Chronic, idiopathic diffuse colitis is a well-recognized clinical and pathological entity in captive rhesus monkeys. A group of six rhesus monkeys were diagnosed with clinically debilitating, chronic diarrhea. Histologically, colonic tissues were characterized as chronic, moderate to severe colitis and typhilitis, with diffuse mononuclear inflammation of lamina propria, reactive lymphoid hyperplasia, and multifocal microabscesses. Colonic tissues were cultured for Salmonella spp. and Shigella spp.; all results were negative. Samples were negative for Clostridium difficile A and B toxins, and special stains of colonic tissue for acid fast bacteria were negative as well. In addition, the 6 diarrheic monkeys tested were negative for serum IgG antibodies to Herpes B virus, STLV, SRV and SIV. Colonic tissue from two clinically normal monkeys from the same colony were also subjected to microaerobic culture. Microaerobic cultures obtained from all 8 monkeys and grown at 37°C and 42°C, revealed pinpoint or spreading colony growth on antibiotic impregnated media. Bacteria were identified as gram-negative, oxidase positive, and urease negative. However, of the nine strains characterized biochemically, two separate biotypes (corresponding to different species by 16S rRNA analysis) were identified. One biotype from diarrheic monkeys and the second biotype from nondiarrheic animals differed by catalase activity, ability to reduce nitrate to nitrite, and sensitivity to cephalothin. Complete 16S rRNA analysis of 5 of the 9 strains characterized biochemically indicated that the organisms isolated were two novel Helicobacter spp. By electron microscopy these novel helicobacters had spiral morphology with bipolar sheathed flagella. This is the first report describing novel Helicobacter spp. being isolated from inflamed colons of rhesus monkeys. Studies dissecting the causal role of Helicobacter spp. in initiation and progression of chronic colitis in macaques may prove useful in understanding the etiopathogenesis of inflammatory bowel disease in humans.
Helicobacter mustelae has been linked to chronic gastritis, peptic ulcers and gastric cancer in ferrets. In contrast to H. pylori, the outer surfaces of H. mustelae is covered by an extensive array of 8.5 nm rings made of a protein designated Hsr. These surface rings are potentially analogous to the classical S-layer found ubiquitously among prokaryotes; functions ascribed to S-layers include roles in protection, cell adhesion, surface recognition and virulence. To examine the importance of Hsr in the pathogenesis of H. mustelae, two isogenic Hsr mutant strains were constructed by disrupting the Hsr gene. Ferrets determined to be specific-roles in protection, cell adhesion, surface recognition and viral infections will develop IBD by 1 year of age. We wished to determine that are infected with H. pylori or H. mustelae will develop IBD.}

**PS04 Novel Helicobacter Species Identified from Random Source Dogs**

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*Helicobacter canis* first reported in diarrheic and normal dogs in Europe has been only reported once in the U.S. from a liver of a puppy with hepatitis. To ascertain whether *Helicobacter* spp. were present in dogs used for research purposes, 5 conditioned random source dogs used in an acute cardiopulmonary bypass experiment were euthanized and liver, bile, cecae tissue, and pancreas were subjected to microaerobic culture and PCR using a 1.2 kb *Helicobacter* genus specific PCR assay. Three dogs had a catalase and urease negative, but oxidase positive gram-negative microaerophile isolated from the cecum. By 16S rRNA analysis, all three bacterial isolates were identified as a novel *Helicobacter* sp., clustering with *H. canis*. All five liver samples were positive for *Helicobacter* sp. based on PCR results; pancreas and bile samples were negative. Chronic hepatitis was also noted histologically in these animals. The significance of enterobacterial helicobacters in causing overt disease or interference with ongoing research using helicobacter-infected dogs requires further studies.

**PS05 Isolation and Characterization of Helicobacter sp. from the Gastric Mucosa of Two Dolphin Species, Lagenorhynchus acutus and Delphinus delphis**

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Dolphins are maintained in captive settings for research, rehabilitation and educational purposes. Most of the current research focuses on bioacoustics, immunological studies and infectious diseases. Gastric ulcerations of unknown etiology have caused significant morbidity in these dolphin colonies for decades. Considerable speculation exists as to whether dolphins have *Helicobacter* spp.-associated gastritis and peptic ulcer disease (PUD). Stranded dolphins that subsequently died were assessed for the presence of *Helicobacter* spp. Novel *Helicobacter* isolates were identified by culture and PCR in the gastric mucosa of 3 of the 8 dolphins. Gram-negative bacteria with a fusiform morphology were isolated from the glandular stomach. Biochemically, the bacteria were urease, catalase and oxidase positive. Spiral organisms were detected by Warthin Starry stain. Histological sections evaluated in two infected animals revealed a multifocal lymphoplasmacytic gastritis and mucous epithelial hyperplasia. Similar lesions are observed in some *Helicobacter pylori*-infected humans. Pure culture of the bacteria from two dolphins were classified by 16S rRNA analysis. It was determined that the bacteria clustered with gastric helicobacters and represent a novel *Helicobacter* sp.: most closely related to *H. heilmannii*. These findings suggest that a novel *Helicobacter* may be playing a role in the etiopathogenesis of gastritis and gastric ulcers in dolphins. To our knowledge, this represents the first isolation and characterization of a novel *Helicobacter* sp. from a marine mammal and emphasizes the wide host distribution and pathogenic potential of this increasingly important genus.

**PS06 H. bilis Infection Accelerates the Development of Inflammatory Bowel Disease in Multiple Drug Resistance (mdr1a) Deficient Mice**

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*mdr1a* deficient mice spontaneously develop inflammatory bowel disease (IBD) and the frequency of disease increases with age and is associated with microbial status (C.M. Panwala. J Immun.161:5733, 1998). Approximately 25% of *mdr1a*-/- mice that are *Helicobacter* spp.-negative and housed under SPF conditions will develop IBD by 1 year of age. We wished to determine if infection with *H. bilis* would accelerate development of IBD in these mice. Approximately 108 *H. bilis* organisms or broth were given to 6-week-old female *mdr1a*-/- and FVB/+ mice (Taconic Farms, Germantown, NY) by oral gavage (3 times). Mice were housed under SPF conditions and were monitored for weight gain/loss and symptoms of disease. All *mdr1a*-/- mice infected with *H. bilis* developed overt signs of disease by 12 weeks post-infection, whereas disease was not observed in control mice. This study suggests that *H. bilis* might provide a model for the study of IBD pathogenesis and drug resistance.
loss and diarrhea. *H. bilis*-infected mdr1a-/- mice developed diarrhea and weight loss at 3–9 weeks post infection and had histologic evidence of IBD in cecum, colon and rectum. Hence, IBD developed in all (10/10) *H. bilis*-infected mdr1a-/- mice with evidence of mild IBD in 1/5 broth mdr1a-/- mice; no IBD developed in broth FVB+/- (0/5) or *H. bilis*-infected FVB+/- (0/10) mice. Histopathologic lesions of *H. bilis*-induced IBD consisted of crypt hyperplasia and branching, severe inflammation with obliteration of normal architecture, crypt abscesses and mucosal ulceration which affected the cecum, proximal, mid and distal colon, and rectum. Flow cytometric analysis of mesenteric lymph nodes (MLN) from *H. bilis*-infected mice showed increased cellularity with increased numbers of B cells, CD4+, and CD8+ T-cells in mdr1a-/- mice relative to FVB+/- controls. Also, MLN from infected mdr1a-/- mice showed increased proliferation to soluble *H. bilis* antigen. RT-PCR from RNA of colonic tissue showed increased transcripts for interferon-γ and IL10 from colitic mice. *H. bilis*-induced IBD in mdr1a-/- mice may be a useful model to explore microbial induced IBD. Mechanistic studies of IBD performed in these mice could be complicated by an intercurrent infection with *Helicobacter*.

**PS07 Chronic Infection with Helicobacter felis Is Associated with an Increase in Serum Cholesterol in iNOS Targeted Mutant Mice**

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Acute infection is known to alter serum lipid profiles. Recently, chronic infection has been associated with increased risk of coronary heart disease, and changes in serum lipid profiles have been examined as a possible underlying cause. *Chlamydia pneumoniae* has received the most attention; however, chronic infection with *Helicobacter pylori* has also come under scrutiny. A large epidemiologic study found a significant increase in serum triglycerides and total cholesterol in people who were seropositive for *H. pylori* relative to those who were seronegative. In addition, the HDL:total cholesterol ratio was significantly decreased in the seropositive group, suggesting that the associated risk for atherosclerosis may be related, in part, to modification in the serum lipid profile. Previously, we showed that iNOS targeted mutant mice (*C57BL/6j*129/SvEv *iNOS*) have an increase in total cholesterol when compared with wild type control animals, even when fed a non-atherogenic diet, and have a high incidence of atheromatous lesions. We inoculated a group of iNOS mutant mice with *H. felis* and compared total serum cholesterol in the infected group (n = 15) with that in a group of uninoculated iNOS mutant mice (n = 23). Ten months post-inoculation, the infected group had a significant increase in serum cholesterol over the already-elevated cholesterol levels in the uninoculated group (P = 0.0216). This finding supports the hypothesis that alterations in the serum lipid profile may contribute to the increased risk for coronary heart disease associated with chronic infection, including infection with *Helicobacter*.

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**PS09 Failure to Detect Helicobacter pylori in Gastric Endoscopic Biopsies from Dogs and Cats**

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*Helicobacter pylori*-induced gastritis was recognized in a closed colony of laboratory cats in 1993. This discovery, and the prevalence of idiopathic gastritis in pet dogs and cats, prompted a search for *H. pylori* infection of the stomach of these species. Two millimeter samples of gastric mucosa were collected in the course of endoscopic examination of dogs (n = 44) and cats (n = 25) presented for signs of gastrointestinal disease at two veterinary referral centers. Samples were placed in 30% glycerol and brucella broth and frozen at -20°C pending transport to the laboratory. Specimens were homogenized using sterile technique and plated onto selective media. Plates were incubated for 7 days at 37°C under microaerobic conditions. Additional samples were evaluated using *H. pylori* 26 Kd based PCR primers. All animals

In order to determine whether different *Helicobacter* spp. induce similar forms of inflammatory bowel disease (IBD) in mice, we compared disease phenotype produced by infection with either *H. bilis* or *H. hepaticus*. Approximately 10^6 *H. bilis* or *H. hepaticus* organisms, or broth for control, were given by oral gavage three times to 3–4 week old IL-10 -/- and C57Bl/10J female mice. All mice were monitored clinically for weight loss and diarrhea and evaluated histologically at 3, 7, and 12 weeks post-infection (PI). IL-10 -/- mice developed diarrhea after infection with either *H. hepaticus* or *H. bilis*. 1.5 and 3 weeks PI, respectively. *Helicobacters* spp.-infected C57Bl/10J mice and brothat-infected controls did not develop diarrhea at any point in this 12-week study. *H. bilis*-infected IL-10 -/- mice showed weight loss, while *H. hepaticus* infected IL-10 -/- mice had growth curves similar to controls. Both *H. bilis*- and *H. hepaticus*-induced IBD in the IL-10 deficient mice was characterized by proliferative typhlicolitis and proctitis and increased MHC Class II expression in epithelial cells of the proximal and distal colon. However, *H. bilis*-infected IL-10 -/- mice showed more severe inflammation in the middle and distal colon than those mice infected with *H. hepaticus*. Transmural inflammation, crypt abscesses and mucosal erosions were frequently observed in *H. bilis*-infected IL-10 -/- mice at all time points but were infrequently found in *H. hepaticus* infected IL-10 -/- mice. No IBD lesions were found in *H. bilis*- or *H. hepaticus*-infected C57Bl/10J or broth-inoculated control mice. RT-PCR performed on colonic tissue RNA from *H. bilis*-infected IL-10 -/- mice showed elevations in interferon-γ. We conclude that (1) the IBD phenotype differs between IL-10 -/- mice infected with *H. bilis* or *H. hepaticus* and (2) a cytokine deficiency plays an important role in initiating a microbial-induced IBD.

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were negative by culture for *H. pylori*. Seven dogs and two cats were positive by PCR; this positive reaction is assumed to be cross reactive with other gastric helicobacters. This study and others have failed to identify *H. pylori* as a resident of the gastric microflora of pet dogs and cats. Other gastric helicobacters do infect the stomachs of dogs (*H. felis*, *H. bizzozeroni*, *H. bilis*, *H. salomonis*, and Flexispira rappini) and cats (*H. felis*, *H. heilmannii*), but their association with clinical disease is still under active study.

**PS10 The Detection of PCR Inhibitors When Evaluating Test Articles for the Presence Helicobacter DNA by PCR**

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PCR can be a sensitive and specific tool for the detection of infectious agent nucleic acid. The sensitivity of PCR is infamous for contributing to false positive results, which occur when test articles or reagents are contaminated by a target sequence or when non-specific PCR products are produced. False-negative results can occur for the following reasons: The number of target sequence copies is below the limit of detection for a PCR assay; reagents are not properly tested prior use; extraction procedures fail to successfully recover nucleic acid; or nucleic acid contains PCR inhibitors. Fecal pellets and cecum/colon tissues are common test articles used for the detection of Helicobacter by PCR. While performing routine PCR testing for Helicobacter, our laboratory observed that data obtained for paired fecal pellets and cecum/colon tissue samples was not consistent. Further investigation revealed that when isolated DNA was diluted, many samples that were previously negative became positive and the correlation between the two test article types improved. We suspected that PCR inhibitors were present in the isolated DNA. Our laboratory investigated three commercial DNA isolation kits to determine their ability to remove PCR inhibitors from DNA isolated from the mouse fecal pellets. Fecal pellets were collected from 10 mice originating from a *H. bilis*-positive barrier room and 4 mice originating from a *Helicobacter*-negative barrier room. Kit “A” was a “salting out” method. Kit “B” was a column isolation method. Kit “C” was a new method specifically designed to remove PCR inhibitors from stool samples. Only DNA isolated by Kit “B” was inhibitory to a *H. bilis* PCR without being diluted; 0/10 amplified. Amplification was detected in most samples for all three kits at a 10\(^{-5}\) dilution. An *H. bilis* DNA spike (approximately 10\(^6\) genome copies) was used to test dilutions of DNA samples that were isolated from fecal pellets, cecum/colon, and liver samples from 4 C3H mice originating from an *H. bilis* positive barrier room. DNA that did not amplify by a *H. bilis*-specific assay also did not amplify when the reaction was spiked. Diluting these samples also diluted PCR inhibitors and permitted target sequence detection. Our studies suggest that it is important to use a spike control to demonstrate that isolated DNA does not contain factors that may inhibit PCR assays used for the detection of Helicobacter.

**PS11 Antibiotic-associated Clostridium difficile Colitis in Mongolian Gerbils (Meriones unguiculatus) Treated with Amoxicillin/ Metronidazole/Bismuth Wafers**

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The Mongolian gerbil (Meriones unguiculatus) has become an important model for the study of Helicobacter pylori-associated gastritis, peptic ulcer disease, and gastric adenocarcinoma. We have recently found that some commercially available gerbils harbor naturally occurring *H. bilis* infections. We utilized an existing protocol for the elimination of *H. hepatis* infection in mice to attempt eradication of *H. bilis* in gerbils. This protocol involved the use of commercially available, nutritionally balanced triple therapy wafers containing amoxicillin (3 mg/tablet), metronidazole (0.09 mg/tablet), and bismuth (0.185 mg/tablet) administered as a sole food source for fourteen days. For a preliminary study, five male Mongolian gerbils were fed the wafers. On day seven of treatment, two of the five animals were found dead. Necropsy and histopathology revealed a distended cecum and colon and moderate typhlitis and colitis suggestive of antibiotic-associated *C. difficile* colitis. The diagnosis was confirmed by positive ELISA for *C. difficile* toxins A and B performed on colonic tissue from the two animals. Additionally, anaerobic culture of cecal contents resulted in growth of *C. difficile* from one of the animals. Antibiotic-associated colitis, while common in hamsters, has not, to our knowledge, been reported in the gerbil. Susceptibility of gerbils may vary depending on the animal source and the route or dose of antibiotics administered. Successful elimination of naturally-occurring Helicobacter infections in gerbils may require the identification of a *C. difficile*-free source for gerbils or alternate techniques such as Caeorian eradication and cross-fostering onto mice.

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**PS12 Smooth Muscle Cells Support Persistent Rat Virus Infection**

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Rat virus (RV) is a common parvovirus of laboratory rats which can cause disease or disrupt biological responses that rely on cell proliferation. Persistence is an established feature of RV infection that has the potential to amplify RV interference with research. This study characterized the development of persistent RV infection as a critical step toward determining persistence mechanisms. Sixty-eight 6-day-old euthymic (35) and athymic (33) RNU rats were inoculated oronasally with 100TCID\(_{50}\) of the U-Mass strain of RV. Tissues from 4 to 6 rats of each phenotype were assessed by virus isolation, immunohistochemistry and in situ hybridization (ISH) at 4, 6, 8, and 10 days (acute infection), and 2, 4 and 8 weeks (persistent infection) after inoculation. Selected tissues also were analyzed by Southern blot and serum was assayed for RV antibody. Acute infection in both euthymic and athymic rats featured widespread dissemination of virus whereas persistent infection occurred primarily in arteries and arterioles. Acute vascular infection appeared to begin in endothelial cells, but soon encompassed arterial and arteriolar smooth muscle cells (SMC). SMC subsequently emerged as the primary targets during persistent infection. Viral mRNA was detected in SMC indicating that persistent infection included virus replication. However, only half of the SMC containing viral mRNA stained for proliferating cell nuclear antigen (PCNA), a protein expressed in cycling cells. Furthermore, the intensity of PCNA staining varied among RV mRNA-positive cells. The prevalence of virus-positive cells remained moderate to high among athymic rats through 8 weeks. Hybridization signal decreased in euthymic rats by 2 weeks, coincident with seroconversion and perivascularitis. Thus, host immunity reduced but did not eliminate infection. Virus-positive pneumocytes and renal tubular epithelial cells also were detected through week 8 implying that kidney and lung are sites for virus excretion during persistent infection. The re-
results illustrate the importance of vasculotropism in persistent RV infection and show that RV infected SMC contain products involved in RV replication during multiple phases of the cell cycle. They also indicate that the immune response that develops in rats inoculated during infancy is insufficient to prevent persistent infection.

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**PS13 Prevalence of Viral Persistence in Progeny of Dams with Acute or Persistent Rat Virus Infection**

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Rat virus (RV) can disrupt research using rats by causing disease or disturbing biological responses. Infection in breeding colonies is particularly onerous for at least two reasons: 1) RV can interrupt production by causing severe or lethal infection in utero and neonatal infants and 2) asymptomatic infection, a more common condition among infants, can lead to viral persistence. While it is clear that persistent infection can be induced postpartum, the risk of persistent infection from prenatal exposure to virus is not known. To address this question, the prevalence of persistent infection was assessed in the progeny of dams with either acute or persistent RV infection. Acute infection was induced in 8 Sprague-Dawley (SD) dams by inoculating them oronasally with $10^3$ TCID$_{50}$ of the UMass strain of RV on gestation day 9. Preliminary experiments indicated that these conditions induced a high prevalence of persistent infection with low to moderate fetal mortality. Persistent infection was induced by inoculating 16 two-day-old female SD rats oronasally with $10^3$ TCID$_{50}$ of RV-Umass and allowing them to mature. Dams, sires and their progeny were tested for RV infection by one or more of the following methods: virus isolation, in situ hybridization, contact transmission or serological assay. Both groups of dams were bred to virus-antibody-free males, at which time virologically naive dams were seronegative for RV whereas persistently infected dams were uniformly seropositive. Furthermore, at least 9 of 16 persistently infected dams transmitted infection to their breeding partners. The progeny of acutely infected dams were tested for infection at 3, 8 and 16 weeks postpartum. Nine of 12 rats examined at 3 weeks were virus-positive and all had antibodies to RV. No virus was detected in rats examined at 8 weeks (10 rats) or 16 weeks (10 rats) postpartum. However, 7 of 10 additional rats from this group transmitted infection to contact sentinels introduced at 9 weeks and 1 of 10 transmitted infection to a sentinel cage mate introduced at 15 weeks. The progeny of persistently infected dams were tested for virus in utero (25 fetal rats tested at gestation day 19), at birth (37 rats) or at 3 weeks postpartum (4 rats). None of the rats were virus-positive. Maternal immunity lapsed in 10 offspring held for 16 weeks. They were then housed with contact sentinels, but did not transmit infection and were virus-negative at necropsy. Eleven persistently infected dams, 13 to 17 weeks old, were tested for virus after their litters were weaned. All were virus-positive by virus isolation and/or in situ hybridization. These results indicate that the progeny of rats infected with RV during pregnancy are at risk for persistent infection of variable duration, whereas the offspring of rats with previously established infection are protected, probably due to maternal immunity. However, persistently infected, seropositive dams can transmit infection to their breeding partners.

