would provide lung tissue flushed free of the blood. Current methods often use perfusion techniques involving cannulation of the pulmonary aorta. Herein we offer a simple but highly effective alternative method.

Male CD rats weighing 300-400 g were anesthetized and attached to a respirator via a tracheotomy. A laparotomy was performed and the abdominal vena cava was cannulated. The cannula was then connected to an infusion pump and the external jugular veins were cut. Kreb’s Henseleit solution was then used to flush the blood from the systemic and pulmonary vasculature, providing lung tissue that was relatively free of blood. In addition, other organs such as the liver became pale or whitish, indicating the clearance of blood from those organs.

Good correlation was observed between in vitro cytokine production (IL-12) and in vivo suppression of ovalbumin-induced changes in airway eosinophilia and plasma leakage. This model allowed for in vitro screening of multiple compounds per animal, thus reducing the number of animals needed to demonstrate in vivo activity. Furthermore, it allowed for the separation of active from inactive compounds, thus further decreasing the amount of animal studies needed. The procedure outlined above was developed and was used in accordance with the guidelines set forth by the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee.

P090 Assessment of Commercially Available Environmental Enrichment for Laboratory Mice: Ask the Animal!

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In the field of biomedical research, the demand for standardized environmental enrichment is growing. For laboratory mice, a wide variety of enrichment items is commercially available. Most of these comply with the demand for standardization and hygiene, but whether they actually enhance the well-being of mice is usually not assessed scientifically. In this study, we tested the preference of mice for a paper-based (SS/DR) and a red perspex nest box (TMH), both commercially available. The preference of 15 groups of 3-4 mice (BALB/c, C3H and C57BL/6) for both nest boxes was investigated and compared to preference for a highly preferred nesting material (tissues). Results indicate that mice of all strains showed a strong preference for the SS/DR (P < 0.05), but not for the TMH. Furthermore, 75% of the mice dragged the tissues through the narrow passage from the TMH cage into the SS/DR nest box, while tissues were never combined with the TMH. Preference of 24 mice for the SS/DR in relation to preference for nesting material was then investigated further in an automated 48-h test. Results confirm that mice had a strong preference for the SS/DR (P < 0.001), and showed that 90% of the mice were willing to work to combine nesting material with the SS/DR. Observational data indicate that the SS/DR can be easily manipulated, while the TMH is a more rigid enrichment item. We conclude that the ability to manipulate meets an important behavioral need of mice to exert control over their environment. Thus, the SS/DR and nesting material would be more suitable enrichment items than rigid structures such as the TMH. We also argue that enrichment items should be designed on the basis of knowledge about behavioral needs and data available from enrichment studies. Further, the items should be scientifically tested prior to marketing.

P091 Care of Japanese Quail During Brooding Period

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Japanese quail are routinely being used for research. Newly hatched baby chicks need special attention during the early brooding period...
for temperature control, feeding and watering. Some baby chicks drown in water dishes if not protected. To prevent drowning, the feed of chicks is mixed with water to form a gruel-like mixture. However, feeding of this mixture also needs close watch. If left unattended, the chicks will walk through the feed-water mixture and their toes will become encrusted with feed, forming feed balls of varying size that harden as they dry and are difficult to remove. This leads to difficulty in walking, growth retardation, physical trauma, swelling, necrosis, and loss of digits. To prevent this problem, newly hatched chicks were raised in a wire-bottom brooder maintained at 98-99°F during the first week. The floor was covered with a rough-surfaced paper to prevent straddling and other leg injuries. The chicks were fed turkey starter to which water was added, forming the gruel-like mixture. One group of chicks were provided the feed-mixture in open dishes and the other group was provided protective lids of galvanized hardware cover cloth (½-in. mesh) placed over the dishes to prevent chicks from walking through the feed mixture. The chicks were weighed daily and examined for feed-mixture encrustation build-up on their toes. The birds raised with covered dishes showed slight to no encrustation on toes, whereas the chicks with uncovered dishes developed severe encrustation on their toes. The severity of this clumping was judged on a numerical scale of 1 to 3 (1 = no build-up; 2 = mild build-up, and 3 = severe build-up (about 3-4 mm in size). The results indicate that feed-ball formation on the toes can be prevented by covering the feed-water mixture with wire-mesh lids.

P092 The Use of Bar-Coding and PDA Scanners in Managing an Animal Facility

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Bar codes and personal digital assistants (PDAs) are common productivity tools in many venues. We combined these two technologies to better document and evaluate animal husbandry and veterinary care tasks in a large (7,000 cage) rodent vivarium. Prior to this initiative, caretakers checked off husbandry tasks on a clipped sheet in the animal room, confirmed with a second check by the facility manager. Clinical findings during rounds by technical and professional veterinary staff were written on cage cards and investigators were notified via e-mail later. Animal census data were similarly recorded on paper and reviewed several times over many weeks before investigators were actually billed. Such paper documentation provided no means to easily identify staff performance trends, accelerate payables cash flow, or determine the cost-effectiveness and animal welfare benefits of various operational strategies. New technology consisted of proprietary Palm OS™ and Adelaide™ automation agent software designed by Digital Paradigms, Inc., plus PDAs affixed with bar-code printers and scanners. Unique bar codes were generated for every cage card, cage rack, changing hood, animal room, and employee involved. When a husbandry or clinical task was performed or verified, the bar code of the pertinent cage card, rack, room and person were scanned, and the corresponding checklist was annotated by that person on a PDA. PDAs were downloaded at least daily at specified PC stations and data were compiled. Trend analyses were easily facilitated for testing hypotheses regarding productivity by employee experience and training, by housing (e.g., BSL-2 versus barrier versus conventional), by clinical complications stemming from a protocol, or by overcrowded cages from breeding programs. Census data were easily verified for accuracy and seamlessly transferred to an invoice.

P093 Disease Surveillance Programs in Murine Facilities with On-Site ELISA

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The timely and accurate detection of murine pathogens is essential in contemporary biomedical research. Cost, accuracy, and reproducibility of test results are frequent concerns when initiating an on-site serology program. This study was conducted to evaluate the advantages of on-site serology performed by ELISA versus pathogen surveillance conducted off-site by a commercial vendor. Sentinel mouse serum samples (n = 92) were divided and tested in parallel for murine pathogens [pneumonia virus of mice (PVM), lymphocytic choriomeningitis virus (LCMV), reovirus, Mycoplasma pulmornis, Sendai virus, Thielers' murine encephalitis virus (TMEV), mouse hepatitis virus (MHV), Mouse Parvovirus (MPV) and mouse rotavirus] at UCLA and by an off-site vendor. On-site testing was performed using commercially available test kits according to the kit manufacturer's directions, while serum samples for off-site testing were prepared according to the vendor's specifications. Results from the two testing strategies were compared and a good beyond-chance level of agreement was demonstrated by means of the kappa test (κ = 0.82). Identical test results for 8 pathogens were obtained from on-site and off-site testing; all sera reacted negatively for PVM, LCMV, TMEV, reovirus, M. pulmornis and Sendai virus, while 3 and 16 samples reacted positively for MHV and MPV, respectively. Two samples positive for mouse rotavirus were detected by the on-site ELISA but not by the off-site laboratory. The turn-around time between sample preparation and results availability for the on-site ELISA was 16 h versus 32 h for off-site testing. Significant material and labor savings were associated with on-site testing and resulted in at least 30% savings over off-site testing. This study demonstrates the accuracy and time- and cost-effectiveness of on-site ELISA, as well as its potentially valuable role in achieving more timely and efficient disease surveillance and control programs in contemporary biomedical research facilities.