Supported by PHS grant RR11740.

**PS14 Mouse Pathogenic Escherichia coli (MPEC) from Japan is a Citrobacter rodentium Isolate Typical of Those Isolated from Mouse Colonies in the United States**

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*Citrobacter rodentium* (formerly *C. freundii* biotype 4280 and *Citrobacter* genomospecies 9) is the causative agent of transmissible murine colonic hyperplasia, an infectious disease that causes high mortality in suckling mice and asymptomatic carriage in adult mice. It is the only *Citrobacter* species that possesses virulence factors homologous to those of the human pathogens enteropathogenic *E. coli* and enterohemorrhagic *E. coli*. These virulence factors, encoded on the LEE pathogenicity island, are required for the characteristic pathology seen in these three pathogens. Mouse pathogenic *E. coli* (MPEC) was first described in Japan in the 1960’s as the etiologic agent of infectious megaenteron of mice and caused a disease pathology similar to that reported for *C. rodentium*. Here we report the in vitro and in vivo characterization of MPEC and compare it to a collection of 16 *C. rodentium* isolates using a battery of genetic and biochemical approaches. No differences were observed between MPEC and the *C. rodentium* isolates by repetitive element PCR, DNA-DNA hybridization, biochemical analysis, or DNA sequencing of LEE-specific virulence factors. In vivo comparison of MPEC the ATCC type strain for the species showed similar pathologic lesions in infected animals. Our results allow us to conclude that MPEC is a misclassified *C. rodentium* isolate, and suggest that isolates of *C. rodentium* are clonal.

**PS15 Development of a PCR Assay to Detect Mouse Thymic Virus in Biological Materials**

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Mouse Thymic Virus (MTV) is an unclassified herpes virus of mice that causes extensive thymic necrosis and immunosuppression in infected neonatal mice and establishes persistent infections in the salivary glands of adult mice. Cell lines, transplantable tumors, or other biological materials derived from mice have the potential to be contaminated with MTV. Traditionally, mouse antibody production (MAP) test has been used to test biological materials for infectious agents, however, recently polymease chain reaction (PCR) assays have been developed that detect the genome of pathogenic agents in these samples. The purpose of this study was to develop an MTV-specific PCR assay for use in testing biological materials for MTV. Because there are no published MTV genome sequences, a herpesvirus consensus primer PCR assay was used to amplify 725 base pairs of the MTV DNA polymerase gene. This amplicon was sequenced and primers specific for regions of the MTV DNA polymerase gene were designed and evaluated in an MTV-specific PCR assay. The MTV PCR assay amplified a 182 base-pair fragment from DNA extracted from tissues of MTV infected mice, but no fragment was amplified from DNA derived from tissues of uninfected mice. Also, no amplicon was generated when DNA from mouse cytomegalovirus (MCMV), the other known mouse herpesvirus, was tested. The sensitivity of the MTV PCR assay was compared with MAP testing. Biological specimens were inoculated with homogenates of MTV-infected thymuses and DNA was harvested and subjected to the MTV PCR assay. MAP testing was performed...
by inoculating 10 BALB/c mice with homogenates of MTV-infected thymuses and testing for the generation of MTV-specific serum antibody. The results indicate that the MTV PCR assay is comparable to the MAP test for detecting MTV in biological specimens. Thus, testing biological materials for MTV by PCR is a favorable alternative to MAP-based testing because the use of animals is eliminated and results can be obtained more rapidly and inexpensively.

**PS16 Histopathologic Assessment of Experimental Tuberculosis in Three Species of Nonhuman Primates**

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The objectives of this study were to determine differences in susceptibility and disease progression between three species of Cercopithecines to Mycobacterium tuberculosis by histopathologic analysis and intradermal tuberculin testing. Six Macaca mulatta, six Macaca fascicularis, and five Chlorocebus aethiops were intratracheally inoculated with M. tuberculosis (Erdman strain) and euthanized seven months post-infection (PI) or upon demonstration of persistent coughing, inappetance, or weight loss. Monkeys were intradermally tested biweekly for a response to mammalian old tuberculin and purified protein derivative. At necropsy tissues were collected for histopathologic evaluation. African green monkeys exhibited the greatest susceptibility based on acute (days 44–52) development of fulminant clinical signs, whereas rhesus and cynomolgus monkeys were moderately susceptible with later lesion development (days 120–210). African green monkeys exhibited the greatest susceptibility based on acute (days 44–52) development of fulminant clinical signs, whereas rhesus and cynomolgus monkeys were moderately susceptible with later lesion development (days 120–210). African green monkeys had significantly higher numbers of acid fast bacteria and a diffuse necrotizing pneumonia whereas other species had fewer organisms and discrete multifocal pulmonary granulomas. Intradermal skin testing was not predictive of the severity of lesions. We conclude that African green monkeys are more susceptible to M. tuberculosis infection compared to rhesus and cynomolgus monkeys based on their earlier development of fulminant tuberculosis and increased numbers of intralobular organisms.

**PS17 Proposed New Etiology for “Bloody Nose Syndrome” in Macaques: Fulfillment of Koch’s Postulates**

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At the National AALAS meeting in 1998, we presented a new etiology for “Bloody Nose Syndrome” in cynomolgus macaques. In evaluating outbreaks of epistaxis at our institution we determined via biochemical and fatty acid analyses that these were caused by a bacteria of the Moraxella genus but not Moraxella catarrhalis. We then established a protocol to fulfill Koch’s postulates. In this study, we intranasally inoculated six naïve female cynomolgus macaques. The inoculum was originally cultured from a macaque in our facility that exhibited epistaxis during one of the previously described outbreaks. Prior to inoculation, the isolate was characterized by cellular fatty acid analysis and ribosomal RNA sequencing at commercial laboratories. Approximately two weeks after inoculation, all macaques developed epistaxis from both nostrils, at which point Moraxella was recovered in pure culture from the nasal cavities. After isolation, this organism was again sent for cellular fatty acid analysis and ribosomal RNA sequencing. Three of the six macaques cleared this organism from their nasal cavities while three macaques remained positive by nasal culture up to two months after inoculation without further evidence of any clinical signs. We are currently attempting to identify a carrier state that recrudesces in the presence of stress by immunosuppressing infected macaques treated with antibiotics and infected macaques that have cleared the infection naturally. Ribotyping and comparison to three medical and environmental databases of sequenced bacteria definitively placed this isolate in the genus Moraxella but could not be speciated with a high degree of confidence. This isolate was phylogenetically similar to Moraxella lincolnii, M. osloensis, and M. lacunata. In this study, we have successfully fulfilled Koch’s postulates as well as proven that while this organism is in the genus Moraxella, it is not Moraxella catarrhalis which has been traditionally associated with this syndrome in the literature. We must also consider that this isolate could be a newly described species of Moraxella but cannot definitively prove this without complete genomic sequencing of the organism.

**PS18 B Virus Free Macaques: Serological Test Results and the B Virus Status of the Animal**

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The demand by the biomedical research community for B virus-free macaques is growing. The availability of B virus-free macaques has never been better, but still falls short of the current demand. Providing resources depends on the definition of an SPF animal, a B virus-free animal, and a serologically negative animal. Currently, the community defines a B virus-free macaque by the serological status of the breeding colony, since virus identification is a more complex issue. Using a combination of serological tests, both true and false seroreactivity have been noted in animals in the NIH supported B virus-free colonies as late as 10 years after the onset of surveillance. We have conducted retrospective studies using all tested macaques that have exhibited seroconversion in this group. We have classified increasing stages of seroreactivity based on test results. We have identified patterns of seroreactivity that are likely to progress to a positive diagnosis, permitting early identification of an infected macaque.

Supported by NIH grant P40 RR05062.

**PS19 Latex Sensitivity in a Macaca mulatta**

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Hypersensitivity to latex is a hazard among health care workers and laboratory personnel but has not been reported in laboratory animals. Allergic dermatitis associated with a latex sensitivity was diagnosed in an adult Macaca mulatta. It presented over several months as multiple episodes of a pruritic macular-papular rash affecting the axillary and inguinal regions, forearms, thorax, and neck and was accompanied by conjunctivitis. Therapy with topical moisturizers and systemic antihistamines alleviated acute signs but a mild dermatitis and dermal lichenification persisted. Skin scrapings, fungal culture of hair and dander, and fecal parasitology were negative for causative infectious agents.
No corneal defects were detected by ocular fluorescein staining. A thyroid profile (T3/T4) was normal; a CBC revealed cosinophilia (1040). Microscopic changes in two punch biopsies of the skin indicated chronic, nonsuppurative cosinophilic dermatitis. A thin-layer rapid use epicutaneous test (allergen patch test) did not provoke a delayed-type hypersensitivity reaction to 24 known contact allergens. Allergen-specific IgE testing (modified RAST test) using six monkey chew food additives were also negative. However, RAST testing to latex revealed a strong positive result (0.74 KU/L) as compared to negative values (<0.25 KU/L). A skin prick test was performed using a latex supernatant. From 0–24 hours the latex and saline control inoculation sites were similar; at 48 and 72 hours, the latex site was significantly inflamed whereas the control site had only mild inflammation. Two weeks later, the latex site was devoid of erythema but was lichenified and flaky compared to the saline site. Vinyl gloves were substituted for latex gloves for direct contact and all medications were discontinued. A marked decrease in erythema, pruritus, and lichenification was noted. A repeat skin biopsy fourteen weeks after the original biopsy revealed a normal epidermis, however, chronic active nonsuppurative, perifolliculitis persisted. These findings indicate that latex induces allergic dermatitis in nonhuman primates and should be included in the differential diagnosis for atopic dermatitis.

**PS20 Post-transplant Lymphoproliferative Disease-Like Syndrome in Two Cynomolgus Monkeys (Macaca fascicularis)**

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Post-Transplant Lymphoproliferative Disease (PTLD) is a neoplastic condition that occurs in about 1–10% of human patients on immunosuppressive therapy after receipt of a solid-organ transplantation. In this report, we describe a PTLD-like syndrome in two 5-year-old female cynomologus monkeys who received a skeletal myoblast transplant and tacrolimus, an immunosuppressive macrolide at a dose of 1.5 to 2.5 mg/kg/day. The first monkey became lethargic, dyspneic with respiratory rales, and dehydrated five months post-transplantation. A large non-painful abdominal mass was detected by palpation in the left anterior quadrant in the region of the stomach. On postmortem exam, there were multifocal, nodular, white masses involving the stomach, pancreas, spleen and kidneys. The second monkey was found hypothermic, emaciated and dehydrated after 12 months of treatment. The lungs were firm and diffusely white, and white nodular mass was present in the left kidney. The small intestinal wall was thickened, mesenteric lymph nodes were enlarged, and the liver was pale. Microscopically, masses and tissue infiltrates in both monkeys were diagnosed as lymphoma. Taken together, the history of transplantation, immunosuppressive treatment and the presence of lymphoma suggest post-transplant lymphoproliferative disease. To the authors' knowledge, there is no reported case of this syndrome in cynomolgus monkeys.

**PS21 Identification of Differentially Expressed Genes in Pacific Blue Shrimp (Penaeus stylirostris) in Response to Challenge with White Spot Virus (WSV)**

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Intensive shrimp aquaculture is widespread throughout Asia and Latin America. Viruses pose a significant threat to cultured shrimp populations and the viability of the industry. Since 1994, White Spot Virus (WSV) has emerged as the most significant viral pathogen in both Asia and Latin America. “White Spot Disease” results in high morbidity and mortality with infected animals often developing characteristic white foci of epidermal necrosis on the carapace. Using mRNA differential display, we attempted to isolate and identify host genes that may be involved in the pathogenesis of WSV infection in experimentally challenged Pacific Blue Shrimp (*Penaeus stylirostris*). Laboratory-maintained juvenile shrimp were injected with tissue homogenate from confirmed WSV infected animals. DNA and RNA were extracted from the hepatopancreas of moribund animals 30–36 hours post-injection. WSV infection was confirmed and quantitated using virus specific DNA primers and the Perkin-Elmer Gene Amp 5700 sequence detection system with SYBR Green PCR. RNA fingerprinting was performed using cDNA synthesized from DNasel treated total RNA and one base anchored oligoT primers (H-T11M, where M = G, A, or C). PCR amplification was performed using 16 arbitrary primers in combination with H-T11M primers. Amplified cDNA was evaluated by 6% denaturing polyacrylamide gel electrophoresis. Thirty-three potential differentially expressed cDNAs between 52–726 bps were identified by 48 primer combinations and cloned. Currently, 7 of the 35 differentially expressed cDNAs have been sequenced and 2 of the 7 showed similarity to a nuclear-cytoplasmic transport protein and an IL-17 receptor protein in the GenBank database. Differential expression of these cDNAs was reconfirmed by SYBR Green PCR using total RNA from WSV infected and control shrimp tissue. This study demonstrates the utility of using mRNA differential display and quantitative PCR techniques to evaluate host gene expression in response to microbial pathogenesis.

**PS22 Enhancement of Breeding Strategies through Efficient DNA Extraction and Processing of Mouse Tissues**

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Maintenance of a variety of transgenic and congenic mouse strains requires the purification of DNA. We previously used the conventional “salt-out” technique for extraction of DNA from mouse tails for subsequent use in thermal cycling reactions. This labor-intensive process required three sample tubes per mouse and over two hours of research time. Labeling and organizing of multiple microtubes allows for the chance of human error. A mouse that is positive for a desired genotype may be switched with one that is negative. The desired mouse may be removed from the animal line, thus slowing breeding progress by limiting the number of animals available to carry the genotype forward. Additionally, the mouse with the undesired trait may be mated, producing genetically undesirable offspring that increases per
PS23 Effects of Fenbendazole on Ethanol Consumption of Rats

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Fenbendazole is an attractive choice for the treatment of pinworms in groups of animals involved in operant behavior studies. Laboratory animal facilities across the United States are often tormented with concerns about how to effectively and efficiently treat pinworm infestation in rodent colonies without adversely affecting ongoing research projects. Rodent infection with *Syphacia obvelata, S. muris or Aspicularis tetraptera*, although relatively nonpathogenic, may cause serious clinical illnesses such as diarrhea, impaction, dehydration or decreased growth rate. Concerns exist about the effects of these illnesses on research. However, concerns about the effect of fenbendazole treatment on research also exist, leading to investigators who may be reluctant to treat. This study was designed to address that reluctance and statistically determine the effect of fenbendazole treatment on rats involved in self-administering ethanol. A colony of approximately 90 Long-Evans rats, involved in a study to determine the role of the mesolimbic dopamine system on ethanol-consumption regulation, were found to be positive for *Syphacia obvelata* during routine health monitoring procedures. The investigator agreed to treat a small, randomly selected group of the colony to determine the behavioral effects of fenbendazole on rats that self-administer alcohol. Body weight and alcohol consumption were monitored in 8 rats previously operant-trained to lever press for ethanol. In this study the control and treatment group consist of the same set of animals. The eight rats were cellophane tape-tested prior to treatment and found positive for pinworms. Body weight and lever response control data were collected and recorded while the rats were fed a standard diet of rat chow. After a two week period, the same group of rats was then fed fenbendazole-treated feed at 150 ppm for a targeted dose of 8.0–12.0 mg/kg/day for one week; followed by a diet of rat chow for one week; and then one additional week of fenbendazole-treated diet. The rats were tape-tested two weeks after the end of the treatment and no ova were observed. The mean pre-treatment body weight was 484.9 grams and ethanol consumption was 0.634 g of 10% ethanol/kg BW. The mean body weight after treatment with fenbendazole-medicated feed was 500.2 grams and ethanol consumption was 0.715 g of 10% ethanol/kg BW. We found that animals treated with fenbendazole-medicated feed did not significantly differ from non-treated animals in body weight or alcohol consumption. At the conclusion of this study, animals were not killed, and therefore, necropsies were not performed. However, post-treatment tape testing did not reveal pinworm ova. These data suggest fenbendazole is an attractive choice for the treatment of pinworms in groups of animals involved in operant behaviors because the treatment is effective and does not significantly affect ethanol-consumption behavior. As an antiparasitic, fenbendazole offers a wide safety margin, ease of administration and excellent ovicidal activity.

PS24 Effects of Fenbendazole on Ethanol Consumption of Rats

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PS25 The Challenges of Eliminating Mouse Hepatitis Virus (MHV) by Quarantine and Stop Breeding

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An endemic MHV infection was confined to two rooms of a 13,500ft² animal facility for over twenty years. The two rooms housed a breeding colony containing several mouse strains including, in recent years, some transgenic animals. When the infection began to spread within the facility, a decision was made to eradicate the virus. Mice in this facility were historically free of other pathogens except for an occasional outbreak of pinworms or MPV. Several methods for eradication were considered including depopulation, embryo transfer, natural delivery with cross-fostering, cesarean rederivation, and stop breeding. Project cost, irreplaceable strains of mice, and the presence of several long-term aging studies all influenced the formulation of a managerial plan. The initial plan for this facility was to stop breeding for six weeks. At the end of the six-week quarantine period, a clinically ill mouse was determined to be MHV positive. A second six-week stop breeding period was initiated, and included exposure of all mice to MHV infected bedding. The successful eradication of MHV was achieved by gaining investigator compliance, the use of sentinel mice, proper sample collection, appropriate use of diagnostic testing methods (ELISA and PCR), and the identification and removal of a persistently infected group of mice.
Maternal administration of perphenazine was used to decrease the incidence of neonatal mortality in a group of DBA/2 IL12+/- mice. This strain has a high incidence of neonatal death due to cannibalism and maternal abandonment. Perphenazine was administered in the drinking water (4mg/kg) for one week beginning on the day of parturition. Of the ten litters included in this trial, six dams received perphenazine in the drinking water and four did not. The seven-day neonatal survival rate was 81% for litters in the treatment group compared to 15% in the untreated controls. Perphenazine is a phenothiazine-class tranquilizer that has been used in humans as an anti-psychotic and anti-emetic with minimal sedative properties. It has been used to induce lactation in galactic mares, and to improve maternal instincts and promote fostering in dogs and ewes. Perphenazine is known to enhance prolactin secretion. Increased prolactin may interact with estrogen and progesterone to induce maternal behavior. Increased prolactin may also lead to lowered levels of dopamine that effect behavioral changes in the dam. It appears from this preliminary data that perphenazine may be useful in increasing neonatal survivability in mice that have a high incidence of cannibalism and maternal abandonment.

**PS27 Causes of Water Bottle Leakage in Mouse Cages**

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Due to a perception by several investigators that our facility had a large number of leaky water bottles, we began an intensive screening process to determine the most common causes of this problem. Technicians were instructed to give all malfunctioning water bottles to the facility QA technician for inspection. These bottles were systematically checked, the cause of leak determined and logged, and any faulty equipment was discarded. The percentage of total bottles was formulated for both barrier and nonbarrier housing areas by examining census data, caging densities, and cage changes occurring each month. The number of bottles used/month was divided by the number of leaky bottles during that period for an overall frequency of leaks/month. The percentage of leaky bottles during a six-month period was .093%. This ranged from a high of .201%/month to a low of .013%/month and a significant downward trend was noted during this time period. The reasons for leakage in the 45 bottles examined were divided into seven categories: very small crack or hole (26.7%), deformed bottle neck (24.4%), bedding in tube/bottle (22.2%), undetermined (13.3%), minute fissure in rubber stopper (8.9%), sticky ball bearing (2.2%), or too much bedding in cage (2.2%). The defects in the bottle and stopper were uniformly very minor and accounted for 60% of all leaks during the six-month period. Removal of these otherwise normal appearing parts from general use was likely a major contributor to the decrease in incidence noted over the reporting period. It was also noted that problems with bedding in the sipper tube were correlated with certain strains of mice, and that an increase in the leakage rate occurred subsequent to prolonged heat exposure during a faulty autoclave run. Continued surveillance and discarding of defective parts, and provision of more enrichment to strains that tend to push bedding into sipper tubes were policies instituted on the basis of these findings. Absorbent paper bedding was offered to investigators as an alternative to hardwood chips for cages of valuable breeding animals to better guard the health of these animals.

**PS28 Preference of Single Housed Rats for a Solid-Bottom or Wire-Bottom Stainless Steel Cage Floor**

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Extensive debate persists over the effects of cage design and material on rodent health and well-being. We modified a stainless steel multiple-animal wire-bottom rat cage by covering one-half of the floor surface with a solid plate of stainless steel. The stainless steel plate was attached to a strain gauge and measured the amount of time the rat spent on the plate or on the wire-bottom side of the cage. Food and water access was equally provided on both sides of the modified cage. The preference for floor type by six male and six female single-housed Sprague-Dawley rats was quantified during a four-hour period in the day and a four-hour period in the night for each 24-hour interval during 30 consecutive days. Flooring preference was positively correlated to the time of the day. At night, rats significantly (P < 0.05) preferred the wire-bottom side of the cage. The measure of the total time spent on each floor type demonstrated an overall preference to be on the wire-bottom floor (P < 0.05). Study results may assist in the selection of housing conditions for rodents.
PS31 Pathogenesis of Avian (H5N1) Influenza Viruses in Ferrets

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Between May and December 1997, an outbreak of avian influenza A (H5N1) virus in Hong Kong caused eighteen human cases of respiratory illness with six fatalities. The viruses isolated from humans had molecular and biologic features characteristic of highly pathogenic avian influenza viruses. Since avian influenza viruses were previously not recognized to cause respiratory disease in humans, it was important to understand the pathogenesis of these viruses in mammalian species. Ferrets are naturally susceptible to influenza A and B viruses and have been widely used to better understand influenza virus pathogenicity and immunity. We used the ferret model to compare the pathogenicity of two H5N1 viruses, A/Hong Kong/483/97 (HK/483), a virus isolated from a fatal case, and A/Hong Kong/486/97, a virus isolated from a case with mild disease. Ferrets were housed under BSL3+ conditions and were infected with HK/483 (n = 14), HK/486 (n = 19) or mock infected (n = 6). Clinical signs, temperatures, and weights were assessed daily. Nasal washes, fecal swabs, and blood samples were collected on odd-numbered days. Ferrets were euthanized on days 1, 3, 5, and 14. Histologic and immunohistochemical analyses were performed on tissues from the major organs and nasal turbinates. Viral titers in these tissues were also determined in eggs. All ferrets became febrile and lethargic, lost weight, and developed nasal discharge and diarrhea. Ferrets infected with HK/486 developed neurologic signs, and one ferret died. Viral titers in nasal washes peaked early in the infection, and were undetectable by day 11. Both viruses were isolated from multiple tissues, including the nasal turbinates, lungs, brain, liver, spleen, intestines, and heart. The pathogenesis of these viruses in ferrets shared characteristics seen with infections in humans, chickens, and mice; however, there were some significant differences between ferret and mouse models. These data suggest that the ferret is a good mammalian model for further studies on pathogenesis of and immunity to avian H5N1 influenza viruses.

These studies were performed following review and approval by the Centers for Disease Control Animal Care and Use Committee.
Venous thromboembolism is a national health problem, occurring at a constant rate over the past 20 years, with an annual incidence over 250,000 cases. Reliable animal models are essential to improve our understanding of the basic pathophysiology of venous thrombosis and coagulation biology, and to direct future therapeutic options. The olive baboon (Papio anubis) is an ideal model for studies involving balloon catheter occlusion of the caudal vena cava and right iliac vein. This model is useful in evaluating the pathophysiology of venous thrombosis due to the similar vasculature and coagulation pathways between baboons and humans. In this model, thrombus forms in the caudal vena cava in 70% of cases and at the balloon sites and right iliac vein in 75%–100% of cases. The rat (Rattus norvegicus) is also a good model of stasis induced venous thrombosis, created by ligating the vena cava just caudal to the renal veins. Consistent thrombus formation occurs in 90% of cases done by this method. Both animal models are designed to evaluate venous thrombosis over varying time periods. Thrombus formation and resolution can be evaluated by ultrasound, magnetic resonance, and contrast imaging. These models facilitate the evaluation of hematologic parameters, coagulation functions, histopathology, and the regulatory role that chemokines, cytokines, and adhesion molecules play in the development of venous thrombosis.

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**PS34 Zebrafish (Danio rerio): A Model for Studying the Genetic Basis of Disease**

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Zebrafish are one of the most utilized aquatic species for developmental and genetic studies. Currently, there are no reliable viral disease models that employ this organism. To elucidate the specific roles that genes play in piscine viral pathogenesis, we developed an infectious disease model for zebrafish utilizing spring virema of carp virus (SVCV). After spawning a wild type strain of zebrafish, 160 were reared to adults and were moved into an aquatic biohazard level-3 facility. Replicate groups of 10 fish were exposed, via immersion, to varying concentrations of SVCV, which had been grown and titered in epithelioma papulosum cyprini cells. Clinical signs became evident approximately seven days after viral exposure. In zebrafish, SVCV produced anorexia, listlessness, multifocal epidermal petechial hemorrhages and death, similar to that observed in naïve cyprinid species in Europe and Asia. Viral isolation and PCR assay of tissue from infected fish revealed high levels of virus. Histopathologic lesions include diffuse branchits, multifocal hepatic necrosis, and melanomacrophage proliferation in the gills, liver, and kidneys.

The future use of this disease model in newly developed stocks of mutant zebrafish will be of assistance in studying the genetic basis of viral pathogenesis and potential genetic differences.

Supported by NIH grant T32 RR07019.

**PS-35 Use of Transdermal Fentanyl Patches in Rabbits for Postoperative Analgesia**

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A vital component of veterinary medical care is prevention or alleviation of pain associated with surgical procedures. Ensuring that analgesia is administered consistently and at blood concentrations sufficient to provide an adequate level of comfort can be difficult to achieve. Long-acting opioids such as buprenorphine have gained widespread popularity; however, buprenorphine is a partial agonist and its action at the kappa (k) receptor reportedly causes a dysphoric effect in humans. Fentanyl is a potent opioid analgesic, with significant action at the mu (μ) receptor associated with euphoria, analgesia, and sedation. Due to fentanyl’s short acting duration, it was not used for pain management until the development of a transdermal delivery system (Duragesic®, Janssen Pharmaceutica). Transdermal fentanyl patches have been found to be effective in dogs, cats, and goats. This study was undertaken to evaluate the pharmacokinetics of absorption and physiologic effects of transdermal fentanyl in rabbits. Rabbits were randomly assigned to treatment or control groups (n = 4 per group). For treatment group rabbits, a 25 µg/hr transdermal fentanyl patch was applied per manufacturer’s instructions. Blood samples were collected and the following parameters assessed at time points (t) of 0, 12, 24, 36, 48, 60, 72, 84, and 96 hours: body temperature, heart rate, respiratory rate, appetite, fecal and urine output, and level of alertness. Body weight was measured at the beginning and end of the experiment. Plasma was extracted and kept frozen at -20°C. Fentanyl levels were assayed using a radioimmunoassay. Two rabbits in each group had skin biopsies taken after patch removal (t = 72) for histopathology. The mean plasma fentanyl level from 4 rabbits with a 25 µg/hr transdermal fentanyl patch was 0.78 ng/ml at 24 hrs. The mean area under the curve concentration for fentanyl versus control rabbits was 63.2 and 9.51, respectively. Using Wilcoxon rank-sum test, this was statistically significant with P = 0.029. There was a wide variability between individuals (as seen with other animal studies) with peak levels of 1.8–2.2 ng/ml in 2 of the 4 rabbits. After patch removal at 72 hours, levels rapidly declined to baseline by 96 hrs. No significant changes (using Wilcoxon rank-sum test) were seen in heart rate, respiratory rate, body temperature, and behavior. Weight loss was noted in the fentanyl-treated group, but was not statistically significant. Histopathology showed mild superficial and perivascular dermatitis in both rabbits, most likely a reaction to the hair clipping. The patch did not seem to elicit any additional pathologic skin changes. One factor that may impact fentanyl absorption is rate of hair regrowth. In a separate group of 4 rabbits that received fentanyl patches, initial clipping had been performed by a reaction to the hair clipping. The patch did not seem to elicit any additional pathologic skin changes. One factor that may impact fentanyl absorption is rate of hair regrowth. In a separate group of 4 rabbits that received fentanyl patches, initial clipping had been performed by a reaction to the hair clipping. The patch did not seem to elicit any additional pathologic skin changes. One factor that may impact fentanyl absorption is rate of hair regrowth. In a separate group of 4 rabbits that received fentanyl patches, initial clipping had been performed by a reaction to the hair clipping. The patch did not seem to elicit any additional pathologic skin changes. One factor that may impact fentanyl absorption is rate of hair regrowth. In a separate group of 4 rabbits that received fentanyl patches, initial clipping had been performed by a reaction to the hair clipping. 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Dual energy X-ray absorptiometry (DXA) is a relatively new technique that has clinical and research applications for the non-invasive evaluation of bone mineral density and total body composition. DXA scanning was used by our laboratory to accurately measure changes in feline bone mineral density, fat and lean muscle mass composition. Whole body or regional analysis can be performed. Normative data was generated for a group of 20 adult female domestic shorthair cats that were analyzed by DXA scanning technique. The cats weighed 3.5 to 6.0 kg and ranged in age from 3 to 7 years. The average bone mineral density (whole body) was 0.614 ± 0.060 g/cm², with a range of 0.378 to 0.650 g/cm². The average fat and lean muscle mass (whole body) were 14.8% and 85.2%, respectively. Total body fat composition ranged from 5.3 to 29.0%; total body lean muscle mass ranged from 71.0 to 94.7%. Advantages of DXA scanning include rapid scan time, repeatable measurements over time, low radiation exposure, ease of operation, and a relatively low equipment cost.

The manner in which bone cells sense physical forces and subsequently transduce them into utilizable biological signals is poorly understood. The question is particularly important however, as it is known that mechanical stimulation is essential for maintenance of a structurally adequate skeleton. Deterioration of this perception/response mechanism with advancing age may play an etiologic role in age-related bone loss and fragility fractures. According to the National Osteoporosis Foundation there are 1.5 million osteoporosis-related fractures each year in the United States. In fact, 1 in 2 women over the age of 50 will suffer a fragility fracture in her lifetime. The total costs of osteoporosis including hospitalization and long-term domiciliary care are estimated at $14–20 billion and will only continue to increase as society’s longevity increases. Due to the inherent difficulties encountered during in vivo evaluations of the cellular, molecular, and mechanical behavior of bone, the majority of research has been conducted using in vitro experiments. However, there has been tremendous inter-laboratory variability in experimental results. This lack of consistent reproducibility may be related to the absence of several essential factors experienced by bone cells in vivo including an appropriate osteoprogenitor cell population, blood supply, and mechanical strain environment. We have designed a canine model in which controlled mechanical forces are applied to trabecular bone growing within a large volume in vivo tibial bone chamber. Bone tissue grows into the chamber through large infiltration portals via a well-synchronized intramembranous process, resulting in a microenvironment characteristic of “normal” trabecular bone in vivo. A unique feature of the bone chamber design is that it can be activated to apply a controlled compressive force on the tissue growing within the chamber. The loading mechanism works by the application of hydraulic fluid pressure against a piston, which then applies direct mechanical force to the tissue in the chamber. This tissue can then be biopsied, after which the animal is allowed normal cage activity while a second volume of bone is allowed to infiltrate the chamber. The loading/biopsy protocol can be repeated several times within an individual animal, which reduces the total number of animals required for a given project and minimizes experimental variability. Two other features of the model include the ability to “load” animals on a daily basis and to manipulate specific components of the bone cell transduction process prior to the application of a load stimulus via subcutaneous teflon lines and access ports. The dogs are fully conscious during loading and minimal restraint is required. Most importantly, the implants have been maintained for up to one year without the complication of infection, due to aseptic placement and adequate veterinary care.

Water is the most abundant component of the body, accounting for up to 60% of its weight. Total body water (TBW) is divided into intracellular fluid (ICF) and extracellular fluid (ECF) compartments. In healthy individuals, these compartments are tightly regulated. However, in pathologic conditions such as cardiovascular disease, renal disease, obesity, immunodeficiency, sepsis, and trauma, alterations in body and fluid composition take place due to dysregulation between fluid compartments. For these reasons, monitoring fluid changes and body composition is a medical concern. Multi-frequency bioelectrical impedance analysis (MFBIA) is a methodology for determining fluid distribution and body composition. Very little research has been performed to assess the utility of this technology in the nonhuman primate. Accurate determinations of body fluid compartments typically rely on invasive, time-consuming, expensive dilution methods. We performed a study to evaluate MFBIA as a method to determine fluid distribution and body composition in the nonhuman primate. In the same subjects, results were compared with estimates obtained with standard dilution methods and dual energy x-ray absorptiometry (DEXA). A total of fifteen adult male and female rhesus monkeys weighing 3 to 20 kg were evaluated. Impedance measurements were collected using a Xitron 4200 analyzer with frequency ranges of 5kHz to 1MHz. Associations between dilution, DEXA and impedance measurements will be determined. Preliminary data suggest that MFBIA is a rapid and reliable noninvasive method of estimating fluid distribution and body composition in nonhuman primates.

The Brown Norway (BN) inbred strain of rat is often used for research into allergen-induced airway disease despite the occurrence of a strain-related pneumonia which has been briefly reported, but has not been well documented. We describe the morphology of eosinophilic granulomatous pneumonia in the BN rat, provide extensive evidence that the lesion is not infectious in origin, and discuss the microscopic differentiation of this lesion from the novel infectious disease, Rat Respiratory Virus (RRV). During 1998 and 1999, routine screenings of lungs

PS36 Dual Energy X-Ray Absorptiometry of the Domestic Cat for Bone Densitometry and Whole Body Composition Measurements

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PS37 A Novel Canine Hydraulic Bone Chamber Implant Model for Investigations of Bone Cell Signaling and Orthopedic Disease

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The manner in which bone cells sense physical forces and subsequently transduce them into utilizable biological signals is poorly understood. The question is particularly important however, as it is known that mechanical stimulation is essential for maintenance of a structurally adequate skeleton. Deterioration of this perception/response mechanism with advancing age may play an etiologic role in age-related bone loss and fragility fractures. According to the National Osteoporosis Foundation there are 1.5 million osteoporosis-related fractures each year in the United States. In fact, 1 in 2 women over the age of 50 will suffer a fragility fracture in her lifetime. The total costs of osteoporosis including hospitalization and long-term domiciliary care are estimated at $14–20 billion and will only continue to increase as society’s longevity increases. Due to the inherent difficulties encountered during in vivo evaluations of the cellular, molecular, and mechanical behavior of bone, the majority of research has been conducted using in vitro experiments. However, there has been tremendous inter-laboratory variability in experimental results. This lack of consistent reproducibility may be related to the absence of several essential factors experienced by bone cells in vivo including an appropriate osteoprogenitor cell population, blood supply, and mechanical strain environment. We have designed a canine model in which controlled mechanical forces are applied to trabecular bone growing within a large volume in vivo tibial bone chamber. Bone tissue grows into the chamber through large infiltration portals via a well-synchronized intramembranous process, resulting in a microenvironment characteristic of “normal” trabecular bone in vivo. A unique feature of the bone chamber design is that it can be activated to apply a controlled compressive force on the tissue growing within the chamber. The loading mechanism works by the application of hydraulic fluid pressure against a piston, which then applies direct mechanical force to the tissue in the chamber. This tissue can then be biopsied, after which the animal is allowed normal cage activity while a second volume of bone is allowed to infiltrate the chamber. The loading/biopsy protocol can be repeated several times within an individual animal, which reduces the total number of animals required for a given project and minimizes experimental variability. Two other features of the model include the ability to “load” animals on a daily basis and to manipulate specific components of the bone cell transduction process prior to the application of a load stimulus via subcutaneous teflon lines and access ports. The dogs are fully conscious during loading and minimal restraint is required. Most importantly, the implants have been maintained for up to one year without the complication of infection, due to aseptic placement and adequate veterinary care.

PS38 Evaluation of Multi-Frequency Bioelectrical Impedance Analysis in Nonhuman Primates

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PS39 Eosinophilic Granulomatous Pneumonia: A Strain-Related Lesion of High Prevalence in the Brown Norway Rat

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The Brown Norway (BN) inbred strain of rat is often used for research into allergen-induced airway disease despite the occurrence of a strain-related pneumonia which has been briefly reported, but has not been well documented. We describe the morphology of eosinophilic granulomatous pneumonia in the BN rat, provide extensive evidence that the lesion is not infectious in origin, and discuss the microscopic differentiation of this lesion from the novel infectious disease, Rat Respiratory Virus (RRV). During 1998 and 1999, routine screenings of lungs

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of BN rats at the Charles River Laboratories, Inc., Diagnostic Laboratory sampled 10 separate VAF colonies (8 barrier rooms on three continents and 2 isolators of cesarian-derived rats). Eosinophilic granulomatous pneumonia was observed in 101 of 112 BN rats (89%). In rats where age and gender was known, the condition was observed in 27 of 33 rats 6–12 weeks old and 22 of 24 rats 8–10 months old. Macroscopically, lesions appear as multifocal tan, gray, or red, 1–3 mm foci scattered throughout the lungs. Microscopically, lesions consist of interstitial and peribronchiolar aggregates of macrophages with numerous eosinophils and, often, numerous multinucleated giant cells of both Langhans and foreign-body types. Lymphocytes are occasionally present, as are neutrophils, but neither is a major component of the pneumonia. Special stains, including Warthin-Starry, Grocott’s methenamine silver, acid-fast, and Brown and Brenn, were negative for organisms. Serologic and microbiologic testing was uniformly negative for all known rodent pathogens. Other rat strains housed in the same barrier room facilities and isolators (gnotobiotic foster mothers) as affected BN rats were invariably free of similar pulmonary lesions, supporting a non-infectious etiology for this finding. Although generally invariably free of pulmonary lesions, supporting a non-infectious etiology for this finding. Although generally invariably free of similar pulmonary lesions, supporting a non-

The mdx mouse, a model of Duchenne’s muscular dystrophy, contains a point mutation in the dystrophin gene, which results in a stop codon and premature termination of translation. In vivo studies have demonstrated that suppression of premature stop codons can be mediated by aminoglycosides. Aminoglycosides cause a misreading of the RNA code resulting in the insertion of substitute amino acids allowing read through of stop codons. We reasoned that in vivo treatment of a systemic genetic disease resulting from a stop mutation might be ameliorated by aminoglycoside therapy and decided to test this hypothesis in the mdx mouse. Using allometric scaling, male mdx mice were treated with 100%, 200% and 400% of the calculated gentamicin sulfate dose subcutaneously once daily for 14 days. Results assessed the ability of gentamicin to restore dystrophin production as measured by both dystrophin-associated glycoprotein complexes and tetanic force generation were used to evaluate function. Serum creatine kinase, muscle histology and dystrophin production were used to evaluate structure. The localization of dystrophin within membrane-bound dystrophin-associated glycoprotein complexes and tetanic force generation were used to evaluate function. Serum creatine kinase was significantly reduced and dystrophin production and its membrane association with sarcoglycan were restored. There was increased protection from damage caused by eccentric contraction. Renal histology, a measure of gentamicin-induced nephrotoxicity, was normal. To our knowledge, this is the first demonstration that aminoglycosides suppress stop codons in vivo and suggests a unique treatment option for systemic diseases caused by point or missense mutations.

PS40 Antibiotic-Induced Expression of Dystrophin in the mdx Mouse Model of Duchenne’s Muscular Dystrophy

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The mdx mouse, a model of Duchenne’s muscular dystrophy, contains a point mutation in the dystrophin gene, which results in a stop codon and premature termination of translation. In vivo studies have demonstrated that suppression of premature stop codons can be mediated by aminoglycosides. Aminoglycosides cause a misreading of the RNA code resulting in the insertion of substitute amino acids allowing read through of stop codons. We reasoned that in vivo treatment of a systemic genetic disease resulting from a stop mutation might be ameliorated by aminoglycoside therapy and decided to test this hypothesis in the mdx mouse. Using allometric scaling, male mdx mice were treated with 100%, 200% and 400% of the calculated gentamicin sulfate dose subcutaneously once daily for 14 days. Results assessed the ability of gentamicin to restore dystrophin production as measured by both structure and function of striated muscle. Serum creatine kinase, muscle histology and dystrophin production were used to evaluate structure. The localization of dystrophin within membrane-bound dystrophin-associated glycoprotein complexes and tetanic force generation were used to evaluate function. Serum creatine kinase was significantly reduced and dystrophin production and its membrane association with sarcoglycan were restored. There was increased protection from damage caused by eccentric contraction. Renal histology, a measure of gentamicin-induced nephrotoxicity, was normal. To our knowledge, this is the first demonstration that aminoglycosides suppress stop codons in vivo and suggests a unique treatment option for systemic diseases caused by point or missense mutations.