P094 A Humane Refinement to an Animal Facility Pest Control Program: Evaluation of “Weekend Survival Packs” for Trapping Wild Rodents

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Wild rodents harboring pathogenic agents in or around animal facilities represent a serious health risk to research animals. We recently reported that wild mice harbor mouse hepatitis virus (MHV) and that this strain of MHV was identified in experimental mice housed in two animal rooms in our animal research facility. To monitor for and eliminate wild rodents around animal facilities requires an effective pest control program. Live trapping of rodents and daily monitoring of traps are essential for determining an accurate micro-
brial profile of the trapped animals. This report evaluates two types of transgels and a homemade “weekend survival pack” for their ability to humanely maintain trapped mice in a healthy condition until the animals are examined for microbial pathogens. The two types of transgels, Napa Nectar Jr. (4-oz. pack) in a vacuum-sealed plastic bag, and Aqua-Jel (3-oz. pack) in a vacuum-sealed plastic bag, and a novel “weekend survival pack” (consisting of 1 oz. of Napa Nectar plus 2 g of peanut butter or bacon bits to attract rodents, in a small, thin plastic bag) were each placed in two types of humane rodent traps with see-through lids, Victor “Tin Cat” and Ketch-all traps. Several small pin-size holes were punched in the small plastic bag of the “weekend survival pack” to facilitate access by trapped rodents. The traps containing one of the three sustainability sources were placed in various locations in our animal facility, feed and bedding storage areas, and in other buildings on our campus where wild mice had been sited. Traps were checked daily for mice. Trapped mice were confirmed to be wild mice by genetic assays. Adult wild mice were able to chew through the two types of transgel packs, but some young, small mice did not chew through these packs. In contrast, all mice were able to chew through the “weekend survival pack” composed of a very thin plastic bag with small holes. The two types of transgels maintained the mice in a healthy condition when the bags were slit open. It was concluded that the novel “weekend survival pack” provides a humane approach for trapping wild rodents around animal facilities. With this simple refinement, it is not necessary to monitor traps every day on a weekend or on holidays. Trapped mice can be maintained humanely so that a microbial assessment can be conducted during normal working hours. This refinement can contribute to an effective and economical pest control program.

P096 Rat Preferences for Commercially Available “Simulated Burrows”

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One objective of this study was to determine whether rats preferred to spend more time inside or outside “simulated burrows,” which were placed in their home cages. We also wished to determine if rats preferred one type of simulated burrow over another. Six male Sprague-Dawley and six male Spontaneously Hypertensive rats, individually housed in large polycarbonate cages, were the subjects for three experiments. In the first experiment, rat preferences for red Rodent Retreats (RR) for rats (Bio-Serv) or Rat Shacks (RS, Shepherd Paper) were compared during the light cycle across 5 days. Following 2 days of housing in barren cages, preference for the red RR versus open bedding was tested, and during the third week preference for RS vs open bedding was examined. When both RR and RS were present in the cage, rats of both strains were observed in RR ~90% of the time whereas they were found in RS ~5% of the time. When rats had access to only RR they spent > 95% of their time in this simulated burrow. However, when rats had only RS in the cage, they spent ~50% in this simulated burrow. We concluded that rats prefer to spend their time during the light phase of the photoperiod in RR compared to RS or on open bedding.

P097 Sound Levels Inside Individually Ventilated Mouse Cages

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The increased use of mice in animal research has forced facilities to maximize mouse housing space. One solution is the use of individually ventilated cage (IVC) systems. One drawback of the IVC rack systems is the possibility that noise from blower motors could have a negative impact on the mice. To assess the impact of ventilation system noise on mice, sound levels were measured inside IVC mouse cages. An impulse sound level meter, coupled with an octave filter set, was used to conduct sound readings. Sound levels were tested in two types of IVC systems: supply and exhaust of cage air using the building ventilations system (LSI), and use of individual blower units mounted on the top shelf of the rack (MSRB). In-cage and room background readings were taken with laminar flow hood cage changing stations (LFHs) on and off, and with the rack blower motors on and off (MSRB only). For both systems, the readings taken inside the cage were not substantially different from the room background levels. For LSI, overall sound readings inside and outside the cage ranged from 72-75 dB with the LFHs turned off, and between 80-84 dB with the LFHs on. In LSI, the LFH contributed significantly to the sounds perceived at the lower frequencies of 63, 125, and 500 Hz (room levels were 43-54 dB when off, 65-76 dB when on), but these are sub-audible frequencies for mice. In-cage noise was less than 50 dB for sounds at 2000 and 8000 Hz, which represent audible frequencies for mice.
The MSRB data revealed total noise at 84-87 db despite the on/off status of blower motors and LFHs. It was suspected that room air ventilation noise from the ceiling diffuser was the most significant contributor to this value. For sounds audible to mice, there was greater noise at the 2000 Hz range when the blower motors were running, from 38-40 db up to 48-61 db. These results indicate that overall noise audible to mice is in an acceptable, non-harmful decibel range, and that the use of laminar flow hoods is a more substantial contributor to the sounds perceived within a mouse cage (and within a room) than the IVC system itself.

P098 The Relative Safety of Repeated Measure Designs in Safety Pharmacology Studies Performed by Contract Research Organizations

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Due to the nature of CROs (contract research organizations) and the need to limit cost to the clientele while still producing reliable and valid data, animals are subjected to repeat dosing designs within their experimental lifetime. The impact of repeated testing in Safety Pharmacology studies on the normal homeostatic cardiovascular, hemodynamic, hematologic, and core body temperature measurements across the experimental lifetime of beagles (Canis familiaris) was examined. The physiological parameters were compared to judge long-term effects of multiple study exposure. A post hoc longitudinal review of the following parameters were conducted in 5 female and 5 male surgically implanted, telemetered dogs over the course of at least 1 year: ECG (RR, PR, QRS, QT, QTc), hemodynamic (systolic and diastolic venous pressure and mean arterial pressure), hematological (basic clinical chemistry and hematology parameters) and mean body temperature. All data from each animal were compared from the initial study of implantation against the final study for that animal. Longitudinal data from each dog demonstrated minimal long-term effects from exposure to the series of test article/washout period intervals. All animals showed normal patterns of circadian rhythms across study designs over time and all cardiovascular hemodynamic parameters are within normal ranges throughout the experimental life of each animal.

P099 Reproductive Performance of FVB and B6 Mice in Various Caging Systems

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Investigators at our institution were reluctant to utilize ventilated caging systems for their breeding colonies based on subjective information from colleagues. Some of the breeding problems they were concerned about included reduced litter size, early pup mortality, and infertility as a result of the use of ventilated caging systems. In order to alleviate the investigators concerns, the study was designed to provide objective reproductive performance data for two ventilated cage systems (A and B) and a standard static micro-isolator caging system. Breeding pairs (n = 25) of 4- to 6-week-old FVB and C57BL/6 mice were placed into each cage type. Reproductive performance was evaluated by the total number of pups born, number of pups per litter, and average pup mortality rate over a 16-week period. The number of pups in each cage unit was recorded at birth, 3, 7, 14 and 21 days. Reproductive performance in all cage systems was significantly better in the FVB strain (mean: 24 offspring during 14 weeks) than in the B6 strain (mean: 14 offspring during 16 weeks) of mice, similar to what other reports have published. Although system “A” had a higher mean pup production per caging unit for both strains, there was no statistically significant differences in the number of generations or litter size between the cage systems. Pup mortality varied from a mean of 7.1-13.2% per cage unit with no significant interactions between caging system or mouse strain. These findings helped us determine the choice of caging system for our breeding colonies and provided valuable objective data to the investigators on the breeding performance of FVB and B6 mice in several different caging systems.

P100 A Community Collaboration Brings Mutual Benefits

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Over the past 9 years, Pfizer has collaborated with a number of local organizations that focus on the special needs of disabled people in our community to identify individuals qualified for employment in our facilities. Currently, we have five “special needs” associates that have integrated successfully into our operations in Kalamazoo, Michigan. To achieve this, we have partnered with three local organizations, Universal & Universal, Pathways and MRC Industries, to select, schedule, supervise, train and coach adults with disabilities, enabling them to work in our cage wash facility. This has required some flexibility and accommodation on the part of Pfizer, but on the whole, the process changes required to make this work have been very reasonable. Typically, schedules have had to be more flexible, duties have had to be simplified somewhat, and some minor work-station adjustments have been necessary. But by working together to make these accommodations, we have created a real win-win situation for all of the individuals and the organizations involved. The individuals we employ receive valuable and educational work experience and learn to identify and accept their strengths and limitations. They benefit not only from the additional monetary compensation, but also from the sense of worth and personal satisfaction that comes from completing a project or task, and most importantly, the sense of normalcy associated with obtaining a job, going to work and earning a paycheck. In turn, Pfizer has discovered a very cost-efficient way to meet staffing needs and increase productivity, while enhancing our relationship within the community. While there may be differences in our various business operations, there are likely many similarities in terms of the needs and opportunities that exist within our communities. We encourage others to explore and tap into these unique resources to the mutual benefit of the individuals involved, the business operations, and the community as a whole.