P41 Newborn Endothelin Receptor Type B Mutant (Piebald) Mice Have a Higher Resting Anal Sphincter Pressure than Newborn C57/BL6 Mice

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Endothelin receptor type B mutant (Piebald) mice are particularly susceptible to developing toxic megacolon in association with a lack of ganglion cells in their distal colon. Piebald mice have several different genotypes and phenotypes. Piebald recessive (Ednrb<s-l>/Ednrb<s-l>) homozygotes may develop megacolon, but usually have a normal lifespan, whereas piebald lethal (Ednrb<s>/Ednrb<s-l>) mice usually die by two weeks of age. Heterozygotes are more variable in their degree of colonic involvement. In human infants with Hirschprung’s disease, aganglionosis of the distal colon is associated with hypertonicity of the anal sphincter. We hypothesized that newborn piebald mice would have a higher resting anal sphincter pressure than newborn wild-type mice. We also describe a reliable and reproducible technique for measuring the anal sphincter pressure in any age mouse. Ednrb<s-l>/Ednrb<s-l> breeding pairs were purchased and bred in our animal facility. Pregnant time-dated C57/BL6 mice provided control newborns. One-day-old newborn mice (n = 9 for piebald mice, n = 7 for C57/BL6) were evaluated for resting anal sphincter pressure. Under the operating microscope, a 24-gauge open tip epidural catheter was placed into the anus until a deflection was noticed on the polygraph pressure monitor (approximately 3–5 mm). The catheter was left in place until the tracing reached a plateau; the pressure was recorded at this point. The catheter was then removed and the pressure recording was allowed to return to zero prior to the next measurement. Three consecutive measurements were made for each mouse. Mean values for each group were determined and compared using the Student’s t-test. The resting sphincter pressure for newborn C57/BL6 mice ranged from 2.3 to 18.7 mm Hg with a mean of 13.3 ± 6.9 mm Hg. In piebald mice, the resting sphincter pressure ranged from 14.0 to 34.0 mm Hg; the mean was 22.7 ± 7.4 mm Hg. This difference in mean resting sphincter pressure between C57/BL6 and piebald was statistically significant (P < 0.0001). We conclude that newborn piebald mice demonstrate a higher resting anal sphincter pressure than wild-type controls. Furthermore, we describe a reliable and reproducible technique for measuring the resting anal sphincter pressure in newborn mice. This technique may be modified to measure anal sphincter pressure in any animal. The finding that piebald mice, which are susceptible to megacolon, have hypertonicity of their anal sphincter is similar to human infants with aganglionosis of the colon. Therefore, piebald mice may provide a useful animal model for the study of Hirschprung’s disease.

This work was supported by the Benjamin Fisher Endowed Chair in Pediatric Surgery.
PS42 Evaluation of Weight Loss as an Alternative Endpoint to Mortality in a Neonatal Rat Sepsis Model

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Previous studies have used hypothermia, the inability to ambulate, altered sleep patterns or weight loss as predictors of mortality in various animal species; however, little is known about the accuracy of these parameters in neonates. The current study evaluated weight loss as a predictor of mortality in a neonatal rat sepsis model. Three-day-old Wistar rats were injected at 0, 6 and 24 hours with 20% lipid emulsion (0.2 mL, i.p.), then challenged with *Staphylococcus epidermidis* (0.2 mL of 10^8, 10^11 or 10^12 cells/mL ATCC strain 35984, s.c.). Animals were weighed prior to bacterial challenge and at 6, 24, 48, 72, 96 hours. Animals who were alive at the 96-hour time point were considered survivors. Percent weight loss was determined for individual animals by using the last available weight and pre-challenge weight. Neonates challenged with lower numbers of bacteria had higher survival rates (76.9% and 46.7% for the 10^8 and 10^11 bacteria/mL doses, respectively) and longer survival times (time to 50% mortality >96, 50.28, and 37.35 hours for the 10^8 [n = 13], 10^11 [n = 15], and 10^12 [n = 15] bacteria/mL doses, respectively). By using the percent weight loss of the first animal(s) to die, a prediction of survival was made for the remaining animals. There was a significant difference (P < 0.05) in percent weight loss of animals that did not survive when compared to rats that did survive the full observation period. In this bacterial sepsis model, the percent weight loss of the first animal(s) to die predicted the mortality of the remaining animals with an accuracy of 100%, 87.5% and 75% for the 10^8, 10^11 and 10^12 bacterial doses, respectively. Therefore, in this neonatal rat sepsis model, percent weight loss could be used as a predictor of mortality. As such, this predictor may prove useful in pilot studies for chronic sepsis to more quickly identify the bacterial doses that induce a desired level of morbidity, however, this is not a substitute for actual measured endpoints in treatment studies.

PS43 Two Novel Surgical Techniques for the Investigation of Fatty Acid Transport in Rats: Preparation of the Inguinal or Epididymal Fat Pad for Perfusion In Situ

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Transport and utilization of fatty acids (FA’s) by tissues, particularly white adipose tissue (WAT) is important in the study of cancer and cachexia. Previous investigations from this laboratory showed that omega-3 FA’s, in addition to the circadian neurohormone melatonin, inhibited total fatty acid (TFA) uptake and in turn regulated the linoleic acid-dependent growth of hepatoma 7228CCTG in Buffalo rats. Here we detail two novel surgical techniques for study of lipolysis in the inguinal and epididymal fat pads in vivo and during perfusion in situ. The surgical procedures preserve a continuous blood flow to the host tissues. The inguinal fat pad was perfused via the superficial caudal epididymal arterial/venous system; the epididymal fat pad was perfused via the epididymal branch of the internal spermatic arterial/venous system. These two WAT tissues differ significantly in terms of FA uptake and release, and glycerol release (P < 0.001). We measured arteriovenous TFA differences across isolated inguinal and epididymal fat pads in fed (6.47 ± 1.33, n = 82, and 0.92 ± 0.12, n = 12, mg uptake/min/g tissue) and fasted (9.69 ± 3.26, n = 69, and 0.80 ± 0.05, n = 12, mg release/min/g) rat controls, respectively. Venous glycerol release from inguinal and epididymal fat pads were 1.97 ± 0.27 and 0.82 ± 0.05 mg release/min/g tissue in fed and 3.25 ± 0.16 and 1.55 ± 0.05 mg release/min/g tissue in fasted rat controls, respectively. Values for arterial and venous whole blood pH during inguinal and epididymal fat pad perfusions were not significantly different (P > 0.05). Mean arterial and venous pO_2 for all perfusions were 150.0 ± 13.2 and 34.0 ± 7.4 mm Hg, respectively, showing significant uptake (P < 0.001): mean arterial/venous pCO_2 was 31.3 ± 6.6 and 58.7 ± 5.2 mm Hg, respectively, reflecting a significant CO_2 release (P < 0.001). Mean arterial/venous hematocrits were 45.0 ± 1.4 and 46.0 ± 1.2%, respectively, and were unchanged during the course of these perfusions. Venous blood flow rates for inguinal and epididymal fat pad perfusions were 83.3 ± 1.7 and 36.8 ± 1.0 mL/min, respectively. To our knowledge, the surgical techniques described here are the first methods for perfusion of both isolated inguinal and epididymal fat pads in situ in the laboratory rat. These models provide unique opportunities for the investigation of lipid transport, hyperlipidemia, cancer and cachexia in vivo and during perfusion in situ.

PS44 Inflammatory Bowel Disease with Increased Cytokine Expression in NF-kB Deficient Mice

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Severe inflammatory bowel disease (IBD) was observed in *Helicobacter hepaticus*-infected mice lacking genes for Nuclear Factor kappa B (NF-kB). In this study, p50- and p65-deficient mice on a mixed 129 x C57BL/6 were derivered by uterine embryo transfer and maintained as specific pathogen free for known helicobacter species. At age 6–8 weeks, mice of each genotype were infected by gastric lavage with three doses of 2 x 10^7 Helicobacter hepaticus bacteria in broth with glycerol only. At six weeks postinfection, typhlocolitis was significantly more severe in p50-/-p65-/- mice and p65+/-;p50-/- mice than in the infected wild type control mice. At age 20 weeks, p50-/-p65-/- mice were significantly more severe than in p50-/-p65+/-;p50-/- mice, p50-/-p65-/- mice and presumed wild type control mice. The number of foci of inflammation at age 20 weeks was significantly higher in each of the infected mouse strains compared to the infected wild type control mice, suggesting that both p50 and p65 may have a significant role in inhibition of inflammatory gene expression compared to controls using RNase protection assays. Increases in INF-g was significantly more severe in H. hepaticus-infected p50-/-p65-/- mice and p65+/-;p50-/- mice than in the infected wild type control mice. Uprgulation of proinflammatory cytokines was suggested by increased IL-12 expression in cecal and colonic biopsies compared to controls using RNase protection assays. Increases in INF-g suggested a pathogenic Th1 response and increases in IL-1b and IL-6 indicated involvement of lamina propria macrophages. The initial observation of IBD in NF-kB deficient mice was unexpected because NF-kB transcription factors have been implicated as promoters of IBD. This data suggests that p50 and p65 may have a much broader role in inhibition of inflammatory gene expression than previously appreciated.
An eight-year-old female New Zealand White rabbit was reported for multiple small, firm cutaneous nodules over her mandible. This rabbit was used to produce polyclonal antibodies against human monocyte chemotactic protein. Physical examination was unremarkable other than the mandibular masses, which were freely movable upon palpation. Differential diagnoses included abscesses, neoplasms, and granulomas. One of the nodules was ulcerated, and a small amount of caseous material was expressed. A swab of the interior of the nodule was submitted for bacterial culture, and *Actinomyces israelii* was obtained. This lesion was treated with debridement and irrigation with chlorhexidine solution. This wound healed uneventfully, but the nodules remained. Excisional biopsy of one of these lesions was recommended, but the laboratory staff declined due to the age of the animal. Several weeks later another ulcerated wound containing caseous exudate developed on the lateral hock. A bacterial culture of this wound was submitted and results were negative. The ulceration was treated in the same fashion as the mandibular lesion, however this time the rabbit also received systemic oxytetracycline. The lesion improved, but never resolved completely. Three months later the rabbit was reported for lethargy and anorexia. On physical examination, she had diminished body condition and nonpainful buphthalmia. A complete blood count revealed a nonregenerative anemia. Three days of supportive care failed to bring about an improvement in her condition, so the laboratory staff agreed to euthanize the rabbit following a final blood collection. During transport and restraint the rabbit became tachypneic and progressively dyspneic and died. Severe pleural effusion was discovered on gross necropsy. Other gross lesions included generalized subcutaneous edema, multiple large mediastinal masses, a mass in the caudal-dorsal abdomen, two masses on the serosal surface of the stomach, and generalized lymphadenopathy. The lesions, including the cutaneous mandibular nodules, were identified as malignant lymphoma by histopathology. Lymphoma is not uncommon in the rabbit, but it is usually seen in juveniles and young adults. Cutaneous lesions are not commonly associated with the disease in this species.

### PS46 Hematuria in a Four-Year-Old Male Beagle

PA Werner*, DC Morfitt, CL Medina

An eight-year-old female New Zealand White beagle was reported for multiple small, firm cutaneous nodules over her mandible. This rabbit was used to produce polyclonal antibodies against human monocyte chemotactic protein. Physical examination was unremarkable other than the mandibular masses, which were freely movable upon palpation. Differential diagnoses included abscesses, neoplasms, and granulomas. One of the nodules was ulcerated, and a small amount of caseous material was expressed. A swab of the interior of the nodule was submitted for bacterial culture, and *Actinomyces israelii* was obtained. This lesion was treated with debridement and irrigation with chlorhexidine solution. This wound healed uneventfully, but the nodules remained. Excisional biopsy of one of these lesions was recommended, but the laboratory staff declined due to the age of the animal. Several weeks later another ulcerated wound containing caseous exudate developed on the lateral hock. A bacterial culture of this wound was submitted and results were negative. The ulceration was treated in the same fashion as the mandibular lesion, however this time the rabbit also received systemic oxytetracycline. The lesion improved, but never resolved completely. Three months later the rabbit was reported for lethargy and anorexia. On physical examination, she had diminished body condition and nonpainful buphthalmia. A complete blood count revealed a nonregenerative anemia. Three days of supportive care failed to bring about an improvement in her condition, so the laboratory staff agreed to euthanize the rabbit following a final blood collection. During transport and restraint the rabbit became tachypneic and progressively dyspneic and died. Severe pleural effusion was discovered on gross necropsy. Other gross lesions included generalized subcutaneous edema, multiple large mediastinal masses, a mass in the caudal-dorsal abdomen, two masses on the serosal surface of the stomach, and generalized lymphadenopathy. The lesions, including the cutaneous mandibular nodules, were identified as malignant lymphoma by histopathology. Lymphoma is not uncommon in the rabbit, but it is usually seen in juveniles and young adults. Cutaneous lesions are not commonly associated with the disease in this species.

### PS47 Abdominal Masses in an Aged Rhesus Macaque, *Macaca mulatta*

NA Rodriguez*, KD Garcia, TA Hewett, JD Fortman, RM Bunte

In September 1997 an adult (27 years old) female captive-born rhesus macaque was received from another research institution and introduced into the rhesus colony at the University of Illinois at Chicago. All animals undergo quarantine procedures before entering the conditioned colony. The animal had normal blood work (CBC and serum chemistry), five negative TB tests, normal quarantine chest radiographs, a fecal exam that revealed E. coli and Entamoeba histolytica (cysts) and a relatively normal quarantine physical exam (severe arthritis was noted in the stifles). Approximately one year from the initial introduction, October 1998, the animal was observed to have diarrhea. The physical exam revealed normal TPR, moderate weight loss, a large abdominal mass (3–4 cm) at the pelvic inlet, and mucus and blood were noted in the feces. An ultrasound and radiographs were performed. The ultrasound revealed a cystic to hyperechoic structure in the lower abdomen. The radiographs depicted increased soft tissue density in the lower abdomen that displaced the colon dorsally and gas-distended bowel loops. Differential diagnoses were neoplasia, endometriosis, foreign body obstruction and gastrointestinal intussusception. An exploratory laparotomy was performed. A large mass was found encompassing the reproductive organs, with the descending colon and urinary bladder adhered to the mass. An intestinal stricture was also noted in the distal jejunum. Due to the size of the mass and the extensive involvement of vital organs, surgery was not practical and the animal was euthanized. The pelvic mass, the intestinal stricture and representative tissues were submitted for histopathology. A presumptive diagnosis of leiomyoma and intestinal adenocarcinoma was made.

### PS48 Gastric Dilatation in Surveillance Mice

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**Signalment and History:** Three hundred and thirty two sentinel Tac. (SW) FBR mice are placed in the MIT Murine Health Monitoring Program at eight weeks of age. These animals receive dirty bedding weekly from resident colony mice. The surveillance program was designed based on a rotating schedule in which every six months two sentinel mice are removed for necropsy and replaced by two new mice such that there are always four surveillance mice in one cage. The mortality rate of sentinel mice was normally 15% per month. In September 1998 the rates dropped to 5% (5/322; 1.5%) and the second half (36/332; 10.8%) show an increase in the second half of 1998. Dilatation of the stomach was a common pathologi-
cal finding (11/41; 26.8%) during the last six months of 1999. The average age of affected animals was 6 months.

**Clinical Signs:** Lethargy, dehydration, body condition score of 2 or less, hunched posture, abdominal distention and in some cases acute death.

**Diagnostic Tests:** Serology, parasitology, protozoology, and bacteriology as part of the regular surveillance screening. In addition, histopathology. *Clostridium perfringens* toxin assay of gastrointestinal contents, and gastric and colonic bacterial cultures were performed.

**Necropsy and Histopathology:** Grossly, dilated stomachs full of ingesta and pale discoloration of the kidneys. Microscopically, there was diffuse gastric mucosal hyperplasia and/or hyperkeratosis. The cause of dilation of the stomach could not be determined by histopathology. Kidney changes were consistent with chronic nephropathy.

**Conclusion:** The dominant pattern observed during necropsies was emaciation, stomach full of food and in most cases evidence of fasting noticed by a small amount of ingesta in the remainder of the intestinal tract. These findings correlated in time with an increased morbidity and mortality during the second half of 1999. Possible causes include infection, inflammation, degeneration, obstruction, neurologic causes, behavioral causes and husbandry changes.

**PS49 Lethargy and Hypothermia in a Rhesus Macaque**

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A 10-year-old male rhesus macaque was presented for lethargy of less than one day duration. The animal had been positive for antibodies to simian retrovirus, but was in good health up until this time. He was being used for a food choice study, and had had his food ration decreased one week prior to presentation. On physical examination, he was moderately lethargic and hunched down on the bottom of his cage, but still required sedation. On physical examination, he was moderately lethargic and hunched down on the bottom of his cage, but still required sedation. At that time he was hypothermic (90.8°F), about 5% dehydrated, and had weak pulses. Chest sounds were difficult to auscultate, and abdominal palpation was within normal limits. Hypoglycemia was suspected based on the history and clinical signs, and he was given 300 cc warmed LRS and 7 cc 50% dextrose intravenously. He sat up and appeared to be responding quickly to this treatment, and was returned to his cage with a heat lamp. During the next few hours, he again preferred to lay on the bottom of his cage, but was still alert enough that moving him without sedation would be a challenge. Bloodwork submitted prior to treatment subsequently revealed normal blood glucose levels. Other hematological parameters were within normal limits other than a leukocytosis of 21.5 x 10^3 cells/ml and an elevated PCV of 54%. Because of concerns that anesthesia could be detrimental to his condition, it was decided that further diagnostic tests and body temperature evaluation would be performed without sedation. Unfortunately, within minutes of being removed from his cage, he developed generalized tremors and died spontaneously. Gross examination revealed a 720 degree volvulus at the root of the intestinal mesentery. The involved bowel was diffusely hyperemic and distended. The abdominal cavity contained about 20 cc of dark brown fluid and had numerous adhesions and fibrinous strands. This is the first reported case of spontaneous mesenteric volvulus in a nonhuman primate.

**PS50 The Diagnosis of a Subcutaneous Mass with Associated Epidermal Ulceration in a Siberian Hamster (Phodopus sungorous)**

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A one-year-old female Siberian hamster (Phodopus sungorous) was examined when a mass with an associated skin lesion was noted during routine animal husbandry. The mass was firm, white, and approximately 1 cm in diameter. The overlying skin was ulcerated with rounded edges and no associated discharge. The animal was successfully nursing a two-week-old litter and was normal except for the mass and ulceration. When the litter was weaned, the mass was unchanged, and the investigator requested euthanasia. A necropsy was performed. Microscopic evaluation of the mass revealed that it was covered with normal epidermis. There was evidence of local invasion into the subcutis. The dermis had been replaced with discrete, expansive, multilobulated, non-encapsulated tissue composed of nests and cords with a population of large, homogeneous, round cells. The cells were characterized by a large open nucleus with one to two nucleoli and large amounts of deeply basophilic, granular cytoplasm. It was well vascularized with a few mitotic figures. From these observations, it was determined that the mass was consistent with a mast-cell tumor. This is the first reported spontaneous subcutaneous mast cell tumor in a Siberian hamster.

**PS51 Cervical Swelling in a Lamb (Ovis aries)**

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**Signalment and History:** A two-week-old ewe lamb was presented for a swelling in the neck region of over one week’s duration. Tail docking was being done by the elastrator method. The lamb was nursing well.

**Physical examination:** The lamb was bright and alert. The ventral surface of its neck had a nonpainful, 10 x 10 cm paramedial swelling. By palpation, the subcutaneous mass was firm, round to elliptical, and slightly mobile; the overlying skin was normal. Differential diagnoses included abscess, caseous lymphadenopathy, goiter or embryonic defect.

**Diagnosis workup:** An aspirate of the mass, obtained and stained with Gram’s stain, revealed gram-positive cocci and gram-negative rods.

**Surgery:** Intraoperatively, the mass had a slightly lobulated appearance. It was ovoid except for an attached caudal tailpiece. Blunt dissection and vessel ligation were necessary to free the mass from the surrounding adventitia. On the dorsal surface, a large duct or vessel was triple-ligated. After resection of the mass, the lamb’s recovery was uneventful.

**Gross and Histological Results:** The center of the mass contained caseous material and pieces of vegetation; one piece of the foreign body was 10 cm long. Using light microscopy, the primary mass was seen to be a cyst lined by non-keratinized stratified squamous epithelium, similar to oropharyngeal epithelium. Continuity of the cyst lining was disrupted by areas of erosion and ulceration; massive suppuration and necrosis were present in the lumen. The cyst wall had numerous primitive epithelial ducts that merged into areas with normal-appearing thyroid follicles. Granulation tissue encapsulated the cyst. The caudal tail of the mass was composed of thyroid tissue.

**Conclusion:** These findings are consistent with a thyroglossal duct and attached cyst that have been contaminated by a foreign body. Embryologically, the thyroid primordia migrates caudally from the floor of the primitive pharynx to form the thyroid lobes.
PS52 Anemia in a Macaque

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A 13-year-old, 9.3 kg female rhesus macaque was reported for exceptionally heavy menses and partial anorexia of two days’ duration. Prior experimental manipulation included ovariectomy and subcutaneous estrogen implant placement two years earlier. Two weeks prior to the onset of menses, a bone scan had been performed. On initial examination, the animal was found hunched on the bottom of her cage but alert and responsive. Physical examination while sedated revealed pale mucous membranes and an enlarged and firm uterus on rectal palpation. A CBC revealed a microcytic, hypochromic anemia with a hematocrit (HCT) of 8%. A peritoneal tap, survey radiographs, and barium enema were then performed. Except for the enlarged uterus, all results were negative. After further palpation of the uterus, a mucopurulent vaginal discharge was noted. Differential diagnoses for the enlarged uterus included endometriosis, endometritis, and pyometra. Differentials for the anemia were anemia of inflammatory disease, chronic blood loss anemia, and bone marrow suppression secondary to the estrogen implant. The estrogen implant was removed and symptomatic treatment with enrofloxacin and iron dextran was begun. Within two weeks of initial presentation, the hematoctit had increased to 27%. At that time, medications were discontinued. A heavy menses was observed again the next month. The HCT remained at 27% but declined to 20% over the next week, once again with marked microcytosis and hypochromasemia. Despite the declining HCT, the animal remained in a clinically stable condition. Vaginal bleeding was apparent intermittently for the next month. During that time, a retroviral panel, serum iron assay, coagulation profile, and bone marrow tap were performed. Serology was negative for SIV 1, 2, and 5, but positive for STLV. Serum iron was low (22 mg/dl) and total iron binding capacity (TIBC) was elevated (511 mg/dl). The coagulation profile was normal and the bone marrow tap was nondiagnostic. An exploratory laparotomy was performed to evaluate the uterus and identify the cause of the persistent bleeding. A hysterectomy was performed and a 0.5 x 0.5 x 1 cm cystic mass that was adhered to the pelvic peritoneum was removed. The uterine wall was thickened and ulcerated on the mucosal surface, and the uterine lumen contained a large blood clot. On histopathology, the mass was identified as endometrial tissue. The uterus was characterized by significant myometrial hypertrophy with areas of pleomorphism and the mucosal surface had significant inflammation. After surgery, serum iron levels increased to 122 mg/dl and TIBC decreased to 445 mg/dl. The HCT stabilized at 37%. Pathologic findings were consistent with dysfunctional uterine bleeding related to endometriosis. The implantation and later removal of the long-term estrogen implant may have contributed significantly to the clinical presentation of this case.

PS53 Abdominal Mass in a Ferret

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An adult male ferret was reported to the veterinary staff by one of the animal caretakers for being thin and showing aggression. Physical examination revealed an extremely emaciated ferret, weighing only 800 grams, with a large, firm palpable mass in the abdomen. After consulting the investigator it was discovered the animal had lost a significant amount of weight over the past several weeks. The PI commented that the drop in weight was directly correlated with a recent change in diet. Initial diagnostics included CBC, serum chemistries and radiographs. Hematologic results showed a severe anemia (15%) and thrombocytopenia (49.6 x10^9/ul). Chemistry results were normal. Radiographs revealed a large homogeneous mass in the abdomen. The differential diagnosis list included neoplasia, splenomegaly, and possibly foreign body. Further diagnostics included a barium swallow radiographic series to assess gastrointestinal patency. No blockage was seen in the gastrointestinal tract. Due to the ferret’s poor state of health, the ferret was euthanized and a necropsy was performed. The gross findings included a greatly enlarged spleen (16 x 4 cm) and enlarged mesenteric lymph nodes. Histopathological examination revealed splenic tissue which consisted of marked extramedullary hematopoiesis with primarily erythroid and megakaryocytic differentiation. The final diagnosis of splenomegaly was made by ruling out the other differentials and finding characteristic microscopic lesions on histological examination.

Note: We are doing the final stains to determine the exact type of tumor.

PS54 Outbreak of Paralysis in Young Swiss Webster Mice

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An outbreak of paralysis among 16 to 20-week-old CFW Swiss Webster sentinel mice occurred in an SPF facility. Animals were purchased from an approved vendor. Two months after arrival and over a period of four weeks, 6 mice out of a batch of 400 presented with progressive hindlimb paralysis with no other clinical signs. The sick animals were from four different rooms. Complete necropsy was performed in five mice. Moderate enlargement of lymph nodes was seen in all five animals, and four showed moderate splenomegaly. The paravertebral muscles of three of the mice showed swelling and gray discoloration. Serum samples from two mice were sent to a commercial lab for routine screening, and two more samples were sent to the vendor for review by their laboratory. All results were negative for the most common murine pathogens. Histopathology. The most outstanding pathological finding was a massive infiltration of the lumbar muscles with lymphoid cells. This infiltration had virtually replaced all the muscle fibers surrounding the spine, invaded the spinal roots, and the epidural space. In addition, moderate to severe infiltration of small lymphocytes was found in several organs, including but not limited to, liver, lung, kidney, pancreas, and salivary glands. What is your diagnosis? How would you explain this cluster of cases in a four-week period? The serological results ruled out infection with pathogens causing posterior paralysis, e.g. GDVII and Polyoma virus. Histopathology revealed Lymphosarcoma causing compression of the spinal cord. There are two leading features to this outbreak: its unusual epidemiological presentation and the localization of the lesions. Two possible causes of this outbreak are: 1) a cluster of closely-related mice inheriting the same genetic condition. 2) a retroviral infection. Abelson murine leukemia virus (A-MuLV) has been reported to cause paravertebral masses. Murine leukemia viruses can cause suppression of humoral and cellular immunity as well as complicating endpoints in aging studies. Little is known about retroviral status in many commercial colonies and few users re-
port the presence of spontaneous lymphosarcomas. This outbreak points out the possibility of purchasing animals that carry an endogenous retrovirus. It also emphasizes the need of diagnosing and reporting clusters of posterior paralysis or lymphomas in mice to assess the prevalence and significance of retroviral infections in commercial colonies.

**PS55 Head Tilt in a Guinea Pig**

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A 9-month-old male guinea pig was presented to the University of Missouri Veterinary Medical Teaching Hospital with chronic mucopurulent ocular discharge and a corneal ulcer. Despite antibiotic therapy, the eye became swollen, the guinea pig stopped eating and became lethargic. The guinea pig subsequently developed nasal and ocular discharges, a head tilt to the left and ataxia. At necropsy, there was a pale nodular mass in the left caudoventral brain cavity. The lungs were adhered to the thoracic body wall and contained several firm masses. Histopathologically, the brain mass was a meningial abscess with pairs or chains of Gram-positive cocci bacteria. Morphological features of these bacteria were consistent with *Streptococcus pneumoniae*, a frequent pathogen of guinea pigs, and *S. pneumoniae* was cultured from the nasopharynx of this animal. The lung lesions were characterized by marked peribronchiolar alveolar dilation with fibrosis and focal mineralization. Dilated alveoli were lined by hyperplastic epithelium and contained proteinaceous material. No bacteria were found in association with these lesions. *Streptococcus pneumoniae* usually causes fibrinopurulent lesions of serosal surfaces such as pleura, pericardium and peritoneum. This case represents an unusual presentation of this bacterium as it is only rarely associated with abscess formation.

**PS56 Environmental Enrichment for Calves with Artificial Organs**

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Calves are not often mentioned in manuals and publications involving environmental enrichment of laboratory animals. Our facility uses 3–6-month-old Jersey breed calves in both short- (30-day) and long-term (60-plus-day) studies with implanted artificial organs. After implantation of the device, these animals are housed in portable, 4 x 6 x 4 ft (W x D x H) aluminum, tube-gate pens. In order to decrease the chance of a calf chewing power cables, catheters, or other monitoring equipment, their head is tethered to the front of the cage, using standard harnesses. Movement can be further constrained by limiting the tether site to 4–6 in. along a bar on the front panel of the pen. Our primary concern was that limited movement does not allow for exercise or stimulation. While this is not an issue for animals housed for 30 days, long-term animals can become unruly and aggressive. In order to keep an animal entertained for short periods of time we began to utilize toys as focal points for oral satisfaction and to channel aggressive behavior. It has been our experience that virtually any “toy” hung within eyesight of the animal would work. We have used empty gallon milk or water containers, rubber “tug-of-war” dog toys, chew rings, long strips of Velcro and ventilator tubing. Other toys that the animals respond well to are large, hard plastic balls, heavy-duty Kong toys, and hanging ropes or chains. The animals also seem to enjoy playing with their food bowls after or between feedings; therefore we leave the bowls in the pens at all times. Since we have instituted using these toys for the animals, we have not had a single line or cable chewed by the animals. Furthermore, incidents of aggression have decreased. In addition to the toys, our facility utilizes a treadmill to give the calves short periods of exercise up to twice weekly. Our final, and perhaps most important, solution to bovine enrichment is that of physical contact. Most of the animals seem to enjoy a good, vigorous scratch with lengthy attention paid to the areas around and under the harness straps. Personnel are monitoring the animals 24 hours a day, so toy use can be closely monitored; however, the positive affect on the animals and staff is obvious.

**PS57 A Novel Approach to Group-Housing Male Cynomolgus Macaques in a Pharmaceutical Environment**

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The R. W. Johnson Pharmaceutical Research Institute (a Johnson & Johnson Company) is committed to exceeding the minimum requirements for non-human primates as established by the Animal Welfare Act & The Guide.

According to Dr. Viktor Reinhardt, “psychological well-being” can be stated as: “having the option to actively express species-specific behavioral patterns that were formerly inhibited due to a lack of appropriate stimuli” and “experiencing less fear/distrss in the presence of care personnel.

In any environmental enhancement plan, we must include "specific provisions to address the social needs of nonhuman primates of species known to exist in social groups. It has been demonstrated that paired companions spend more than 20% of their active time hugging, grooming and playing with each other. Twenty percent of our primates are maintained in a single-housed environment. Of those single-housed animals, 40% exhibited moderate to marked degrees of self-directed activity; i.e., hairpulling. By contrast, none of the pair or group-housed animals exhibited these behaviors.

Our goal was to provide increased socialization in a group of juvenile cynomolgus male macaques. Through a stepwise process, we transitioned these animals from a single cage environment to pair housing, and finally into a large enrichment unit, where they have been successfully maintained for over one year. We firmly believe that these primates are now more receptive to handling and training, and will therefore be better animal models, as noted by a marked decrease in vocalization and self-directed behavior during pole/collar capture and chair restraint procedures.

1United States Department of Agriculture 1991
2Reinhardt 1990, 1991b
3Britz-Heidbrink, Inc

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Several groups of rhesus macaques with five to seven group members were trained as a whole with positive reinforcement training techniques to 1) go to the front of their enclosure, 2) go to a designated station area, and 3) touch a designated target. Time invested by the trainer to achieve reliable performance by the monkeys was assessed. Five groups (n = 27 monkeys) took on average 390.2 minutes (range = 62.0–999.0 minutes), during a mean of 30 sessions (range = 8–71) to be trained to reliably go to the front of the enclosure. To train 26 monkeys to go to their individual stations, it required on average 386.2 minutes (range = 117–924) during a mean of 30 sessions (range = 12–66). Training the same 26 animals to touch a target while at their station required an additional 241 minutes of training time on average and an additional mean of 16.4 sessions. While some (n = 14) monkeys remained at their stations, others (n = 12) required more concentrated training to learn to stay. To achieve 100% compliance on this task required more effort on the part of the trainer during the sessions, but did not increase the overall training time (mean = 617.2 minutes). While the average amount of time to train nearly all animals in a group to cooperate with the trainer was about 10.4 hours from start to finish [targeting requiring the most training effort (mean = 627 minutes)], there are many potential benefits of such an initial investment. These may include increased accessibility to the animals for activities such as assessing 1) general health, 2) wounding, and 3) infants on mothers. Achieving enhanced accessibility to the group may also present additional training opportunities such as presenting a thigh for injection, or hands, feet, tail, back, or mouth for inspection. In facilities where rhesus are housed in large social groups and separation of individuals for relatively minor procedures is not an option, the initial investment of time in training groups may be beneficial in achieving health and management goals in a more timely, efficient, and less stressful manner.

PS59 Validation Program for Nonhuman Primate Enrichment Devices Ensures Effective Sanitation

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Validation programs help ensure appropriate sanitation of devices utilized in nonhuman primate (NHP) environmental enrichment programs. We designed a validation program to assess the effectiveness of mechanical equipment such as rack washers and autoclaves, or manual procedures and chemical disinfectants, to sanitize a variety of NHP enrichment devices. Standard operating procedures were established for collecting microbiologic samples from each type of sanitized device. Sterile cotton swabs were used to collect samples from 1 in² area(s) on each device. Two selected sites on each device were generally evaluated. The swabs were transferred to RODAC® plates and incubated for 72 hours at 35°C. This method is particularly effective for irregular-shaped devices, which can be difficult to directly RODAC plate. Bacterial growth was monitored daily and the number of colony forming units (CFU) per plate was recorded at 72 hours. Based on APHA guidelines, we defined acceptable bacterial density as less than 15 CFU/plate on three consecutive platings. These procedures helped us identify enrichment devices, such as natural wood logs and plastic balls, that were not effectively sanitized by our standard techniques and thus required alternate methods of disinfection or sterilization. Validation programs which ensure adequate sanitation of enrichment devices can aid in prevention of disease transmission between nonhuman primates via enrichment devices.
Assessment comprised exposure of filters loaded with a nominal level of 10° Bacillus subtilis spores to the chlorine dioxide/air mixture of the microbiological challenge room for defined time periods during the exposure/holding phase. The filters were then dissolved and spore enumeration performed. Samples were obtained from each quartile of the decontamination process. Quartile spore log reduction values were summed to yield the estimated value for the 120-minute cycle holding phase. This study documents the efficacy of using a low (1mg/L) concentration of chlorine dioxide to achieve a consistent level of spore inactivation over an extended period. In each of three evaluations, the predicted reduction of Bacillus subtilis spores exceeded 15 log. These findings support the utility of gaseous chlorine dioxide for the decontamination of rooms and other large, sealed volumes.

PS62 Web-Based Auto-Tutorial for Barrier Training

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The maintenance of pathogen-free mouse colonies not only requires the use of appropriate facilities and equipment, but also sufficient training of personnel who use the facilities and equipment to house the mice. Failure of one or more staff to adhere to the prescribed appropriate standard operating procedures (SOPs) could result in the introduction of pathogens into the facility. How does an institution provide training in a manner that meets the needs of the investigator in a timely manner and provides assurance that the users of the mouse facilities are adequately trained? The Animal Resources Center at The University of Chicago has developed an electronic media auto-tutorial that includes a written evaluation that electronically transmits test scores back to the Animal Resources Center. The program is web-based and available to faculty and staff at the University. The auto-tutorial graphically illustrates with minimal text each step of the SOPs. Test scores are entered into a personnel database. After successfully completing the auto-tutorial, the new investigative staff are required to complete a practicum/tour with the supervisor of the facility. We use the practicum/tour to evaluate the staff’s ability to perform procedures as they were trained. The practicum/tours are scheduled when requested. After successfully completing the auto-tutorial and the practicum, they are given access to the barrier facility. This program has provided detailed training and evaluation of staff using the barrier facilities. With this system, there is minimal delay in getting staff trained. This training program has resulted in a high degree of compliance with the SOPs.

PS63 Laboratory Training and Continuing Education

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Training is an integral component of any animal care and use program. Aspects of training are covered in the Animal Welfare Act as well as in the Guide for the Care and Use of Laboratory Animals. The American Association of Laboratory Animal Science has a well-developed plan for training, and documentation of training, of animal care technicians. Training research staff has often been more problematic. Issues of compliance and meaningful participation can arise. We will briefly discuss how the training program for research staff at Dartmouth College has evolved. We plan to discuss the process of scheduling continuing education sessions with different laboratories within a department throughout the year. This offers a valuable opportunity to establish the ARC’s position as a resource to PI’s and their staff. We will discuss the development of our current program. While the core of each continuing education session is reinforcement of basic ARC policies and animal welfare issues, we have utilized the veterinary staff, animal care staff and researcher’s comments and concerns to customize these sessions. We feel this approach better meets the needs of both the ARC and the research staff. We have a very simple documentation and evaluation system, which allows a great deal of flexibility. We will also discuss the resources that help to make the program successful. In summary, we feel that the system we currently have in place helps assure compliance with regulatory training requirements, while remaining flexible enough to meet the individual needs of the research staff in an academic environment.

PS64 Augmenting Animal Care with Staff from an Outsourcing Contractor Using a Team Approach

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During recent years there has been an “explosion” of the number of mouse breeding colonies at academic institutions. At The University of Chicago, the mouse barrier facilities have doubled during the last five years. Mouse breeding was conducted in each barrier facility resulting in inefficiencies with a high risk for introducing pathogens. A committee of investigators and Animal Resources Center staff at The University of Chicago cooperatively developed a program to centralize mouse breeding and to pattern the program used at commercial rodent vendors to maintain well-managed pathogen-free breeding colonies. In addition to the recommendations for facility improvements and new cage equipment, the committee concluded that access to the facility must be limited and that facility must be supported by staff experienced in managing breeding colonies. The committee also concluded that the existing animal care staff lacked a sufficient number of qualified staff to support the breeding facility and recommended that the animal care staff be augmented with contracted employees from an outsourcing contractor. Prior to finalizing a contract with an outsourcing contractor, The University reached a cooperative agreement with Union officials representing the animal care staff. The staff from the vendor followed the standard operating procedures established by The University of Chicago Animal Resources Center and have offered suggestions for improvements. Currently, the staff supporting the facility provides a complete breeding service that includes tissue sampling and maintaining breeding and procedure records on a network database. The team approach for this project has received widespread support by the Investigators at The University of Chicago. The paper reviews the economic issues, the integration of augmented staff into the animal program, and the introduction of new technology associated with the changes made in maintaining mouse breeding colonies at The University of Chicago.
Our 25,000-square-foot, nine-employee Animal Care Unit (ACU) reported a yearly average of 11 ergonomic-related injuries between 1992 and 1998. These injuries constituted 38% of the total reported injuries. The injury rate and associated lost work time were considered unacceptable and led to a complete ergonomic evaluation and strategies to affect change. Measurements were taken to determine forces necessary to lift, pull, or push various pieces of standard animal facility equipment. Videos were taken of animal care staff during the performance of tasks associated with ergonomic injury. Meetings were conducted to evaluate task performance as well as to solicit input from ACU employees. As a result of these evaluations, changes in equipment, equipment design, and procedures were made to reduce the likelihood of ergonomic injuries. Although improvements were noted, continuing ergonomic injuries warranted additional approaches. As part of a comprehensive program of ergonomic evaluation, a certified health and fitness trainer was consulted to review and evaluate the ranges of motion typical in daily animal husbandry. The trainer interviewed each employee individually, determined pre-existing health problems, divided staff into 2 groups based on fitness level, and developed a strength and flexibility program tailored to each group. A program was launched that was designed to reduce or eliminate ergonomic injuries and lost time and required a minimum of space, equipment, and time. Equipment consisting of stability balls, medicine balls, stretch bands, and dumbbells was purchased for $550. A 390-ft² room was identified, and staff were scheduled during work hours on two nonconsecutive days per week for 1-hour sessions. Following implementation of this program, no ergonomic-related injuries were reported in the ACU in 1999. Initial assessment of flexibility over a 15-week period indicated that 3 of 5 participants demonstrated substantial improvement in measured flexibility. The program was deemed cost-effective (approximately $20,000 for the equipment and trainer) since a single OSHA injury can result in a $100,000 outlay. Regardless of monetary outcome, the program was effective because of team building among the participants, improved personal fitness, health, and flexibility.