P101 A Guide to Efficiently Running an Animal Receiving Dock

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Receiving and distributing laboratory animals, or “new arrivals,” in an efficient manner is an integral part of the daily routine in an animal facility. If animals are not distributed in a timely fashion, the workload of the room technicians may be delayed and investi-
P102 A Program for Comprehensive Transgenic Facility Training for Postdoctoral Veterinarians

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There is an increasing need for development of transgenic models of human disease within the field of genetic engineering. As innovative technologies emerge, such as retrovirus-mediated transgenesis and stem-cell manipulation, lack of expertise and inefficiency in the production of transgenic animals are becoming major drawbacks within the field. The objective of this teaching tool was to implement a systematic guide by which postdoctoral veterinarians acquired conceptual understanding and skills to produce transgenic animals and manage transgenic resources. Over the course of an intense 12-week period, graduate veterinarians become familiar with nomenclature, methods of embryo biology, surgical techniques and surveillance of health status in transgenic colonies. The training is structured into four sections: animal colony management, embryo retrieval and implantation, embryo injections, and unassisted production of a transgenic animal. A vast array of surgical and supplementary manipulations that support maintenance of transgenic resources are also reviewed. Proficiency in specific tasks such as administration of exogenous hormones, collection of fertilized eggs, uterine and oviduct transfers, cryopreservation of embryos and in vitro fertilization must be demonstrated upon completion of training. Emphasis is placed on emerging technologies and coursework accompanies each applied session in the transgenic facility. For further analysis, genotyping is performed on chimeras produced. Ultimately, the program provides laboratory animal veterinarians with the knowledge and expertise to interface productively with growing opportunities in the field of genetic engineering.

P103 Analyzing Breeder Trends of Genetically Engineered Mice (GEM) in a Laboratory Animal Facility

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It is common to experience poor reproductive performance when breeding mice due to factors such as husbandry, macro- and micro-environment, and the influence of genetic manipulation which can result in the loss of the mouse strain. The analysis of breeding efficiency in colony mice assists in identifying critical production trends due to these factors. This aids in the identification of breeder cages operating at less than optimal production, thus supporting the recommendations made for increasing pup production. Breeding fecundity of each line is analyzed using computer software and compared to published values. This is followed by individual discussion with the investigator to implement breeding strategies and to identify lines in which there is evidence of genetic influence on the breeding performance. The tracking of breeders benefits both the animal facility and the investigator by utilizing the breeders and space more efficiently and identifies lines that are no longer being used by the investigator and are candidates for cryopreservation. This system can prevent the loss of valuable animal models due to low fecundity, saves space and labor resources, and offers a unique assessment of genetically engineered mouse breeding colonies and their reproductive performance.

P104 Business Continuity Planning—A Matrixed Approach

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A business continuity plan (BCP) is a detailed description of the steps to be taken if a catastrophic event threatens your institution’s ability to continue with business. The development of a BCP (in generic terms, a disaster plan) is a critical component of a well-managed business. In large research animal programs with complex infrastructure needs and dependence on support bodies external to the program, it is critical to define expectations prior to a disastrous event. The time to consider what priority the research animal program will receive is not when the site is in a full disaster scenario. At that time, multiple critical operations may be impacted, thus requiring significant resource to restore business. Appropriate pre-planning and strategic partnerships with support functions are of utmost importance for ensuring minimal business interruption and expedient return to normal operating conditions. Crucial to this planning is the actual exercising or role-playing of the strategies, routine testing of staff understanding, and establishment of agreements with partners. These actions, when supported by management, will further provide good assurance that the BCP is current and effective. A plan for business continuity, created by veterinarians and animal facility managers without pre-determined support from the groups that must respond alongside, may be ineffective. This poster will present how the laboratory animal science division of one major U.S. pharmaceutical company developed its disaster plan in concert with the company’s corporate R&D Business Continuity Plan.
Housing ferrets has proven to be a challenge at our facility. They are master escape artists and put rigorous demands on the husbandry staff. We initially modified some stainless steel guinea pig cages to house adult ferrets. We were housing them in groups ranging from 4-6 animals, which worked out well. However, some of the research being conducted with the ferrets required 6-week-old animals. Young ferrets were even more of a challenge because they were able to slip through gaps in the caging, making it quite difficult to contain them. The adult cages were not adequate for young animals, so we then modified some plastic rabbit cages to try and fit these young animal’s needs. Again, we encountered various unexpected challenges, such as difficulty monitoring food and water consumption and trying to maintain a clean living environment for them. To resolve these issues we reduced the size of the groups to 2 animals and housed them in PC rat boxes (19 × 10 × 8 in.). This allowed us to observe them more clearly, although it did increase the husbandry required. In addition to the housing challenges, these young ferrets were weanlings that were not acclimated to dry ferret chow or automatic watering systems. We tried many different combinations of feed and feeding regimens before finally evolving to our current process that transitions these weanlings from mother’s milk (at the vendor facility) to soft, moist food in our facility, and finally to standard dry ferret diet. Our current procedure also addresses the nutritional requirements of the animals, and decreases husbandry labor as much as possible. Currently, with our housing and husbandry processes in place, we are quite successful at maintaining these young animals as a healthy and valuable research model.
World primates. Accommodating both New and Old World primates into our research facility has proved challenging, however. First, it was necessary to house each species separately to prevent transmission of communicable diseases. This separation was critical because diseases that may remain asymptomatic in one species can be fatal to another. Of particular concern were viruses, such as herpesvirus, for which there are limited treatments and high morbidity rates. To prevent disease transmission, we devised stringent standard operating procedures (SOPs) that addressed personal protective equipment procedures to inhibit cross-contamination of housing areas, specific transportation routes of animals, and modifications of transport devices to contain waste and restrict exposure to aerosol contaminants. Aerosols were contained by a HEPA material cover that was placed over the New World monkey’s restraint chair during transport through Old World space. Training of the New World cebus required restraint chair modifications due to their small size and anatomical features such as hunched posture, prehensile tails, and short necks. One such adaptation included the use of thinner, reinforced plexiglass for the neck restraint. Additional equipment modifications were necessary, such as padding capture poles to prevent oral trauma caused by pole biting. Also, because they remained pair-housed while on the training protocol, monitoring weight, water intake, and food consumption ensured that each animal in the pair received adequate nutrition. Our paper discusses considerations necessary for implementing a successful training program of New World primates in a facility housing Old World species. We elaborate on prophylaxis of inter-species communicable diseases, effective SOPs and procedures to ensure staff compliance, and modifications of training equipment and techniques.


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Requests for specific pathogen free (SPF) animals have become standard for housing our research animals. One major challenge of establishing and maintaining an animal holding facility as SPF is to construct a building which will substantially reduce the ability for introduction of unwanted pathogens. Routes of vermin and pest access along with environmental contamination created during construction must be addressed prior to housing animals. The process of preparing a SPF animal facility should begin at the pre-construction phase. Building design and finished material selection are critical components that need to be addressed in advance. Post-construction inspection should further identify and correct areas of possible introduction of bacteria and pest harborage. Following construction completion, the facility is sanitized according to a common industry standard: removal of gross debris and three applications of an appropriate disinfectant, creating an acceptable environment to house animals. The adequacy of this decontamination process in reducing bacterial growth and pest infiltration was assessed pre- and post-sanitization treatment by bacterial swabs along with an integrated pest management program. The bacterial swabs and contact plates used to identify and monitor bacterial species were a better monitoring method in contrast to randomly placed pest traps which are primarily suited to monitor insects. A comparison between the new animal facility and an operating animal facility was performed, revealing mixed cultures in the existing animal facility, including coliforms. Only gram-positive bacteria were identified in the new facility. The monitoring also identified key areas that are difficult to keep clean due to construction design or traffic patterns. Adequate bacterial management in an animal facility begins with proper construction detail and is maintained during the animal facility’s existence through routine disinfection.

P110 Helping Technicians Cope with Grief: A New Program to Offer Support and Comfort to Employees and Local Branch Members

L Bell*
University of Colorado Health Sciences Center, Denver, CO

Although the UCHSC Animal Care and Use program recognized the emotional toll that euthanizing study animals can have on techni-
cians, we did not have a very successful program in place to assist them with their grief. Our standard practice was to offer opportuni-
ties for the technicians to speak with a counselor. Since very few tech-
nicians ever took advantage of this opportunity, we began to look into other ways to help our employees with the grieving pro-
cess. We found a local vendor who was supplying a product called “Comfort Pals” to area veterinary hospitals as a means of helping clients who had to euthanize their companion animals and thought the product, a plush animal with a backpack filled with inspirational cards, would serve our needs as well. In addition, our local branch chose to begin sending a small memorial donation to the AALAS Foundation for branch members who lost a loved one. We have been using the Comfort Pals for approximately 8 months and the employees have told us that they appreciate the recognition and support we are providing. We have received similar comments from branch members since we implemented the memorial donation pro-
gram for our local branch members.