When employees begin life in a new work environment, they face not only the pressures of learning a new job, but also learning the ins and outs of a university or institution while adjusting to life in a new town. The stress of so much “newness” can impair the employee’s ability to adjust to and learn the new job. At our institution, we developed a peer-mentoring program to provide counsel and support to new animal care staff members. New employees are mentored by a team of coworkers who are not members of their daily work group. The mentoring sessions coincide with the probationary period (90 days) and occur on a weekly, then biweekly schedule. Mentoring sessions last approximately one hour with the new employee’s work being covered by his/her supervisor or other coworkers so that he/she may feel free to take that time away from a work assignment. Oversight of the program is provided by the administrative assistant (author). Initially, the administrative assistant meets with the new employees and their mentors to explain program guidelines and suggest mentoring activities and sites. She also sends E-mail reminders to mentors and supervisors when sessions are to take place, records when sessions occur throughout the duration of the program, meets with new employees halfway through the program to see how it is working for them and if the mentor match is compatible, and follows up after the program is officially done with questionnaires for both the mentor and the new employee to critique the program. Any concerns regarding the program are brought to the husbandry supervisory team for discussion and resolution. Active participation and buy-in are key to the success of this program, which has the full support of husbandry management and the unit director. Although the process is involved, the results of this program have proven worthwhile. At least one new employee, fearful of taking her concerns to her supervisor and ready to quit, sought help from her mentor. As a result of their communication, the employee was moved to another work assignment where she was very successful, and she has remained as a valued employee. Other mentor relationships have been equally successful, fostering confidence and growth in new employees, and rewarding mentors with new opportunities to teach and gain respect. The mentoring program has promoted and strengthened this organization’s team philosophy and foundation.
Swine are an increasingly popular alternative species in cardiovascular research. For research, miniature swine combine advantageous physiological characteristics and small size. We compared limb and axial leads for quantification of electrocardiographic (ECG) parameters in conscious miniature swine (Ellegaard-Gottingen) (n = 16). Individual swine (3–6 months, 5.6–16 kg) were comfortably positioned in a sling and standard veterinary ECG leads were positioned on the limbs (lead II). In addition, adhesive leads were placed on the cardiac apex area, r. mastoid process of the temporal bone, and sacrum (Nehb-Spöerri [axial] lead system). ECG traces of 10 second duration were collected using a Cardiotest EK 53R (Hellige, Germany) instrument; representative Lead II and axial ECGs for each animal were analyzed using a Cardiotest EK 53R (Hellige, Germany) instrument; representative Lead II and axial ECGs for each animal were analyzed manually (mean ± SD). RR, P, PR, and QT durations were similar. S-wave amplitudes were -0.14 ± 0.03 mV (II) and -1.35 ± 0.10 mV (axial); mean diastolic systolic quotients (DSQ) were 1.07 ± 0.14 (II) and 0.93 ± 0.16 (axial); and average modal QRS vector values were -9.3 ±11.8 (frontal plane) and -107 ± 4 degrees (sagittal plane). QRS duration tended to be slightly longer, T-wave amplitude more positive, and modal vector more consistent using the Nehb-Spöerri axial lead system. Because of the vertical axis of the base-apex cardiac vector in the chest of the miniature swine, traditional limb electrocardiography may be sub-optimal for evaluation of QT interval; an axial lead placement is preferred.

A new wound healing pig model was developed by excision of 2 x 2 cm full thickness skin in the skeletally immature pig. The wounds and wound healing that occurred during use of this model were characterized. The consistency of this animal model for the molecular analysis of wound healing was assessed with quantification of total RNA yield and comparison of mRNA levels for type III collagen from skin samples derived from different locations on the pigs. The pattern of change at the molecular and cellular level during the healing in this animal model was defined, as well as the bacteriological profile. mRNA expression for relevant genes was assessed by semiquantitative RT-PCR using porcine specific primer sets and RNA isolated from normal skin and samples at various times post-wounding. Analysis of cellular change was assessed by DNA quantification and histology of tissue sections. Quantitative bacteriology was performed using standard techniques and selective plating techniques. The results demonstrated that wound re-epithelialization in this animal model took an average of 18 days. The results also show that RNA and DNA content paralleled each other throughout the healing process and the observed changes in the pattern of RNA and DNA content of the scar tissue were consistent with the increasing wound cellularity. The mRNA levels for collagen I, collagen III, HSP47, IL-1, TGF-b, MMP-1, -2 and -9, TIMP-1, -2, -4, PAI-1, and versican were significantly elevated at specific times after wounding; the mRNA levels for biglycan and fibromodulin were not changed; however, mRNA levels for TIMP-3 were depressed. The result of the bacteriology study indicated that the total bacterial count in this animal model reached 10^7 CFU/gram, with the highest number at day 7 post-wounding. Pseudomonas aeruginosa and Staphylococci aureus were the most common bacteria detected in this model. The model may serve as a standardized method for investigating the effect of various agents on wound healing which could subsequently be confirmed through clinical experience. These studies also suggest that skin wound healing is a series of complex matrix-cell interactions that involve cellular migration and inflammation, followed by proliferation of fibroblasts with new collagen synthesis, and lastly remodeling of the scar tissue. Further definition of this model should identify unique points in the healing process which lead to the development of rational approaches to improve skin wound healing.

Mucosal and systemic candidiasis of endogenous origin was studied in a novel animal model, the immunosuppressed gnotobiotic (IGB) newborn piglet. Past studies in the gnotobiotic (GB) piglet have demonstrated that many organisms, such as diarrheogenic Escherichia coli, Shigella sp., Cryptosporidium parvum, and Enterococcus bieneusi produce pathology resembling the human disease. In this study, immunosuppression was achieved with 25 mg/kg methylprednisolone 1M and 15mg/kg cyclosporine PO daily. Intragastric inoculation of IGB piglets (n = 8) with the wild type strain of Candida albicans (SC5314) led to the development of extensive mucosal infection and to gastrointestinal colonization with dissemination to multiple internal organs including heart, lung, liver, kidney and regional lymph nodes. Histopathology and immunohistochemistry demonstrated the presence of multifocal colonization of C. albicans in these organs and unique invasive gastrointestinal lesions. A C. albicans strain (CKY138) lacking two putative transcription factors, Efg1p and Cph1p, has been reported to be defective in filamentous growth and avirulent in the mouse model. In the IGB piglet model (n = 9), this strain was able to colonize the gastrointestinal tract with some filamentous forms. However, it showed reduced ability to produce invasive lesions and to disseminate as compared with the wild type. These results illustrate that the IGB piglet model for candidiasis exhibits the mucosal and disseminated forms of disease seen in humans and allows the in vivo evaluation of putative virulence factors of C. albicans.
P04 Development of a Porcine Isolated Heart Model to Evaluate Aortic Root Motion

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Information on the natural motion of the aortic root is either incomplete or contradictory. In order to accurately model the aortic valve complex and to design new prosthetic valves that more closely imitate a natural valve, it is necessary to investigate the motion of the aortic valve, annulus, coronary sinus region and aorta in a dynamic system. The isolated working heart model was chosen as the test system because it is representative of the in vivo state yet it is easily manipulable and accessible for measurements. The system was modified to allow direct perfusion of the coronary arteries without interfering with aortic root motion. Hearts from 70–100 kg domestic swine were harvested following cold cardioplegia. The coronary arteries were dissected and cannulated with 10 Fr. arterial cannulae external to the coronary sinuses. The heart was attached to a perfusion apparatus consisting of a roller pump, a fluid reservoir, and an oxygenator/heat exchanger that warmed and oxygenated the perfusate delivered to the heart. The heart was perfused with Minimum Essential Medium (MEM), a tissue culture growth media, containing salts, amino acids, vitamins, glucose and a colored pH indicator. To this solution was added sodium bicarbonate, sodium pyruvate and insulin. The sodium pyruvate served as an energy source for the heart in addition to glucose and the insulin facilitated glucose uptake by the cardiac muscle cells. It was critical to assure that the pH of the perfusate was between 7.2 and 7.3 prior to perfusion. Following perfusion with the 37°C MEM, the fibrillating heart was converted to sinus rhythm by treatment with lidocaine HCl added to the perfusate and electrical defibrillation. A 32 Fr. right angle cannula was placed in the left atrium to supply fluid to the left side of the heart. The aorta was connected to a fluid capacitor and a resistance clamp to provide physiologic systemic compliance and resistance. Once the heart was beating regularly, data was collected by various modalities. A mercury strain gauge was placed around the aortic root to measure circumferential expansion. Pressures were measured from a catheter placed in the apex of the left ventricle and from the aortic outflow adapter. Ultrasound was used to determine the identity of the suspected scavenger, the effects of bones location was made. The bones were then analyzed to determine the identity of the suspected scavenger, the effects of the weather on the remains and the percentage of missing bones. The animals were observed by our students and specimens were collected at specific times to correlate with the natural decomposition process. The animals’ remains were collected at the end of one year and a map of the remaining bones location was made. The bones were then analyzed to determine the identity of the suspected scavenger, the effects of the weather on the remains and the percentage of missing bones. The students using this model are left with a more complete picture of the decomposition process and the expectations of remains discovery over time. This poster represents the various stages of decomposition of the animals and the biological information gathered from the use of these animals as a teaching technique.

P05 An Alternative Model for Teaching Microsurgery

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Animals have been widely used as a training model for teaching microsurgery. However, unlimited access to the animal laboratory is not always possible and increased scrutiny of the ethical use of laboratory animals is a major factor to consider when teaching microsurgery. In such situations an alternative method is required for microsurgical training techniques, such as medical grading tubes, surgical gloves and the PracticeRat (Sharpoint, Reading, PA). These alternative teaching tools provide an ideal training model and present an alternative for teaching microsurgical techniques. They are readily available and abundant in the laboratory. In addition, these alternative tools can be used to provide initial training prior to a clinical situation. A practice card designed from surgical gloves and medical grading tube was used to practice simple sutures. The training progressed to a more difficult level using medical grading tube for end-to-end anastomosis, end-to-side anastomosis, side-to-side anastomosis and free graft placement. This alternative proved to be challenging for the trainees but improvement of hand coordination was observed. The alternative models familiarized trainees with the instruments and with developing a surgical approach before moving on to more clinical settings. The use of surgical gloves and medical grading tubes resulted in the significant reduction in the numbers of laboratory animals used for teaching microsurgery as well as reduced training costs.

P06 Use of Laboratory Animals in the Teaching of Forensic Biology and Anthropology

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At Ferris State University, the criminal justice program requested that the biology department teach two courses in forensic biology. Toward this goal, we have developed an animal model system to facilitate the teaching of forensic biology to criminal justice students who have little if any training in biology. The field work in these courses deals with the placement of laboratory rats or locally obtained pigs to mimic the conditions under which actual police officers and crime technicians would observe in the course of their job. The animals are euthanized according to our local animal care committee’s recommendation and are placed outdoors in various ecological situations and in various state of coverings. The animals were observed by our students and specimens were collected at specific times to correlate with the natural decomposition process. The animals’ remains were collected at the end of one year and a map of the remaining bones location was made. The bones were then analyzed to determine the identity of the suspected scavenger, the effects of the weather on the remains and the percentage of missing bones. The students using this model are left with a more complete picture of the decomposition process and the expectations of remains discovery over time. This poster represents the various stages of decomposition of the animals and the biological information gathered from the use of these animals as a teaching technique.

P07 Sexual Development in Male Rats: Role of Penile Nitric Oxide Nerve Fibers

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The objective of this study was to determine the possible contribution of different neuromodulators to the development of penile erection in rats in juvenile rats. Penile NADPH positive
staining have mainly been associated with nerve fibers in male rats. NADPH is a co-enzyme important in the synthesis of nitric oxide (NO) which has been shown to be of major importance to produce penile erections. Other neurotransmitters involved in penile erections are: vasointestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY). These modulators of erections were measured in juvenile rats to establish developmental changes related with known periods of sexual behavioral changes. Thirty Sprague-Dawley (SD) male rats were divided according to different ages ranging between 1–60 d. Before euthanasia and under general anesthesia, a penile mid-shaft specimen was taken for nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase staining as well as immunohistochemistry for vasointestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY). Results show that following NADPH staining the number of positive NADPH structures increased in the corpus cavernosum and raised significantly starting at 30d only. The number of NADPH positive structures in the dorsal nerve was 33.17 ± 18.5 at day 1 and raise steadily to plateau of 941.5 ± 77.8 on day 60. The number of VIP, CGRP and NPY fibers was small and constant (3–15) for the 0-60d period. In conclusion, penile NO fiber growth is important during pubertal development and an important growth spurt occurs at 30 days. This was not observed for VIP, CGRP and NPY. Behavioral studies have shown that penile erection first occur around 30–40d in rats. NO seems would therefore important in the maturation of erectile mechanisms.

P08 A Female Arousability Rat Model: Increases in Clitoral and Vaginal Blood Flow following Nerve Stimulations and Injection of Neuromodulators

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The objective of this study was to establish a rat model for the study of female arousability. Fifteen female Sprague-Dawley rats (Charles River) 250–300g were used. Under general anesthesia (pentobarbitol, 60mg/kg) the dorsal clitoral nerves and pelvic plexus nerve were isolated for the evaluation of clitoral and vaginal capillary blood flow (CBF), respectively. A laser Doppler probe (dia 0.5 mm, Transonic), coupled to a Doppler flowmeter (ALF-21, Transonic), was placed on the clitoral glans and vagina to measure CBF. Electrical pulses were delivered (MacLab 8S stimulus isolator, ADInstruments) using a stainless-steel bipolar hook electrode (Parameters: 2 mA, 20Hz, pulse width 0.2 ms for 5 s). In a group of 5 female SD rats, blood flow was recorded from the clitoral glans following clitoral nerve stimulation with and without sodium nitroprusside (SNP), a NO donor, and L-nitro-arginine methyl ester (L-NAME), a competitive inhibitor of nitric oxide synthase. Results show that clitoral blood flow increased following clitoral nerve stimulation (df1,2 = 12,108, F = 21.4, P < 0.001). Vaginal blood flow was increased following pelvic plexus nerve stimulation (df1,2 = 12,108, F = 4.75, P < 0.001). During electrical stimulation, SNP increased by 50% (t test, P < 0.001), and L-NAME decrease by 80% (t test, P < 0.001), clitoral blood flow in comparison to baseline values recorded during electrical stimulation of the clitoral nerve. In conclusion, the female rat can be used as a model for the study of the physiology, pharmacology and sexual dysfunction relating to blood flow in clitoral and vaginal tissues. Similar to male penile erectile mechanisms, NO is important for the blood engorgement of the clitoris.

P09 Susceptibility to the Induction of Glutathione S-transferase Positive Hepatic Foci in Offspring Rats after γ-ray Exposure During Gestation

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Glutathione S-transferase positive (GST-P”) hepatic foci development was used as a means of determining whether the offspring of gestating maternal rats, which were subjected to genetically-damaging levels of γ-ray radiation, were more susceptible to the development of cancer after treatment with diethylnitrosamine (DEN), a known carcinogen. A single dose of 10, 30, 60, and 90 rads involving whole body exposure to γ-rays was given to pregnant rats (15 animals per group) at day 14, and during postnatal week 4. DEN was intraperitoneally injected to their offspring twice in one week. Thirteen weeks after birth, the rats were sacrificed. Irradiation of maternal rats with 30 rad γ-rays before mating significantly increased both the incidence and the size of GST-P” foci in the livers of both male and female pups, when combined with DEN treatment, whereas other dose levels had no such effect. Using a rat-liver model, the results of this study indicate that a low dose of radiation during the embryonic stage increases the susceptibility to carcinogens. In addition, under certain circumstances low doses of radiation, an externally applied cancer-inducing stimulus, may increase the likelihood of cancer, whereas high doses may not.

P10 A Comparison of Cerebral Preconditioning on Stroke Using DWI and T2 MRI in SHR and SHRsp Rats

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Stroke rates third as a cause of death in the United States. Eighty percent of all strokes are ischemic. Understanding the biochemical mechanisms that occur during ischemic stroke is required for the discovery of compounds to prevent and treat stroke. A previous study by Barone et al. showed that SHR rats that underwent a transient focal ischemia, had a reduction in infarct size when undergoing subsequent permanent ischemia. This phenomena is referred to as “preconditioning.” We hypothesized that preconditioning may not be as effective in animals that have a genetic predisposition to stroke. The current study compared the effects of preconditioning on male SHR and SHR-sp (stroke prone) rats with control groups (having only permanent occlusion), n = 7–9 per group. Rats in the PC group underwent a 10 minute occlusion of the middle cerebral artery (MCA) at day 0 followed by permanent occlusion of the MCA 24 hours later. We evaluated infarct development and size longitudinally with DWI and T2 MRI at 3 time points. Neurologic scoring was performed and correlated to infarct size. Infarct size in the SHR rats was significantly larger than SHRsp rats (P < 0.05) with both DWI and T2 images in the preconditioned groups. The difference correlated with histology and neurologic scores. Results indicate that mechanisms responsible for preconditioning may be less effective at reducing infarct size in stroke prone animals.
P11 Targeted Ablation of the Mouse Skeletal Actin Gene

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The four muscle actins in higher vertebrates show distinct expression patterns and display highly conserved amino acid sequences. It is hypothesized that the muscle isoactins are specifically adapted to their respective tissues and that the minor variations among them have developmental and/or physiological relevance. To assess these issues, the skeletal actin gene was disrupted by homologous recombination. All mice lacking skeletal actin die in the early neonatal period (day 1–9). The null animals appear normal at birth and can breathe, walk, and suckle but, compared to normal littermates, show a markedly lower body weight within 4 days and develop scoliosis. Newborn skeletal muscles from nulls are similar to wild-type mice in size, fiber type and ultrastructural organization. Newborn heterozygous and homozygous null animals show an increase in cardiac and vascular actin mRNA in skeletal muscle, with no skeletal actin mRNA present in the nulls. Adult hemizygous animals show no overt phenotype and an increased level of skeletal actin mRNA in hindlimb muscle. In combination, these data indicate that skeletal actin is not required for survival to birth. Although increases in cardiac and vascular smooth muscle actin may compensate partially for the lack of skeletal actin in null mice, this is not adequate to support skeletal muscle growth and function. Null mice show a loss of glycogen and reduced brown fat that is consistent with malnutrition leading to death.

P12 Investigation of the Role of M₂, M₃ and M₄ Muscarinic Receptors using Receptor Knockout Mice

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The muscarinic acetylcholine receptor family consists of five subtypes (M₁–M₅), but the various functions of the receptor subtypes in the central and peripheral nervous systems have not been clearly elucidated. To study the physiological role of the M₂, M₃ and M₄ muscarinic receptor subtypes, mice lacking functional M₂, M₃ or M₄ receptors (knockouts) were produced by gene ablation methodology (Gomez et al., Proc. Natl. Acad. Sci. USA 96, 1692–1697, 1999; Gomez et al., Proc. Natl. Acad. Sci. USA 96, 10483–10488, 1999). The role of the receptors in agonist-induced effects was determined by administering the non-selective muscarinic agonist oxotremorine (0.03, 0.1, and 0.3 mg/kg s.c.) to knockout or wild-type mice and evaluating agonist-induced salivation, hyperthermia and tremor. Salivation and tremors were measured separately using the same scoring method: 0 = no effect, 1 = moderate effect and 2 = profound effect. Baseline rectal temperature was measured prior to administration of oxotremorine and 30 minutes thereafter. Oxotremorine induced a dose-dependent increase in salivation, decreased core body temperature (hyperthermia) up to 8–9°C, and caused massive tremors in wild-type mice. Oxotremorine-induced hyperthermia was significantly decreased in M₂ knockout mice, only at the intermediate dose in the M₄ knockouts and was not altered in the M₃ knockout mice. Tremor was completely absent in the M₄ knockout mice, but was not appreciably altered in the M₂ and M₃ knockouts. The scoring of salivation was particularly lower in the M₃ knockouts. In a second experiment, we quantified differences in salivation between the knockout and wild type mice. Mice were anesthetized with urethane (1250 mg/kg s.c.) and then treated with oxotremorine (0.1 and 0.3 mg/kg s.c.) 5 minutes later. Small cotton pellets were placed in the mouth of each mouse and were replaced upon saturation with saliva. The cotton pellets were weighed to determine total salivation for each mouse over a 15 minute period. The quantity of oxotremorine (0.3 mg/kg s.c.)-induced salivation was reduced to 22, 58 and 89% of wild type in the M₂, M₃ and M₄ knockout mice, respectively. Thus, these data demonstrate the key involvement of M₂ receptors in muscarinic agonist-induced hyperthermia and tremor as well as the role of M₂ receptors and to a lesser extent M₃ receptors in salivation. Furthermore, they demonstrate the value of using gene ablation technology to clearly assess the physiological role of the individual muscarinic receptors in mice.