P111 Hand-Raised Infant Baboons Gain Weight More Rapidly Than Maternal-Raised

RF Wolf*, B Valentine, A Born, GL White
University of Oklahoma Health Sciences Center, Oklahoma City, OK

Infant baboons were removed from their dams less than 24 h after birth for the purpose of creating a specific pathogen free (SPF) colony. The infants were hand-reared by a small group of human caretakers. Nursing diet was Similac mixed at label concentration; solid food was introduced at 6 weeks, and the animals were weaned at 4 months. Weights of 35 hand-reared animals were compared with those of 76 infants who were left to nurse on their mothers in our conventional colony. Colony animals were raised in a multiple caretaker home, multiple female indoor-outdoor facility and were allowed to naturally wean themselves. Weights for the 2 groups (mean ± 1 standard deviation) were the same at birth (hand raised = 0.92 ± 0.13 kg; colony raised = 0.91 ± 0.10 kg). Although there was no statistical difference between the groups at 1 month of age, there was a trend for the formula-fed infants to be slightly heavier. At 2 months of age, the hand-reared group weighed more than the mater-
nal-reared (1.65 ± 0.22 kg and 1.39 ± 0.12 kg respectively; P < 0.05). The hand-reared infants remained heavier (P < 0.05) at 4 months (2.37 ± 0.30 kg and 1.83 ± 0.21 kg) and 5 months (2.66 ± 0.25 kg and 2.11 ± 0.21 kg), but at 6 months of age there was no longer a significant difference. At 8 months of age there was virtually no difference between the 2 groups. These data reveal that Similac is an excellent milk replacer to use when hand-raising baboons. Infant
baboons receiving Similac grew at a slightly faster rate than those left to nurse on their dams. However, the weight difference is transient, disappearing soon after weaning.

**P112 Ideas on Building Teamwork through Technician Encouragement and Incentives**

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Facility management and technicians must work together to promote an environment of mutual respect and teamwork. This is accomplished through several special incentives and morale-boosting activities throughout the year. On a monthly basis, technician birthdays are recognized. Each month a technician is identified and responsible for providing a birthday ‘surprise’ to those having birthdays that month. These celebrations can include cake and ice cream, a specially made dessert, lunches, and cards. Another way technicians recognize each other’s good work is “Happy Grams.” These are special e-mails sent to acknowledge and congratulate a technician who goes above and beyond their job description. During the holiday season, technicians participate in a Secret Santa game and exchange gifts at a holiday potluck. The managerial staff also coordinates several special events, which express their appreciation of the technical staff. For example, monthly Tech Talk luncheons are scheduled. Tech talks luncheons involve a presentation given by an investigator on projects currently being run in the animal facilities. These luncheons not only serve to reward the technicians, but also encourage them to feel invested in the project and science they contribute to through animal husbandry and technical work. Technician Appreciation Week is also a much-anticipated time for investigators to participate by providing breakfasts and lunches, for the hard work performed throughout the year. During this time, they serve the entrees. Appreciation gifts, bowling parties, and time are given out to those who have not recorded any STA (short term absence/sick time). These same incentives plus testing fee reimbursement are also rewarded to those making event and concert tickets are given out to those who have not attended a class. Other incentives include a birthday ‘surprise’ to those having birthdays that month. These celebrations can include cake and ice cream, a specially made dessert, lunches, and cards. Another way technicians recognize each other’s good work is “Happy Grams.” These are special e-mails sent to acknowledge and congratulate a technician who goes above and beyond their job description. During the holiday season, technicians participate in a Secret Santa game and exchange gifts at a holiday potluck. The managerial staff also coordinates several special events, which express their appreciation of the technical staff. For example, monthly Tech Talk luncheons are scheduled. Tech talks luncheons involve a presentation given by an investigator on projects currently being run in the animal facilities. These luncheons not only serve to reward the technicians, but also encourage them to feel invested in the project and science they contribute to through animal husbandry and technical work. Technician Appreciation Week is also a much-anticipated time for investigators to participate by providing breakfasts and lunches, for the hard work performed throughout the year. During this time, they serve the entrees. Appreciation gifts, bowling parties, and time are given out to those who have not recorded any STA (short term absence/sick time). These same incentives plus testing fee reimbursement are also rewarded to those obtaining AALAS certification. These ideas have been implemented in an attempt to build teamwork, and reward hard work, and have been an overwhelming success.

**P113 Implementation of a System for Review and Approval of Departures from Standard Operating Procedures and Institutional Policies in an Animal Resource Program**

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1Unit for Laboratory Animal Medicine and 2University Committee on the Use and Care of Animals, University of Michigan Medical School, Ann Arbor, MI

Compliance with federal regulations, national standards, and institutional policies and procedures is required to conduct biomedical research utilizing animals. However, it is not uncommon for circumstances to arise that require departures from regulations, policies, and practices. To accomplish this, animal resource programs must develop mechanisms to appropriately document and review these variations from standard practices. Our program, with an average of 140 variations (termed exceptions at our institution) per month, was plagued with problems with the legacy handwritten exception process, such as slow approval time, inconsistencies, and the inability to retrieve specific approved exceptions. A team consisting of the husbandry managers and supervisors developed a system to simplify the exception process. Factors such as the duration for review and approval, consistency, and network accessibility were considered. A decision tree was created to determine which groups (husbandry, veterinary, IACUC) should review and approve exceptions. To ensure that exceptions are only reaching the hands of those individuals whose endorsement is required, all exceptions are submitted via e-mail to the husbandry manager, who evaluates which regulation, policy, or practice is being affected and directs the exception request to the appropriate group. Exceptions to facility standard operating procedures (SOPs) require only the manager’s approval, who signs the request and returns it to the person submitting it. Exceptions to facility SOPs require only 24 h for review and approval. All other exceptions are forwarded to the veterinary resident and then the faculty veterinarian for their review and approval, taking roughly three days. Veterinary exception requests are forwarded to the IACUC office where an IACUC designate reviews the request, verifies it has been previously approved by the IACUC, and returns it to the team supervisor. If the exception request has not been previously approved by the IACUC, the investigator is required to submit the request as a modification of his/her approved protocol for review and approval by the IACUC. Any requests that constitute departures from either the Guide or USDA standards that require IACUC approval are placed on the agenda for the next IACUC meeting. In one week’s time, an exception that does not require IACUC approval can complete the entire process from submission to IACUC office verification. The original signed copy of the exception form is posted in the animal room. Electronic copies of approved exceptions are saved on our network server. Each team has its own folder, where exceptions are saved by room number and a descriptive title. Computer posting allows wider access to staff members, makes renewal time more efficient, and enables our institution to analyze trends for necessary changes to facility SOPs. This revised system has vastly improved the exception review process for our animal resource program by producing concise and easily accessible exceptions in an expedient manner.

**P114 Increasing Efficiency of an Animal Care Program with the Implementation of a Bar-Coding System**

AN Loewen*, CR Katz

Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI

In order to charge investigators for animal care services, a system of cage counting has to exist. At our facility, census sheets were kept for each animal room containing customer name, their billing category and account number. Investigators may have multiple account numbers, multiple cost centers, or both in the same room. Our facility had an average of 17,000 inventory sheets generated monthly. It was the daily responsibility of the animal care technicians to count each cage in their rooms and record additions, subtractions, and current cage count on the census sheets. At the end of the month, all of these sheets were submitted to the main office. In the past, all sheets had to be manually entered into the billing system. It took 3 people a week to accomplish this task. Mathematical errors complicated this process. Last year we began implementing a bar-coding system for counting cages. With the bar-coding system, each cage card is assigned a unique bar-code and directs the exception request to the appropriate group. Exceptions to facility standard operating procedures (SOPs) require only the manager’s approval, who signs the request and returns it to the person submitting it. Exceptions to facility SOPs require only 24 h for review and approval. All other exceptions are forwarded to the veterinary resident and then the faculty veterinarian for their review and approval, taking roughly three days. Veterinary exception requests are forwarded to the IACUC office where an IACUC designate reviews the request, verifies it has been previously approved by the IACUC, and returns it to the team supervisor. If the exception request has not been previously approved by the IACUC, the investigator is required to submit the request as a modification of his/her approved protocol for review and approval by the IACUC. Any requests that constitute departures from either the Guide or USDA standards that require IACUC approval are placed on the agenda for the next IACUC meeting. In one week’s time, an exception that does not require IACUC approval can complete the entire process from submission to IACUC office verification. The original signed copy of the exception form is posted in the animal room. Electronic copies of approved exceptions are saved on our network server. Each team has its own folder, where exceptions are saved by room number and a descriptive title. Computer posting allows wider access to staff members, makes renewal time more efficient, and enables our institution to analyze trends for necessary changes to facility SOPs. This revised system has vastly improved the exception review process for our animal resource program by producing concise and easily accessible exceptions in an expedient manner.

**P115 Guide to the IACUC Approval Process**

Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI

In order to charge investigators for animal care services, a system of cage counting has to exist. At our facility, census sheets were kept for each animal room containing customer name, their billing category and account number. Investigators may have multiple account numbers, multiple cost centers, or both in the same room. Our facility had an average of 17,000 inventory sheets generated monthly. It was the daily responsibility of the animal care technicians to count each cage in their rooms and record additions, subtractions, and current cage count on the census sheets. At the end of the month, all of these sheets were submitted to the main office. In the past, all sheets had to be manually entered into the billing system. It took 3 people a week to accomplish this task. Mathematical errors complicated this process. Last year we began implementing a bar-coding system for counting cages. With the bar-coding system, each cage card is assigned a unique bar-code and corresponding number. The information encoded in the bar-code includes primary investigator name, billing category, account number, protocol number, source, date of arrival, and housing location. Bar
codes are automatically assigned to each cage card when the appropriate requisition is generated. The cards are activated on the census when receipt of the order is confirmed. When a lab removes a cage from the room, the cage card has to be returned to the appropriate animal care supervisor so the card can be deactivated and charges stopped. Every two weeks, all cages are manually scanned in each animal housing room. Scanned data is uploaded to the PC to allow reconciliation between the database census figures and the actual room counts. The biggest advantage of the bar-coding system is the time our animal care technicians save by not counting cages every day. This allows them to devote more time performing duties directly related to animal care such as enrichment. The monthly billing process is more efficient and accurate, which saves time for the office staff. Being computerized also allows staff members wider access to census counts and the cages can be tracked on a room-by-room basis.

P115 Kicking Abs: A Novel Approach to Technician Enrichment

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In the stressful and demanding environment of animal research, technicians are constantly searching for an outlet to help improve mental and physical well-being. Technician burnout is a common problem that often leads to high turnover. We have developed a program that incorporates an exercise program into our lunch hour to combat stress and fatigue. Three days per week, technicians and supervisors reserve a conference room with audiovisual equipment. Several tapes have been contributed to accommodate different tastes into the workout routine. Cardio workouts are done twice a week, with the remaining session being yoga, Pilates, or an abdominal workout. The workouts usually last 30-35 min, which leaves time for lunch. After several weeks, weight was the only thing lost. What was gained was an increased sense of pride, morale, and overall good will. Not only have we been successful in reaching our physical fitness goals, but a renewed sense of teamwork has also resulted. This exercise program helps relieve stress and anxiety as well as strengthening relationships among co-workers. As new employees are integrated into the group, they are invited to join our exercise sessions. This results in more personalized interactions with the rest of the staff, which helps them assimilate into our team more quickly. Where sluggishness had once prevailed, there is a renewed sense of mental alertness that has contributed to increased productivity and energy throughout the workday.

P116 Less Traumatic Way of Transporting Opossums (Didelphis virginiana) During Cage Changing

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Opossums are not domesticated animals and create special challenges in the vivarium. Our purpose was to develop a technique to remove the opossums from their cage (e.g., for experimental manipulation and routine husbandry) with as little stress and injury as possible. We had two problems to address. The first was that the opossums were injuring their tails, claws, and paw pads trying to grip the floor grate of rabbit cage when being removed. The second was that some were very aggressive when trying to avoid human contact.

We developed two very simple and non-intrusive techniques to move them. The first technique utilizes a plastic board and leather gloves. The second technique utilizes a clear plexiglass anesthetic induction chamber with a sliding lid. Using these two methods, we very rarely have minor injuries and the opossums display less stress-associated behaviors during handling. These methods are quick and calm, which benefits both the animals and the technicians.

P117 Meeting the Training Needs of Investigative Staff

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It is clearly stated in the Guide for the Care and Use of Laboratory Animals that “investigators, technical personnel, trainees, and visiting investigators who perform animal anesthesia, surgery, or other experimental manipulations must be qualified through training or experience to accomplish these tasks in a humane and scientifically acceptable manner.” It is the responsibility of management personnel to ensure that everyone entering an animal facility is trained in the regulatory, technical, humane and safety aspects involved in the care and use of laboratory animals. This can pose a formidable task given the wide variation in educational background, technical skills and experience that exists among research personnel. Other complicating issues include time constraints, cultural and language barriers. In order to address these training needs, we have developed a comprehensive training program that requires all personnel that enter the animal facility to complete an on-line training course provided by the Office of Animal Care and Use, a facility-specific orientation provided by the Program Veterinarian or her designee and enrollment in an Animal Exposure Surveillance Program (AESP). Hands-on technical training specific to the techniques described in their Animal Study Proposal is available and is required for invasive procedures. An animal allergy brochure created by the Occupational Health and Safety Office is provided to all employees. In addition, we have developed two training CDs (one that addresses basic mouse biomethodology and another for aseptic rodent surgery), an investigator handbook and a web site that can be referred to as needed for training reinforcement and reference. These training tools assist in ensuring that trainees have a basic understanding of humane laboratory animal care and use, technical methodology and their ethical and legal responsibilities prior to handling live animals. It also works to further achieve the intent of the 3 Rs (refinement, replacement and reduction).

P118 Novel IACUC Outreach Effort to Facilitate Animal Protocol Submissions

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The Institutional Animal Care and Use Committee (IACUC) has oversight responsibility of its institution’s animal program, facilities, and procedures. One of its primary responsibilities is to review and approve animal use protocols submitted by investigators. To facilitate protocol submission and review, many committees offer training to their investigators to explain the process. The training does not generally cover every aspect of the institution’s animal protocol as it relates to the various forms of research that may be performed at the facility. As such, it is not uncommon for an investigator to receive requests for modifications from the administrator or committee after a protocol has
been submitted for review. To help minimize the number of modifications requested, our IACUC assessed the questions raised during the routine review of protocols and communicated this information to investigators. Initial assessment of IACUC questions was done on protocols submitted within a three-month period of time. Modifications requested from the committee during this period were clustered into broad categories (e.g., consistency, alternatives, and personnel), and into subcategories where applicable (e.g., for “alternatives,” omission of years covered in the literature search or search dates). The top five categories of comments and corresponding subcategories returned to investigators were posted to the IACUC’s web site as well as suggestions for limiting the number of comments returned for these reasons. New investigators were encouraged to visit the web site prior to submitting a protocol. Since posting this information to the web site, quarterly assessments of IACUC comments were conducted for one year for trend evaluation. For 3 of the 5 broad categories and 11 of the 17 subcategories, we observed a decreasing trend in the number of comments returned. We continue to assess the comments that are returned to investigators and update the web site as needed.

P119 Positive Effects of Cell Phone Usage in Laboratory Animal Medicine

K Jacobs*

Pfizer-Comparative Medicine, Kalamazoo, MI

Team members within the Comparative Medicine unit explored various options for improving inter-company communications and potential safety issues. Since implementing the use of cell phones in our facility, we have noted a number of significant improvements in both efficiency and personnel safety. These improvements include:

• Improved Safety: Cell phones allow direct and immediate contact with co-workers and safety personnel and allow individuals to stay mobile after making initial contact. They are also useful in communicating with internal and external emergency personnel.

• Improved Communications: With multiple projects taking place simultaneously, prompt and efficient communication is critical. Cell phones allow personnel to request assistance and direct communication with others who are on and off-site. Instant communication with others can allow individuals to remain on-site, obtain immediate answers to questions, prevent possible errors, and quickly resolve any potential issues. Therefore, downtime is reduced. If face-to-face communication is required, cell phones allow for efficient location of individuals without exploring the entire facility. The ability to stay in contact during non-business hours can also be valuable to business operations. Cell phones allow immediate contact with co-workers during non-business hours, while allowing critical communication to take place and permits personnel to remain mobile to complete other duties.

• Improved Efficiency: In some work environments, heavy workloads prevent personnel from retrieving e-mail messages in a timely manner. A cell phone allows for direct contact of project-specific personnel and the potential for resolving issues immediately. In addition, many facilities have a limited number of landline telephones, two-way radios and pagers, which can reduce the mobility and flexibility of the personnel using them.