P13 Unique Lesions in a Transgenic Knockout Mouse Model of Sickle Cell Anemia

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Six male adult mice were necropsied after sudden death during cross-continental transportation and ambient temperatures near 32°F. The mice were double knockouts for the mouse alpha- and beta-globin genes and carried one copy of the human beta-globin sickle cell transgene, HB°. At necropsy, all mice had lesions typical of sickle cell thrombosis due to stress, including splenomegaly, cardiomegaly, and a mottled liver. Histologically, the spleen contained multifocal aggregates of sickle-shaped erythrocytes. The liver had multifocal areas of centrilobular necrosis, and the kidneys showed mild proliferative glomerulopathy and microthrombi. The spleen, liver, and kidney demonstrated increased iron deposition compared to age- and sex-matched single knockout, which carried one copy of mouse alpha- and beta-globin in addition to HB°, and wild-type mice. Subepicardial angiogenesis was observed in the heart and has not been previously reported. Seven double knockout mice (three male and four female) that were euthanized at the end of a terminal experiment involving pulmonary function testing, which involves periods of anoxia, were necropsied. All the mice had lesions identical to the six that died during transport, which suggests that such lesions can occur due to stresses other than hypothermia and transport. Five male and five female mice with single knockouts of the mouse beta-globin gene and five male and four female wild-type mice were necropsied after pulmonary function testing, but were histologically normal. This suggests that only the double knockout mice are susceptible to stress-induced sickling and infarction, which illustrates the importance of husbandry in the handling of this transgenic knockout model of sickle cell anemia.

P14 Phenotypic Evaluation of the Tg-AC Transgenic Mouse: Correlation of TPA-Induced Papilloma Formation to the Responder Genotype

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Tg-AC mice [FVB/N.Tac-Tg(N(v-HA-ras))] carry the activated v-HA-ras oncogene fused to a mouse zeta-globin promoter and exhibit characteristics of genetically initiated skin as a target for tumorigenesis. Test articles applied to these mice can be scored
for their tumor-promoting effect by the observation of dermal papilloma formation. A commonly used positive control is to dose Tg.AC mice with TPA (Phorbol 12-Myristate 13-Acetate). To define the extent of genotype to phenotype correlation, and as a quality control test, Tg.AC mice were topicaly dosed with 2.5ugm TPA three times per week. All mice had been genotyped as hemizygous carriers using a southern blot with a probe specific to mouse zeta-globin (a.k.a. “Responder” genotype). A threshold of three papillomas per mouse was set as a positive result; additional studies demonstrate a sharp increase in the number of papillomas after three appear. 100% of the Tg.AC carrier mice tested (183/183) developed three or more papillomas while none of the similarly dosed FVB controls developed papillomas. Papilloma onset time was typically 7 to 10 weeks following first application. Mice did not display signs of distress following dosing or resultant from the development of papillomas. This assay has proven relatively easy to administer with papilloma formation easily identified by technicians.

P15 The Ectopic Expression of Rubella E2 Gene on Pancreatic Cells Changes IDDM Incidence via Unknown Immunological Mechanisms
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NOD (Nonobese diabetic) mouse is known to be an animal model for human IDDM which manifest autoimmune destruction of the insulin-producing b cells in the pancreas. In spite of much progress in understanding the mechanism of IDDM, the immunopathological cue is not determined yet. Approximately 12–20% people diagnosed with congenic rubella syndrome (CRS) develop IDDM in within 5–20 weeks. Rubella virus antigens or antigens altered by rubella virus on b cells may act as foreign to the host’s immune system, leading to b cell-specific autoimmune pathogenesis. Alternatively, rubella virus might be involved in the induction of antigen-specific cytotoxic T cells that recognize b cell-specific antigens by molecular mimicry. We hypothesized that the autoimmune destruction of b cells be accelerated if ectopic expression of E2 gene is recognized as non-self by host immune system. In order to test the hypothesis, we developed transgenic NOD mice overexpressing rubella E2 gene in b cells of pancreas using rat insulin promoter. This may result in increase in IDDM incidence and/or earlier onset of IDDM in these transgenic mice compared to non-transgenic NOD mice. Of 3 lines generated, two lines show similar incidence of IDDM with NOD mice (more than 75% at 30 weeks of age in our breeding colony) which have been maintained under the same environmental condition. Unexpectedly, however, one of the lines shows extraordinarily low IDDM incidence (15% at 30 weeks of age). These animals have very low insulin scores compared to the other 2 lines and NOD mice. Several studies indicate that functional balance between the two helper T cell subsets is a determinant in establishing islet pathology. There was no consistent difference in the pattern of cytokine gene expression between the 3 lines and NOD mice. We conclude that the transgenic animal with extraordinarily low incidence of IDDM have different mechanism of protection from IDDM. These animals will serve as a good animal model in elucidating the molecular and immunological mechanism of IDDM.

P16 Evaluation of Bilateral Orthopedic Surgeries in Rabbits in an Attempt to Reduce Animal Numbers Used on Study
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Two animal models using rabbits for cartilage research have been compared regarding postoperative analgesia. The first model involved bilateral articular cartilage defects of the stifle joint. These animals were provided buprenorphine for pain management post surgery. Based on ambulation, activity level, food consumption, weight loss, and fecal output, the rabbits on this study exhibited good recovery. The objective of the second model was to induce osteoarthritis by means of anterior cruciate ligament transection and medial meniscectomy. Based on previous literature, need for increased cartilage for RNA, and interest to decrease animal numbers, a bilateral approach was used in this model, the approach to pain management was altered and more intensive postoperative care was provided. Although these efforts improved the overall clinical condition for the animals, weight loss food consumption, activity level, fecal output, and ambulation were evaluated and provided rationale for the recommendation of unilateral surgery for future studies.

P17 The Development and Refinement of Two Animal Models for Testing Bone Therapeutic Agents
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P&GP’s Bone Focus Area is currently searching for bone anabolics for the treatment of osteoporosis and primarily uses two rat models in our stage two and stage three projects: the local injection model (LIM) for screening compounds for anabolic activity, and the ovariectomized rat model (restoration model) for determining efficacy of these compounds. Although these are reliable models, we continue to explore methods that will reduce the number of animals used and refine techniques to optimize results. The LIM involves implanting a cannula (a 20 g needle cut to 6 mm) into the proximal tibial metaphysis and administering daily intraosseous injections. This is a short-term model that requires small amounts of compound, provides a rapid answer, and reduces systemic effects. Recently, we have been exploring modifications to the surgical and dosing techniques to reduce stress and increase consistency, which will require fewer animals per group to demonstrate statistical power. For example, in the past, cannulas were pushed through the cortex into the marrow cavity. We now use a small hand drill to pre-drill holes for cannula insertion which greatly increases precision as well as reducing trauma and extraneous bone in the marrow cavity. In addition, we are developing a positioning platform and drill guide to further ensure consistent cannula placement and reduce surgery time. To eliminate the stress of daily handling and anesthesia for intraosseous injections, we are investigating the use of a time-released polymer that will require only a single intraosseous injection of compound. In a rat restoration study, animals are ovariecetomized (OVX) at six months of age. Sixty days after OVX, animals have reached an osteopenic state and are then treated with test compound or vehicle for 60 consecutive days. This has proven to be a very reliable model with predictable results. Our refinements of this model include reducing the number of animals to four per group to determine the efficacious dose in 50% of animals (based on six dose groups covering a 2 1/2 log dose range). In order to optimize the use of
research animals, we need to improve our studies whenever possible. As shown, we have refined the methods used in the local injection model to reduce stress on the animals, reduce the number of animals used, and increase precision. The restoration model has been modified to use the fewest number of animals possible to obtain the most information, making a reliable model more efficient. We consider it our responsibility to continually search for ways to reduce numbers and refine techniques when replacement isn’t an option.

P18 Rat Meniscectomy Model for Arthritis and Neuropathic Pain
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Osteoarthritis, a degenerative condition involving articular cartilage and remodeling of the subchondral bone, is the most common of all the arthritic diseases. This condition can cause severe, chronic, disabling, and intractable pain that can result in abnormal activity of nociceptive processes. Neuropathic pain is moderate to severe pain of long duration which no longer serves a protective function, and is usually associated with nerve injury. Symptoms include combinations of allodynia, constant pain, lancinating pain, and thermal hyperalgesia. Both conditions have a significant socioeconomic impact. Behavioral studies in meniscectomized rats were performed to evaluate or validate the model for osteoarthritis and possibly pain of neuropathic origin. The procedure involves the removal of the medial meniscus of one knee to induce an osteoarthritic lesion. A total of 60 animals (36 meniscectomized, 12 sham operated, and 12 non-surgery control) were studied. Histological evaluations of the meniscectomized animals at 28, 60, 90, and 120 days indicated evidence of progressive articular and cortical bony degradation similar to pathologic conditions observed in humans. Standard behavioral tests for mechanical and thermal allodynia and hyperalgesia were performed weekly, and significant differences were observed between the three groups of animals up to 120 days post surgery. Drugs used to treat arthritis and neuropathic conditions were studied, and differences were noted in their ability to attenuate the behavioral effects of meniscectomy. These findings demonstrate evidence of a neuropathic component in this model. The pathophysiological mechanisms involved in determining whether primary osteoarthritic conditions have an associated neuropathic component can be used as a basis for determining the effects of this condition in man.

P19 An Evaluation of Well-Being following Intradermal Immunization with Freund’s Complete Adjuvant in Guinea Pigs
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Intra-dermal immunization with Freund’s Complete Adjuvant (FCA) is widely used to induce cell-mediated antibody response in the Guinea Pig Maximization Test. At present both ISO and the US EPA list the sensitization tests in guinea pigs as the preferred method of assessing potential sensitization by materials. The resultant skin ulcers produced by the FCA during the induction phase of testing has been the subject of consideration for potential pain or discomfort/distress suffered by the animals. Our IACUC requested that a review of the procedure for potential pain and distress be performed. Weight and behavioral changes were selected as the parameters for this assessment of pain and/or discomfort. Four test groups were evaluated: Negative Control (n = 15), Test Article (n = 19), Positive Control (n = 5), and Saline (n = 5). Except for the Saline group all groups received FCA (0.1 ml) in addition to saline for the Negative Control group, test extracts for the Test Control, and Dinitrochlorobenzene (DNFB, a potent irritant) for the Positive Control group. The four groups were cared for and treated in the same manner per the maximization testing guidelines in a controlled environment. Hartley young adult male Guinea pigs were used in groups of 4 per cage. Weight and behavioral assessments were taken for all 4 groups during the course of the study (25 days). Weight gain was averaged on a week-by-week basis. Standard Deviation and Student’s t tests were calculated for all groups. Percent body weight gain was consistent among three groups (Saline, Negative, and Test) for the entire study. During week 1 the Positive Control group’s weight gain (%) was approximately 50% less than compared to the other test groups (Saline, Negative, and Test) (P < 0.05). For week 2 the Positive Control Group was slightly below mean from the rest of the groups. By week 3 the weight gain momentum for the Positive control group had reached its peak to almost 20% above the other three groups but by week 4 their percent gain rate dropped slightly below the other three groups. Behavioral changes associated with pain or distress were minimal and consistent in all groups during certain phases of the study; i.e., intradermal injection or bandage application, otherwise all groups exhibited normal behavior throughout the study period. In conclusion, we found that the only animals that received the Positive control showed a significant variance in their weight gain. The animals in the Test and Negative control groups that were treated with FCA were similar to the Saline only group. Therefore, we conclude, based on evaluation of weight gain and behavioral observations, that FCA does not cause significant distress when used in the Guinea Pig Maximization Test.

P20 Neurological/Behavioral Testing within the Chronic Rodent Study
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A new component of chronic toxicity test guidelines (OPPTS 870.4100 and 870.4300) is the enhanced neurological/behavioral assessment. We have developed an approach to accommodate the requirements of this assessment, without compromising our ability to achieve the core goals of the basic guideline chronic rat study. First, our approach assumes that a 3-month neurotoxicity screen (OPPTS 870.6200) will be conducted, in addition to the “chronic” (1-year) neurotoxicity evaluation, as part of the development of a complete toxicological data package. Secondly, the chronic neurological assessment then takes the form of a modified subchronic assessment, consisting of key elements that can be practically incorporated into the study design of the typical chronic rat bioassay. Specifically, during months 6 and 12 of the 12-month chronic rat study, a representative sample (10 rats/sex/level) is subjected to a functional observational battery which includes observations in the home cage, during handling, and in an open field, as well as various reflex/physiologic observations and measurements. Observations in the home cage and open field include various assessments of behavioral activity. In addition, all animals (~25/space/level) are observed weekly outside the home cage utilizing a standard arena. At termination, 5 rats/sex/dose are selected for perfusion and collection of neural tissues for microscopic evaluation. Based on both scientific and practical considerations, landing foot splay, aerial righting, and motor activity in an automated device have been excluded. Overall, this approach provides a sound
neurotoxicological assessment that integrates well with the conduct of the chronic rat study.

**P21 Mouse Bone Marrow Aspirate Techniques: Refining Experimental Methods in Stem-Cell Transplantation Experiments**

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The Fred Hutchinson Cancer Research Center has long been involved in studies regarding transplantation biology toward its mission of eliminating cancer as a cause of human suffering and death. Much work is focused on the investigation of hematologic malignancies and inherited or acquired diseases of the blood and immune systems. Animal models involving bone marrow and peripheral stem-cell transplantation experiments are essential to this research, typically involving total body irradiation in conjunction with immunosuppression regimens via chemotherapeutics. Recently, much research on human hematopoiesis has been done through xenograft experiments in NOD/SCID mice. Analysis of human hematopoietic stem cells engrafting in these mice has historically required euthanasia of graft recipients at serial time points to provide adequate specimens. The bone marrow aspirate (BMA) technique described by S. F. F. Verlinden et al. (Exper. Hematol. 26:627–630, 1998) allows for a reduction in the number of animals required for these experiments and for repeated analysis of the same animal over short and long term studies. This method causes minimal apparent discomfort to mice, high quality and volume of material, and can be accomplished in less than 5 minutes under light levels of anesthesia. Recognizing the advantages of this approach to our research programs, we have adapted it to our facility and made commensurate refinements to our needs. We routinely obtain BMA volumes of 20–25 μl from the femurs of mice anesthetized via 2.5% isoflurane gas (induction chamber followed by nose cone) with excellent survival rates and few complications. Marrow harvest is effected via a 29.5 gauge needle on a 0.3-ml syringe prefilled with 50 μl of EDTA. After surgical preparation of the skin, the needle is directed proximally from the distal end of the femur between the two condyles after manual flexure of the stifte joint. Gentle aspiration produces high-quality specimens, allowing for assessments of cell viability, type, number, and extent of chimerism using flow cytometry. We are currently exploring whether variants of this approach could offer additional possibilities for transplantation biology experiments. For example, since only a limited number of donor cells typically reach the mouse bone marrow after intravenous infusion, we are examining the possibility of utilizing direct intraosseous routes, thereby bypassing the lungs, heart, and other areas where cells may become entrapped. BMA approaches applied to mice have allowed us to reduce the number of animals required per experiment, gain repeated access to bone marrow specimens, and create options for other aspects of stem-cell transplantation research.

**P22 Method of Bone Marrow Collection from the Humerus vs. the Iliac Crest in the Rabbit**

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Bone marrow was collected from the iliac crest in 4-month-old New Zealand White rabbits under anesthesia. This method was used every 8–12 weeks for a six-month period. Collection at the iliac crest site led to the frequent observation of localized bone degradation, and decreased sample volume. Therefore, the method of collection from the humerus was used as an alternative site. When collecting from one humerus approximately 10 ml of bone marrow was obtained. This amount was equivalent to the combined volume when using both the left and right iliac crest. In addition, higher yield allowed alternation between left and right humerus and greater healing time between collections.

**P23 The Use of a Unique Intratracheal Inhalation Procedure to Evaluate the Anti-inflammatory and Pulmonary Functional Effects of Inhaled Drugs for the Treatment of Asthma**

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A unique procedure was developed to dispense site-directed delivery of respirable formulations of aerosolized drugs from metered dose inhalers (MDIs) to the airways and lungs of anesthetized small animals. The procedure has been adapted for delivery of drug to mice, rats and guinea pigs and has been used primarily for the assessment of anti-inflammatory effects of anti-asthmatic drugs. Effects of aerosolized drugs on pulmonary function have also been evaluated. Intratracheal inhalation of aerosolized drug appeared superior to the more conventional intratracheal instillation method of drug solutions or drugs suspended in liquid vehicles. The inhalation method produced good drug distribution patterns in the lungs. Particle sizes were kept generally in a range from 1–2 microns. Sub-acute repeated intratracheal inhalation dosing with CFC or HFA propellant placebos was well-tolerated and no irritation or inflammation in tracheal or bronchial tissues was observed. The glucocorticosteroids (GCS), beclomethasone dipropionate and fluticasone propionate, and the phosphodiesterase IV (PDE) inhibitors, rolipram and RP73401 were compared using the intratracheal inhalation and intratracheal instillation methods in a model of mild pulmonary inflammation. Lung tissue eosinophil peroxidase (EPO) levels were used to indicate eosinophil recruitment to the lungs 48-hours after a pro-inflammatory challenge. The potency of the drugs administered by intratracheal inhalation was greatly enhanced. Although each compound was effective by either dosing method, the potency of the inhaled formulations were ~6 times greater for glucocorticosteroids and ~50 times greater for inhaled PDE inhibitors. The ratio of the potencies for the intratracheally instilled to the intratracheally inhaled form for each drug within a drug class remained approximately the same. These studies indicate that the intratracheal inhalation procedure provides an effective means for site-directed pulmonary delivery of aerosolized drug in small animals. In addition, it appears useful for comparing the pharmacological effects of different inhaled drugs while keeping drug distribution and dose to the lung fairly constant.

**P24 CSA® Clot Signature Analyzer for Assessment of Hemostasis in Dogs**

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The Clot Signature Analyzer (CSA®) is an automated system for assessing multiple aspects of hemostasis function. Fresh, non-anti-coagulated, whole blood is analyzed under conditions of physiological flow and temperature to provide information on platelet function and coagulation in the ‘punch’ channel and thrombus formation in the collagen channel. Our investigations have focused on the ‘punch’ channel. While blood flows through...
the channel (tube), it is punched by a 15 mm needle that results in two fine holes in the channel. As blood passes through these holes, platelets are activated, adhere to the site, aggregate, and close the holes, simulating repair of vascular injury. The time for hole closure is defined as platelet hemostasis time (PHT). Continued blood flow through the channel leads to fibrin generation, clot formation and channel occlusion. The time from start of blood flow to channel occlusion is the measured clotting time (CT). Secondary hemostasis (coagulation) was assessed in dogs with hemophilia A, as well as in normal dogs. The CT for a group of hemophilic dogs was >30 minutes (n = 14) compared to 12.8 ± 1.4 minutes for the normal dogs (n = 16), indicating the ability of the CSA analysis to distinguish between the groups. These CT results correlate with the conventional in vitro assays of clotting activity, Clotting Factor Analysis (FVIII-clot) and Factor VIII Chromogenic Assay (FVIII-chrom). Clotting activity results for hemophilic versus normal dogs were: FVIII-clot—6.2 ± 2.9% vs. 95.8 ± 15.2% and FVIII-chrom—not detectable vs. 115.8 ± 27.9%. Treatment of hemophilic dogs with either canine cryoprecipitate or recombinant antihemophilic factor (rAHI) generally resulted in reduced CTs for several hours following administration with a return to the prolonged clotting times seen in the pre-treatment blood sample. This result also correlated well with increased factor VIII activity detected by FVIII-clot and the FVIII-chrom, as well as with decreased Activated Partial Thromboplastin Time (APTT). Cuticle bleed time (CBT) has traditionally been used as the method for assessment of hemostasis in the hemophilic dog model. CBT has been found to be highly variable, technician dependent, and does not correlate well with the assays of clotting activity. Use of anesthesia during the procedure does not decrease the variability in CBT. Comparison of CT to CBT results in the conclusion that the CT is an easier, less traumatic assay procedure which provides a more consistent measure of hemostasis that correlates well with conventional analysis of clotting activity.

P25 Comparison of Telemetry Thermometry and Manual Rectal Temperature Recordings in the Beagle Dog

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The accurate measurement of body temperature is a critical component of some pharmacology paradigms. Our objective was to determine if there are significant differences in body temperature determinations measured by telemetry transponders, placed either intraperitoneally (i.p.) or subcutaneously (s.c.), and manually obtained rectal body temperature determinations. Eight dogs with multifunction telemetry transponders (4 i.p., 4 s.c.) were utilized for the experiment. Body temperature determinations were made in all dogs with a digital rectal thermometer at thirty minute intervals over six hour periods; telemetric body temperature determinations were made every 2.5 minutes and time-averaged for thirty minute intervals. Body temperature was measured under three different experimental conditions: control study (no experimental intervention), depression of body temperature (propofol-induced anesthesia), and elevation of body temperature (LPS-induced pyrexia). In all studies, i.p. telemetry and rectal body temperatures were significantly higher than s.c. telemetry body temperatures (P < 0.05); i.p. telemetry and rectal body temperatures did not differ significantly. Telemetry provides a useful means for body temperature measurement without the need for physical restraint, and is a more practical means of body temperature determination than repeated rectal temperature recordings. The excellent correlation between i.p. telemetry and rectal body temperature makes i.p. telemetry the method of choice for repeated, long-term body temperature determination.