• Cost-Savings: Monthly costs of other communication tools, such as two-way radios, pagers, landline telephones and e-mail can exceed the monthly cost of cell phones. Cell phone usage can potentially reduce monthly costs and improve financial resources. Utilizing cell phones provides a better means of overall communication than other methods. The issues listed above indicate that cell phone usage significantly improves inter-company communications; safety conditions, reduces inefficiencies and increases cost effectiveness.

P120 Reducing Stress in Individually Housed Sheep

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Sheep are herding animals, depending on their flock for protection and comfort. Occasionally, it is necessary to individually house and separate those sheep that need to fast prior to surgery, for health reasons, or due to specifics in a research protocol. Therefore, when group housing is not an option, steps should be taken to reduce the stress this incites. At our facility, an isolated sheep would become extremely agitated and skittish, show an increase in respiratory effort and becoming uncontrollably vocal for hours, desperately wanting to reunite with its flock. We have found that the incorporation of mirrors greatly reduced the stress of this isolation. A large mirror, approximately 4 × 3 ft., was mounted on the sidewalk of each isolation housing unit. The transfer of sheep into this mirrored enclosure had demonstrable changes in behavior. Vocalization stops completely and the sheep remains completely calm. It seeks out its own mirrored image, stands close and occasionally nudges at its mirrored partner. Consumption of food and water remains unchanged and the risk of injury has been eliminated, as the sheep no longer tries to jump or escape the enclosure. This change in behavior occurs consistently among all isolated sheep. Upon returning to group housing, the animal acclimates with the rest of the flock without incident. The use of mirrors has greatly improved the quality of life for ovine in our research facility. The stress levels are dramatically reduced not only in the isolated sheep, but also the staff in charge of their care. Group housing is ideal, but when conditions require individual housing, mirrors are a delightful enrichment tool.

P121 Research Animal Facility–A Time/Motion Study

DS Crowell*

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Changes in institutional philosophy and policy now require a greater ability to justify, with concrete numbers, existing and future staffing needs. Historically, the number of animal care technicians required was based upon a rough subjective average of the number of cages that were expected to be changed. We needed an objective baseline with which to justify the number of positions budgeted. A time/motion study was developed that would account for each of the activities of the animal care technicians. The study was designed to provide an analysis of each responsibility and incorporate the time needed for completion of all animal husbandry-related tasks as a measure of the time needed to change a single cage. A form was developed to capture the various daily, weekly and monthly activities and the time required to complete each. Because of the size of the facility, the animal care technicians in the research animal facility typically spend their entire day providing husbandry services in a single room. Animal care technicians volunteered or were chosen to complete the forms each day for one-week periods. The information gathering was then repeated by the same technicians, in their respective rooms, for up to six weeks. The results of the data gathered were analyzed to calculate “Productive Work Time,” “Personal and Fatigue” allowances, “Delay” time, and an “Average Time/Box” standard. The baseline created with this data will allow us to justify currently budgeted positions as well as prepare
legitimate labor estimates for future growth. Additionally, this information also gives our supervisors the ability to better evaluate the expected productivity of the animal care technicians.

P122 Streamlining Surgical Suite Operations

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WIL Research Laboratories, Inc., Ashland, OH

With the addition of a new large animal surgical suite at our facility came a new set of challenges—the need for additional technical training, equipment needs and storage, and creating the space and techniques that could be used for a wide range of species and procedures. We addressed these issues starting in the blueprint stages of the surgical area. Great care was taken in designing the layout of the suite. We carefully planned and designed specific storage areas, located support and mechanical areas, and planned for future expansion. Additionally, the space and procedures had to be user-friendly and adaptable. New standard operating procedures were written to cover the conduct of all functions in the suite. A new training program was implemented for surgeons and surgical assistants which includes required reading, examinations, and hands-on practicums. We have utilized technology to full advantage. Instrument pack lists were created with digital photos for easy pack preparation and surgery room set-up. Spreadsheet-based “Preparation Guides” were designed for anesthesia which require only the entry of the species and body weight to determine the anesthesia protocol for individual animals. Our supply inventory and ordering process were also automated. The planning and procedures have been successful. After approximately four years of use, the surgical suite runs efficiently, the technical staff is well trained and has adapted easily to the mix of surgical procedures. New staff and capabilities are quickly integrated into the daily operations, and plans are underway for expansion of the suite.

P123 Successes and Failures in the Development of a SPF Baboon Colony

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Baboons are known to harbor analogs of many of the herpesviruses and retroviruses known to infect humans and other primates. Our objective was to develop a self-sustaining colony of baboons free of all known herpes viruses, four retroviruses and SV 40. These viruses include HSV, VZV, CMV, HHV6, EBV & HHV8, 4 retroviruses (simian foamy virus, SRV/D, SID, & STLV) and SV 40. We utilize serological and PCR to test for the 11 target viruses. We recruit 25 infant baboons per year. Utilizing timed pregnancies, infant baboons are removed from their mothers within 12 h of birth in clusters of 5 infant baboons. Each group of 5 baboons is maintained in separate rooms in a SPF nursery for one year. All infants are repeatedly tested for the target viruses. We recruit 25 infant baboons per year. Utilizing timed pregnancies, infant baboons are removed from their mothers within 12 h of birth in clusters of 5 infant baboons. Each group of 5 baboons is maintained in separate rooms in a SPF nursery for one year. All infants are repeatedly tested for the target viruses. Any baboon that breaks with one of our 11 target viruses is immediately removed from the colony. At one year of age they are moved into large gang cages that will accommodate larger group of juvenile baboons. The SPF baboon colony is maintained under SPF conditions and procedures. We had 9 successes (i.e., infants negative for all 11 viruses) and 15 failures (i.e., infants that broke with one or more of the viruses) our first year in developing the colony. Cytomegalovirus alone was diagnosed in 13 of the failures; vertical transmission was the probable cause of the failures. Two infant baboons were diagnosed with Cytomegalovirus, Rhadinovirus, and Herpesvirus papio 2. We are now testing newly introduced baboon infants by PCR and virus culture for CMV during the initial 3 weeks in the hopes of identifying positive animals earlier. During this time, infants are maintained in separate cages and allowed no direct contact in order to prevent disease transmission.

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P124 The Employee Recognition Program at the Oregon National Primate Research Center

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In the current job market there is a need to attract and retain qualified, dedicated laboratory animal technicians. To facilitate this and increase employee morale, we formed a diverse committee of technicians and management personnel to create and implement an Employee Recognition Program. The program is divided into 4 sections: recognition of our current employees, encouraging career growth, promoting referrals, and building team spirit. The recognition portion of the program consists of two rewards for the technicians: an Employee of the Quarter award, and a “High Five” technician acknowledgement. The High Five recognizes a technician for going that extra mile; a peer, investigator or manager can submit a High Five nomination to acknowledge the extra effort. Secondly, to encourage career growth, we organize classes to assist the technicians in preparing for each of the three levels of AALAS certification examinations. The program covers the cost of study materials, AALAS membership, and the examination. Additionally, there is a financial bonus upon successfully obtaining AALAS certification. We also offer a referral incentive to encourage scouting for new talent. Many of the rewards and incentives are for varying amounts of “Monkey Money,” certificates the employees can exchange for goods at our “Monkey Market.” The last part of the program is team building. Each year we plan summer picnics, organize AALAS International Laboratory Animal Technician Week activities, and arrange a holiday party that is always popular. The activities have provided an opportunity for the technicians to interact with the investigative staff. According to the results of a recent employee survey, the employees feel that morale has improved since the institution of our employee recognition program; 18 of the 22 respondents felt that morale has increased. We are pleased that the program has been successful in achieving our goals of building team spirit and increasing job satisfaction. We plan to continue to offer an employee recognition program at the Oregon National Primate Research Center.

P125 The Impact of “Just-In-Time” on the Verification of Congruency Between Protocols and Grant Proposals

KJ Diven*, PA Matos, JR Owiny

Johns Hopkins University, Baltimore, MD

The Public Health Service (PHS) Policy on Humane Care for Use of Laboratory Animals requires that institutions which have an assurance on file with the Office of Laboratory Animal Welfare verify approval by the Institutional Animal Care and Use Committee (IACUC) of those components related to the care and use of animals.
The logical consequence of this process is the development of a logistics center for mice, keeping the animals in warehouse-type racks in one large hall. Currently these automated systems are readily utilized for storage of various goods in the industry. With ventilated caging it will be an achievable goal to technically modify these systems for rodent housing.

This concept has the following advantages:

- Reduced construction costs for a less complicated building (one large hall with few additional rooms).
- Optimal space use (maximum number of cages per floor area).
- Lower personnel costs due to a high degree of automation for transportation and robots for all cleaning tasks (staff has the complete working time for animal care).
- Ergonomic workplace for animal care technicians with the possibility to sit: all cages and equipment will be automatically transported and robots for all cleaning tasks (staff has the complete working time for animal care).
- Adequate monitoring of all cages by a computerized system with motion sensors and infrared video cameras for the documentation of animal behavior and well-being.

Animal health has been monitored since the colony was established and only monkeys free from Herpes B and tuberculosis (TB) were kept for breeding. The colony continues to be Herpes B- and TB-free and is also negative for other viral diseases, namely SIV, STLV and Retrovirus D. The testing regime in 1988 was reduced so that animals are tested for TB at weaning and at 18-24 months old. Herpes B virus testing also occurs at 18-24 months. Additionally, these juvenile animals have been tested for Retrovirus D since 1993 and SIV and STLV since 2000.

The authors acknowledge the assistance of M. Wood and M. Baskerville in the preparation of this poster.

P127 The Warehouse System: A Model for Future Rodent Housing?

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During the past 15 years, laboratory mouse populations have been growing exponentially due to the progress in transgenic technologies. This process facilitated the construction of modern ventilated caging equipment and has been a major challenge for laboratory animal facilities, which have to provide increasing mouse holding capacity by better space utilization and ongoing construction of new vivarium buildings. Recently, library-style ventilated racks have been manufactured that enable a marked increase in stocking density when used in larger animal rooms.

The logical consequence of this process is the development of a logistics center for mice, keeping the animals in warehouse-type racks in one large hall. Currently these automated systems are readily utilized for storage of various goods in the industry. With ventilated caging it will be an achievable goal to technically modify these systems for rodent housing.

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- Adequate monitoring of all cages by a computerized system with motion sensors and infrared video cameras for the documentation of animal behavior and well-being.

Production data has been collated from the colony since 1982.

Since 1982 the colony has been managed to be self-sustaining, although in 1987, 3 females and 2 males were purchased from another UK rhesus monkey colony and in 1994 4 females and 2 males were transferred from a UK unit which closed.

The output since 1982 has been such that the number of infants born per female per year has been between 0.45 and 0.75 and the number of infants weaned per female per year has been between 0.31 and 0.64. The births per female per year from other captive units are 0.64 [Walker, M.L et al, 1982. Reproductive performance in captive-acclimated female rhesus monkeys (Macaca mulatta). Journal of Medical Primatology 11(5):291-302.] and up to 0.67 [Anderson, D.M and Simpson, M.J. 1979. Breeding performance of a captive colony of rhesus macaques (Macaca mulatta). Laboratory Animals: 13(3):275-281.].

Animal health has been monitored since the colony was established and only monkeys free from Herpes B and tuberculosis (TB) were kept for breeding. The colony continues to be Herpes B- and TB-free and is also negative for other viral diseases, namely SIV, STLV and Retrovirus D. The testing regime in 1988 was reduced so that animals are tested for TB at weaning and at 18-24 months old. Herpes B virus testing also occurs at 18-24 months. Additionally, these juvenile animals have been tested for Retrovirus D since 1993 and SIV and STLV since 2000.

The authors acknowledge the assistance of M. Wood and M. Baskerville in the preparation of this poster.

P126 The Laboratory Animal Board Study Group (LABSG): A Multifaceted Tool for ACLAM Board Preparation

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Preparation for the specialty board examination for the American College of Laboratory Animal Medicine (ACLAM) is an intensive process. Ten years ago, the Laboratory Animal Board Study Group (LABSG) online club was established to provide a forum for journal review for examination preparation. Over the years, the mission of this group has expanded to include practice examinations, practicals, questions from common reference resources such as the Guide and the ACLAM Laboratory Animal Medicine textbook, and summaries and questions from common laboratory animal science journals like Comparative Medicine. This poster is intended to be an introduction to the variety of study aids available through the LABSG online journal review club and the LABSG web page (http://home.comcast.net/~jgmwlr11/). The LABSG resource is an extremely valuable support tool for veterinarians preparing for the ACLAM specialty board examination.

P126.5 The Production from a Rhesus Macaque Breeding Colony

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Macaque have been housed at Porton Down for almost a century. In 1974 it was decided to establish a breeding colony of Indian origin rhesus monkeys (Macaca mulatta). Initially the colony was made up of 22 females and 4 males (MacArthur, J. A. et al, 1978. Establishment of a small breeding colony of rhesus monkeys. Laboratory Animals 12:151-156.) and currently there are 175 females and 10 males in breeding. Animals were imported into the colony up to 1982 when the colony was closed to non-UK bred imports.

Production data has been collated from the colony since 1982.

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The authors acknowledge the assistance of M. Wood and M. Baskerville in the preparation of this poster.
To the authors’ knowledge, such a system does not exist yet. However, we are convinced that it will be realized and revolutionize the future mouse housing in terms of cost efficiency and space utilization for the research institution, ergonomics and work safety for the personnel and improved and documented housing standards for the animals.

P128 Treasure Hunt: How to Motivate Animal Technicians to Complete Additional Room Tasks

AJ Bakowski*

Charles River Laboratories at University of Texas Southwestern Medical Center, Dallas, TX

Do your animal technicians tend to skip over the additional tasks involved in caring for an animal room? Examples of such tasks might include dusting the tops of automatic water lines, cleaning air supply vents or swapping out dirty, unorganized supply boxes. To make these tasks more appealing to the technicians, we instituted a treasure hunt program at our facility. Brightly colored handwritten notes are left inside animal rooms in places that need attention. Each note contains the date of placement, the room number, the supervisor’s name, and the task to be corrected. It also advises the employee who finds the note to see their supervisor. When a note is discovered, the supervisor verifies to ensure the task was completed. If so, the technician is rewarded with a piece of candy from a grab bag assortment. To help track the program’s progress, the supervisor keeps a master list which includes each note’s location and date of placement. Many notes are discovered within a few days of placement; helpful hints are provided when technicians struggle with less conspicuous notes, such as inside a dirty light cover. Since the program’s inception, the time for animal technicians to find each note and complete the task has continued to decrease. The technicians take pride in finding these notes and enjoy the reward that comes with it. This program helps to satisfy room sanitation standards for our department and results in easier preparations for semi-annual IACUC inspections. Future ideas for this program will include other animal care positions and monthly certificates for top achievement. The goal of the program continues to focus on training and motivating technicians to become more aware of all aspects of animal room care while also providing incentive and creative fun.

P129 Use of Hydrogen Peroxide Gas for Decontamination of Viral Infected Areas

R Dattoli*

Wyeth Massachusetts, Cambridge, MA

A common problem for animal researchers and caretakers alike is viral outbreaks within their animal facility. Quarantine of animal holding rooms and other functional areas is a burden to everyone working within the facility. This poster suggests eradication and prevention procedures. Practices for dealing with outbreaks are to set up quarantine procedure within the infected areas and then depopulation followed by decontamination. Past decontamination procedures included using sponge mops and chemical jet sprayers, followed by para-formaldehyde fogging performed by an outside vendor; also, Environmental Health and Safety (EH&S) staff need to be involved. This process was used four times in Cambridge and twice in Andover over 15 months at a cost of $4,066 for each occurrence.

Last December we had an outbreak of mouse parvovirus (MPV). Investigation indicated the virus was likely transported to Cambridge on equipment during relocation. This has led us to believe that our procedures were not effective enough to eradicate the virus. Seeking an alternative method of sanitation that would ensure decontamination of equipment as well as the facility, we chose hydrogen peroxide vaporization. With this process, medical-grade hydrogen peroxide is transformed to a gaseous state and distributed throughout the room. The hydrogen peroxide gassing unit was purchased, and the first sanitation of a viral-infected area was performed in January 2004. Biological test indicators (Geobacillus stearothermophilus) placed at several locations around the rooms were negative following the decontamination process, indicating the conditions were met for killing G. stearothermophilus. These same conditions have shown to kill MPV.

Five weeks post-decontamination, sentinel mice were dispatched from this area for health monitoring for bacteriology, infectious disease (PCR), parasitology, pathology and serology came back negative. Cost analyses related to animal lives and lost research time reflects a substantial savings over time.

P130 Using Advertising to Increase Profit in the Small Animal Diagnostic Laboratory

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Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI

The function of the Animal Diagnostic Laboratory (ADL) has evolved from large to small animal medicine over the years, requiring a reassessment of services and a new vision. Laboratory operating expenses were rising without a parallel increase in revenue, necessitating a change. Our laboratory is in a unique position to offer services that are valuable to principal investigators, but few researchers were aware of their availability. In preparation, the ADL updated its instrumentation to accommodate the smaller volume samples expected in rodent research. Increasing visibility by advertising was the next priority. First, the laboratory staff wrote an article that appeared in a university newsletter distributed to the 2,500 members of the research community who utilize animals in their laboratories, and also to all of the animal care committee members. Next, we concentrated on integrating our activities and pricing into the newly designed departmental web site, ensuring that the information was easily accessible. We also displayed a poster detailing our services at a university research center symposium, an event attended by university biomedical investigators. A brochure has been designed and collaboration with phenotyping services is being investigated. The number of requests for information about our services has increased with each form of advertising. The tallies of the recharge work performed in the ADL for 2002 were compared to the tests performed in 2003. Between 2002 and 2003, the number of CBCs submitted to the ADL for testing on a recharge basis increased by 730% (monthly average increase from 5.2 to 38.0 samples), individual chemistry tests increased by 839% (from 11.7 to 98.2 samples), and chemistry panels by 180% (from 7.0 to 12.6 samples). The increased effort made by our laboratory to advertise their services led to a notable increase in rechargeable services. We have become significantly more visible to the research community and plan to continue in our effort to reach new customers and increase business.
P131 Utilization of a Free-Ranging Monkey Troop to Document Annual Hair Growth and Molt Patterns in Rhesus Monkeys (Macaca mulatta)

P O’Neill-Wagner*

DHHS, PHS, NIH, NICHD, Laboratory of Comparative Ethology, Poolesville, MD

Is the coat condition of nonhuman primates important for animal health assessment? Is there a natural cycle of predictable hair growth and shedding that occurs? Among mammals, relationships exist between the reproductive cycle, the pelage (hair, fur, skin) cycle, and other phases of the annual cycle. Reports for nonhuman primate species have focused primarily on the molt stage, leaving an absence of data about the hair-growth stage. In this study, the initiation of hair growth activity and shedding were documented for a group of 36 free-ranging rhesus monkeys located at the National Institutes of Health Animal Center. Dye marking and shaving techniques were employed to document changes in the animal’s coats. Observations of individual animal hair growth and loss were recorded. For Group 1, over-the-counter hair dye agents were applied to 9 males and 10 female rhesus monkeys in mid-June. Most monkeys shed the dyed hair and completed new hair growth by the end of August. Two nonreproductive females failed to shed, thereby retaining their dyed hair. In October of the same year, 24 animals (13 females and 11 males; Group 2) were similarly dyed. Posterior neck regions of the entire troop of 36 monkeys were shaved. All animals were subsequently observed for shedding and for growth of new hair on a weekly basis for 9 months following shaving. In Group 2 the nonreproductive females and a 1-yr. old male completed their new coat growth within 7 months. By contrast, others began their new hair growth 7 or 8 months after shaving. Alpha and beta males were the last to begin new coat growth. Results suggest that daylight (photoperiod) and hormonal activity (pregnancy and lactation for females, breeding for males) were instrumental in determining hair loss and growth patterns. Winter temperatures had no influence on stimulation of new hair growth.

P132 Large Animal Enrichment for Sheep and Goats

PW Fish*

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Large animals in a research environment housed in confinement buildings present a husbandry problem of how to accommodate natural browsing behaviors of small ruminants and still maintain easy handling during polyclonal studies. The purpose of this project is to design and implement large animal enrichment pens (AEP) that could enhance the normal browsing and climbing behavior of small ruminants therefore providing a more natural environment for the antibody production herd at Abbott Laboratories. The stations/positions are designated wash process at one animal facility and two different stations/positions of the cage wash personnel, University Animal Care has developed and implemented new areas. The schedule covers five different stations/positions of cage wash personnel, University Animal Care, University of Arizona, Tucson, AZ

C Kilcullen-Steiner*, C Johnson, C Richner, T Ruddy, J Brooks

University Animal Care, University of Arizona, Tucson, AZ

To decrease repetitive motion injuries and alleviate the boredom of cage wash personnel, University Animal Care has developed and instituted a station/position rotation schedule within the cage wash area. The schedule covers five different stations/positions of the cage wash process at one animal facility and two different stations/positions at another animal facility. The stations/positions are designated

P133 NIH Primate Concentrate Supplement, A Novel Nonhuman Primate Diet Supplement

DE Barnard1*, C Clarke2

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Macaca mulatta monkeys used in AIDS research and Aotus trivirgatus monkeys used in malaria research may become anorexic and malnourished during a study, which can confound results. Primate research facilities typically make their own in-house diet supplements for monkeys that are anorexic. These in-house supplements are usually very palatable but do not meet the nutritional requirements of the monkeys, which can be detrimental to the monkeys’ health and experimental protocols. A novel primate diet supplement, the NIH Primate Concentrate Supplement (NIH-PCS), was formulated to provide a concentrated form of nutrients and calories for anorexic monkeys. The gross energy, protein, fat, and crude fiber concentrations provided by the NIH-PCS diluted 60% are 2.8 Kcal/g, 20.1%, 10.7% and 1.9%, respectively. A series of palatability studies using three flavors (cherry, raspberry, and cheese) of the NIH-PCS were performed using aotus and rhesus monkeys. The owl monkeys’ mean weight increased from 960 g to 1,070 g and the rhesus monkeys’ mean weight increased from 8.8 kg to 9.1 kg during the study. There were no signs of diarrhea or constipation. The owl monkey palatability factors for the raspberry-, cherry- and cheese-flavored NIH-PCS were 0.946, 0.960, and 0.945, respectively. The rhesus monkey palatability factors for the raspberry-, cherry- and cheese-flavored NIH-PCS were 0.954, 0.948, and 0.902, respectively. The results show that the NIH-PCS is a highly palatable dietary supplement that can provide adequate nutrient supplementation to nonhuman primates.

P134 Improving Cage Wash Personnel Morale

C Kilcullen-Steiner*, C Johnson, C Richner, T Ruddy, J Brooks

University Animal Care, University of Arizona, Tucson, AZ

To decrease repetitive motion injuries and alleviate the boredom of cage wash personnel, University Animal Care has developed and instituted a station/position rotation schedule within the cage wash area. The schedule covers five different stations/positions of the cage wash process at one animal facility and two different stations/positions at another animal facility. The stations/positions are designated...
as dirty side and clean side at the small animal facility and dirty-side dumper/loader, dirty-side disassembler, clean-side orders, clean-side autoclaving and clean-side unloading at the larger animal facility. Since the program has been instituted, we have decreased the incidence of “carpal tunnel syndrome-like” complaints completely as well as significantly reduced other repetitive motion complaints and actual injuries. Other benefits include better overall job knowledge of each aspect of the cage-washing process by the employees; during employee shortages, any member of the cage wash team can fill in at any position at either building. In addition, morale is improved because an employee will only have to be in a “hated” position for 2 weeks at a time and can look forward to not seeing that position for 14 weeks once they complete the rotation.

P136 Effect of Environmental Enrichment on the Reproductive Performance of FVB Breeding Mice

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Environmental enrichment increases the well-being of laboratory animals living in standard cages because it allows for species-typical behavior and cognitive stimulation. There are very few controlled studies describing the effect of enrichment on the reproductive performance in mice. The purpose of this study was to characterize the reproductive performance in FVB breeding pairs provided with two types of commercially available mouse environmental enrichment devices. The study consisted of three groups of 10-week-old FVB breeding pairs. Group 1 (10 pairs) was provided with a transparent microenvironment dome equipped with an activity wheel; group 2 (10 pairs) was provided with the transparent microenvironment dome only; and group 3 (25 pairs) was housed without an enrichment device. Breeding pairs were housed one per static microisolation cage continuously to allow for breeding during the postpartum estrus. Average litter size and average inter-birth interval after the first litter was compared in all groups. Breeding pairs housed without enrichment devices (Group 3) had an average litter size of 7 pups. The average litter size in groups 1 and 2 was 5 pups. The average inter-birth interval for breeding pairs in Groups 1, 2, and 3 were 40, 36 and 32 days, respectively. The results suggest that while the enrichment devices used in this study provided for species-typical behavior, their use is associated with longer inter-birth intervals and smaller litters in FVB breeding mice. Managers and investigators should be aware of this potential effect when considering the use of enrichment devices as part of a standard housing practice in a mouse breeding colony.