P26 An Electro-Imaging System for Canine Ophthalmology Utilizing A Hand-Held Fundic Camera

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An electro-imaging system utilizing a hand-held fundic camera is used to collect the fundic images in the canine. The electro-imaging system consists of a hand-held fundic camera, an IBM personal computer (PC 350), Microsoft Windows NT 4.0, Adobe Photoshop, and a printer. This electro-imaging system is used to store, edit, and print the images captured by the fundic camera. Hand-held fundic cameras are essential for use in the canine. The hand-held fundic camera, Nidek NM 100, digitalizes pictures allowing for the direct transfer of captured images into reports. This electro-imaging system has been successfully applied in canine toxicological studies and is an easy-to-use system.

P27 Transrectal Ultrasound is a Useful, Non-invasive Tool for Evaluation of the Prostate Gland in Male Beagle Dogs

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A non-invasive method was developed to estimate canine prostate gland volume and weight for a dog model of human benign prostatic hypertrophy. Transrectal ultrasonographic (TRUS) examination of the prostate gland was performed in 50 Beagle dogs using a commercially available real-time, B-mode ultrasound unit and a 7.5 MHz transrectal imaging probe. Two perpendicular scan planes were required to obtain prostate measurements in 3 dimensions, therefore the transrectal probe scan was moved from a side-fire to end-fire orientation in order to obtain the required measurements. Prostate volume was calculated by a planimetric method, multiplying length x width x height. A nomogram was used to estimate prostate weight from the ultrasound volume measurements. At the termination of the studies, the excised prostate glands were measured using calipers. Actual prostate gland volume and weight measurements were compared directly with TRUS volume and weight estimates. The experimental results indicated that TRUS permits rapid, accurate, non-invasive determination of canine prostate gland size. TRUS had an advantage over MRI and radiography since a minimum amount of specialized equipment and no special facilities were required for the conduct of studies. This methodology was effectively used to estimate prostate volume and weight changes during the course of two, long-term pharmacology studies.
P28 Total Body Composition Parameters in a Colony of Rhesus Macaques

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Total body composition measurements were evaluated on a colony of 113 Indian-origin rhesus macaques (Macaca mulatta) utilizing dual-energy x-ray absorptiometry (DEXA) technology. The colony consisted of six females and 107 males, ranging in age from two to 28 years, which were maintained on a commercial nonhuman primate diet. The purpose of the study was to obtain and compare total body composition parameters between various aged groups of rhesus. Age groups evaluated were 2–5 yr (n = 52), 6–10 yr (n = 52), 11–19 yr (n = 7) and 20–28 yr (n = 2). Parameters evaluated included fat, muscle, and bone mineral density (BMD). Animals were maintained under isoflurane anesthesia and positioned in dorsal recumbency during the DEXA scanning procedure. Scans were evaluated, and data compiled and analyzed. Results for the entire colony are as follows: Percent body fat for the entire colony ranged between 4% and 36%, with a mean of 9.18 ± 7.39%. BMD for the 113 animals varied from 0.61 to 0.99 gm/cm², with a mean of 0.80 ± 0.09 gm/cm². The data compiled in this study provide valuable normative information on quantitative total body composition of rhesus macaques.

P29 Whole Body Autoradiography of a Central-Acting Analgesic in Rats

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The objective of this study was to determine the distribution of radioactivity of an opioid analgesic in selected tissues using whole body autoradiography (WBA). Two groups of 6 Fischer 344 male rats were administered a single oral dose at 100 mg /kg (Group 1; 6 rats) and 20 mg/kg (Group 2; 6 rats). Animals were euthanized at selected time points post-dose (0.5 to 168 hr post-dose) and carcasses were snap-frozen and processed for sectioning. A series of sagittal and para-sagittal sections were obtained from each carcass and were exposed to highly sensitive film until adequate imprinting of radioactivity was obtained. The pictures obtained were analyzed semi-quantitatively using computerized densiometry. Main findings drawn from analysis of the pictures were: 1) Tissue distribution of drug-derived radioactivity was similar for both the 20 and 100 mg/kg dose levels. 2) At early time-points (0.5 and 2 hr), radioactivity was mainly measured in the stomach and small intestine and at later time-points (6 hr) in the large intestine. 3) Relatively high concentrations of radioactivity (ca 10 to 100 times blood concentration) were detected in the liver, kidneys and the urinary bladder at 0.5, 2, and 6 hr post-dose. These findings suggest that the analgesic may be metabolized and/or stored in the liver and that a significant portion of drug-derived radioactivity was excreted in the urine. 4) Radioactivity could be detected in most of the organs and tissues including lungs, heart, pancreas, spleen and the epithelium of the digestive system. However, relatively higher concentrations were measured in glandular tissues. 5) Significant amounts of radioactivity were measured in the brain and the spinal cord (2±4 times blood concentration). This suggested that drug-derived radioactivity crossed the blood-brain barrier to a significant extent. Background levels of radioactivity were observed in most organs and tissues between 24 and 168 hr post-dose suggesting that the drug and its metabolites were almost totally excreted at these time points. This study shows that WBA may be used to understand the metabolism of novel analgesic drugs in rats and that opioid analgesics affect many organs, not only target tissues such as brain and spinal cord, which may cause secondary side effects. This study was conducted for a Pharmaceutical company that desires to remain anonymous.

P30 A Procedure for Intrathecal Administration of Radiolabeled Compounds in the Rat for Quantitative Whole-Body Autoradiographic Evaluation

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Direct administration into the central nervous system is necessary for compounds that do not cross the blood-brain barrier. Previous methods for quantification of tissue distribution in this compartment were limited. The objectives of our study were two-fold: to develop a simple but reliable procedure to dose a micro-volume of 125I-labeled compound over 5 minutes to the intrathecal space, and to ascertain the optimal concentration and method for quantitative whole-body autoradiography (WBA). The animals were anesthetized and placed into a stereotaxic device with the head flexed slightly downward. No stereotaxic coordinates or previous knowledge of stereotaxic work were necessary to learn this technique. The occipital protuberance and dorsal spine of the atlas vertebrae were identified and the area between these landmarks was cleaned with Betadine and 70% isopropyl alcohol. A butterfly catheter set was modified to reduce the dead volume, provide a “window” for confirmation of aspirated spinal fluid, and provide a means to control a micro delivery over extended time. The manipulator holding the needle was set to an angle of 30 degrees off vertical, which was found to be suitable for all of the animals we tested. Upon entering the cisterna magna, a “flash” of spinal fluid was seen in the microbore tubing. Aspirating approximately 3 μL of spinal fluid with no evidence of air confirmed the needle bevel was completely within the intrathecal space. Different dose concentrations of 125I were delivered to determine the preferred level for WBA. No remarkable clinical observations were noted during this method development. The results of the dosing procedure indicate it is an easily performed method for compound delivery to the intrathecal space of the rat. In addition, this dosing system provided the option to use syringe shielding for high level radio-concentrated doses. Results obtained from the Molecular Dynamics Storm phosphorimager confirm the 125I-labeled test compound was delivered correctly and provided the optimal concentrations to analyze distribution within the central nervous system and to evaluate possible systemic distribution.

P31 Contrast Radiographic Evaluation of Two Different Dog Models for Chronic Biliary Access

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Intravenous cholangiography and cholecystography were utilized to periodically evaluate chronic biliary access and the potential formation of pathophysiologic shunts in two different chronically instrumented dog models. The models were prepared by (1) placement of a T-tube biliary cannula in the common bile duct, or (2)
placement of a biliary cannula in the gall bladder. Post-surgical clinical assessment included survey and contrast radiographic evaluation of anesthetized dogs. The radiopaque contrast agent, 52% iophamidol meglumine solution, was administered intravenously at a dose of 0.6 ml/kg over a 10 minute period. Serial radiographs were taken at approximately 30 minute intervals until optimal visualization of the biliary tract and cannula was achieved. Over the course of several months, diminished bile flow rate was observed in several clinically normal dogs. Primary differentials for decreased bile flow included a normal physiologic decrease in bile production, hepatic injury, biliary obstruction, catheter leakage and/or formation of a biliary shunt. Contrast radiographic evaluation was essential for identifying the shunt formation, which compromises the integrity of the animal model. This technique is a valuable diagnostic tool for the validation and periodic assessment of chronic biliary cannulation models.

P32 Endoesophageal Intubation for Controlled Ventilation in Rabbits: A Rapid and Easy Technique to Establish a Patent Airway
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The endoesophageal tube (EET) was newly developed for controlled mechanical ventilation in rabbits. A technique using EET has been described as a rapid, easy,atraumatic, and reliable method for establishing a patent airway in rabbits. The EET has a distal blocked end for sealing the esophagus, the pharyngeal perforations at the level of pharynx, and an oral cuff for oropharyngeal sealing. The patent airway of rabbits can be accomplished only by inserting EET into the esophagus. The EET is inserted blindly through the oral route without using laryngoscope and advanced until the line indicating the depth of insertion on the shaft lies between the upper and lower incisors. The pharyngeal perforations of EET should lie in the pharynx so that ventilation through EET forces gas out of the pharyngeal perforations and into the larynx and trachea. Proper positioning of the EET and adequacy of ventilation can be ascertained by the capnography, pulse oximetry, and blood gas analyses. This tube may be used for: atraumatic intubation technique for research personnel not trained in endotracheal intubation; compensation after failure to insert a conventional endotracheal intubation; emergency for securing an airway when rapid endotracheal intubation is not possible. Inhalation anesthesia with the EET has been used effectively and successfully in this laboratory.

P33 Intubation in Rabbits Using Capnography
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Endotracheal intubation is very difficult in rabbits because of the prominent incisors, narrow oropharynx, and relatively thick tongue. This may cause significant laryngeal or oral trauma during a conventional blind intubation or laryngoscopic intubation. Therefore, an inexperienced investigator may be more likely to traumatize the delicate tissues of a rabbit’s larynx. A technique is described for an atraumatic and reliable endotracheal intubation using the capnography in rabbits. The endotracheal tube connected to the capnography is inserted slowly and blindly without direct laryngoscopy into the laryngeal cavity until capnographic waveforms emerged on the display. On inspiration of animals, the vocal cords are maximally opened and the tube is inserted into the trachea. The proper positioning of endotracheal tube can be ascertained by watching the emergence of the capnographic waveforms. If the tube is inserted into the esophagus, the capnographic waveforms immediately disappears. This is considered to be the new reliable tracheal intubation method for distinguishing esophageal from tracheal intubation without injuries during intubation.

P34 A Technique to Anesthetize and Intubate Rats for Thoracotomy with Improved Survival and Clinical Outcomes
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For surgical procedures involving rodents, the administration of anesthetic gases is often necessary via endotracheal intubation. We developed an easier technique in the rat with a device that allows animals to receive anesthetic gases through a uniquely devised nose cone, while being intubated. The first group of 75 rats was anesthetized with sodium pentobarbital (45 mg/kg, IP) alone and received 1.25% isoflurane during surgery. Another 92 rats were anesthetized with methohexital sodium (25 mg/kg, IM) for induction, and received 1.25% isoflurane throughout the procedure. Animals receiving pentobarbital required 20 ± 5 min (n = 12) to lose the pedal reflex, as compared to 5 ± 1 min (n = 12) for animals given the combination of methohexital and isoflurane. In the first group, we failed to intubate 10% (8/75) of the rats due to glottidospasm and increased salivary secretions. In the second group, we only failed to intubate one animal. Both groups underwent left ventro-lateral thoracotomy with endotracheal intubation and mechanical ventilation as part of a study investigating ischemically induced heart failure. During the recovery period, 25% (17/63) of the animals treated with pentobarbital could not be successfully weaned from the ventilator. However, all the animals in the second group were weaned successfully. The time needed for animals treated with pentobarbital to return to sternal recumbency was 45 ± 10 min (n = 12) compared to 10 ± 2 min (n = 12) for animals treated with methohexital and 1.25% isoflurane. The overall survival rate of the second group was markedly improved (75%) compared to animals given pentobarbital (32%). In conclusion, this technique reduces stress during induction, and improves the overall clinical outcome of the thoracotomy.

P35 Prevention of Hypothermia During Surgery in Nonhuman Primates: Comparing Warm Air Units to Warm Water Circulating Blankets
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Hypothermia is often an adverse side affect associated with some forms of general anesthesia. It may be particularly pronounced in the very young, geriatric, or debilitated patient and during lengthy procedures. Hypothermia is a common cause of anesthetic deaths. There are various methods used to reduce the degree of patient hypothermia including the application of an external heat source. The purpose of this study was to compare the effectiveness of the warm air blanket to the warm water-circulating blanket. Nonhuman primates that were part of ongoing, IACUC-approved surgical protocols were anesthe-
tized with ketamine (10 mg/kg) IM, intubated and prepped for surgery. Anesthesia was maintained using Isoflurane. The animals were divided into three groups: 1) warm air blanket (n = 10), 2) warm water-circulating blanket with a towel on top of the blanket (n = 10) and 3) warm water-circulating blanket with the animal in direct contact (n = 8). Rectal temperatures were recorded every 20 minutes until completion of the procedure. The three groups were compared using an analysis of variance with pairwise comparisons made using the Bonferroni procedure. The overall significance level was set at 0.05. The mean temperature ± Std. Error at completion of the surgery for group A was 98.3°F ± 0.5, for group B was 95.0°F ± 0.5, and for group C was 95.6°F ± 0.2. The difference between groups A and B and A and C were statistically significant. There was no statistically significant difference between groups B and C. The mean difference ± Std. Error between the finishing temperature and starting temperature (finish temperature – starting temperature) was +0.7°F ± 0.6 for group A, -1.8°F ± 0.5 for group B and -3.2°F ± 0.4 for group C. The difference within each group was not statistically significant for group A but was for groups B and C. Comparing the temperature difference between the groups (finish temperature – starting temperature), there was a statistically significant difference between groups A and B and between A and C, but not between B and C. We conclude that for our study the warm air blanket significantly reduced the level of hypothermia experienced by the animals during the surgical procedure when compared to the use of the warm water-circulating blanket. There was no significant difference between the two warm water-circulating blanket techniques.

**P36 Fluid Loading Provides Protection from the Nephrotoxic Effects of Alloxan in the Porcine Diabetes Mellitus Model**

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Fluid loading was used to decrease the incidence of nephrotoxicity following alloxan administration to Yucatan mini-pigs. Because of its pancreatic islet cell toxicity, alloxan has been used to induce diabetes mellitus in swine. An investigator experienced a significant incidence of acute renal failure in pigs during the first five days following alloxan administration. Initially pigs were given 100–125 mg/kg alloxan intravenously with no additional drugs or supportive care. Two out of six pigs became anorectic, lethargic, and developed azotemia, as evidenced by elevated BUN and creatinine levels. These clinical findings were consistent with acute renal failure. The sick pigs were treated with intravenous fluids and cimetidine (prophylactic treatment for gastric ulcers). Despite treatment, both pigs died. Data from studies utilizing other nephrotoxic cancer chemotherapeutic agents (like cisplatin) suggests that nephrotoxicity can be minimized with prior fluid loading or concurrent fluid administration. Accordingly, the protocol was modified to include administration of one liter of 0.9% sodium chloride intravenously at the time of alloxan administration. After the protocol modification, the incidence of acute renal failure dropped to three pigs out of twenty-three with no deaths. Thus, it appears that fluid loading protects from the nephrotoxic effects of alloxan.

**P37 Non-invasive Blood Pressures of the Yucatan Micropig (Sus scrofa) With and Without Midazolam Sedation**

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The current literature suggests that the effects of midazolam, a water-soluble benzodiazepine, on blood pressure in swine are minimal. The hypothesis of this study was that a light sedative dose would produce a significant decrease in blood pressure in this species. Female Yucatan Micropigs (n = 20), 16–30 kg (22 kg average), age 4–6 months, were individually placed in a swine sling, allowed to acclimate and systolic (SBP), diastolic (DBP), mean blood pressure (MBP) in mm Hg and heart rate (HR) in bpm were measured by oscillometry. The pressure cuff was placed at the base of the tail and five sets of values were recorded at five-minute intervals, beginning at 10 and ending 30 minutes, post-placement. Following a 5–4 day rest period this procedure was repeated with the addition of a dose of 0.5 mg/kg/im midazolam HCl at the time of cuff placement. A paired one-way Student’s t-test was used to compare the means of the five measures between control and midazolam treatment. The mean differences with standard deviation for SBP, DBP, MBP, and HR are 18.9 (3.97 SD), 17.8 (5.27 SD), 18.6 (3.09 SD), 20.7 (3.73 SD), respectively. All four parameters were significantly reduced in the midazolam-sedated group (P < 0.001). The maximum decrease in SBP, DBP, and MBP occurred at 15 and 20 minutes post-dose. The mean values based on the means of the five measures are 128 (12.6 SD), 80 (9.4 SD), 99 (9.2 SD), 135 (17.4 SD), and 109 (15.4 SD), 63 (12.6 SD), 80 (13.6 SD), 115 (15.5 SD), for SBP, DBP, MBP, HR, in the control (n = 20) and midazolam (n = 20) groups respectively. This report concludes that midazolam, given intramuscularly at a sedative dosage, negatively affects cardiovascular parameters, measured with a blood pressure cuff, in sexually mature female Micropigs when compared to values in awake pigs. This is similar to reports in humans.

**P38 Anesthetic Management for Laparoscopic Aortic Surgery in a Porcine Model**

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The goal of this study was to determine an appropriate, safe, and reliable anesthetic technique for a porcine surgical model of laparoscopic infrarenal abdominal aorto-aortic graft implant. Twenty-nine pigs (54.8 ± 2.2 kg, mean ± SEM) underwent full laparoscopic aorto-aortic graft implant. Pigs were grouped into acute studies (n = 15), or survived for either 4 hours (n = 5), or 7 days postoperatively (n = 9). Pigs were premedicated and sedated with atropine (0.04 mg/kg, IM) and tiletamine/zolazepam (6 mg/kg, IM). Anesthesia was induced and maintained with 1–3% halothane in oxygen. Preservative-free morphine sulfate was preemptively administered epidurally (0.1 mg/kg). Body temperature, heart rate, systemic arterial blood pressure, hematocrit, total serum protein, pulse oximetry, end-tidal CO₂ and arterial blood gases were monitored. Hemodynamics were supported by intravenous administration of lactated Ringer’s solution, dobutamine (0.1 to 5 mg/kg/min, as needed), and in two cases, with fresh whole blood. Times until extubation, standing and ambulation were recorded in survival animals. The 7-day sur-
vival animals were also scored for behavior (distress, mobility, attitude, alertness, activity and appetite) at 12, 24, 36 and 48 hours postoperatively. All animals survived the surgical procedure, however, two cases were converted to a laparotomy due to intraoperative surgical complications. Notable findings using this anesthetic technique included moderate respiratory acidosis (highest PaCO$_2$ = 60 ± 3.2 mm Hg), a decrease in mean systemic arterial blood pressure following removal of the aortic cross-clamp (88.2 ± 2.9 mm Hg decreased to 72.4 ± 3.6 mm Hg), a decrease in base-deficit/base-excess following removal of the aortic cross-clamp (5.1 ± 0.4 mEq/L decreased to 1.8 ± 1.4 mEq/L), and persistent posterior limb dysfunction in 1 of 13 survival group animals that was associated with prolonged aortic cross-clamp time (92 min compared to 58 ± 2.9 min in the other 12 survival animals). Only two of the 13 survival pigs required an additional analgesic agent in the postoperative period. Most animals returned to nearly normal behavioral scores within 24 hours postoperatively. Aggressive patient monitoring and support using these anesthesia and preemptive analgesia techniques resulted in excellent physiological and behavioral outcomes.

P39 Sagittal Sinus Cannulation in the Piglet

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Hypoxic ischemic brain injury often results in large numbers of handicapped infants. A limiting factor in studying this problem is a reliable and accurate method of sampling cerebral venous fluid. Studies have shown that sagittal sinus blood samples accurately reflect cerebral venous blood when compared to internal jugular blood. In previous work either a section of bone was removed or a 2-cm diameter Burr hole through the skull was made necessitating more expensive equipment. We have devised a method that is less invasive and cost effective. Using a 2 mm drill bit, a hole is created that passes through the nuchal crest of the skull up to the dura of the brain. A 1.30 catheter is inserted and advanced until resistance is felt. At this point, the tip of the catheter has reached the dura. The catheter is advanced again into the sagittal sinus until blood fills the catheter. Patency was maintained with heparinized saline. The criteria used for a successful procedure were multiple blood samples of 0.4ml for blood gas analysis throughout a 4–5 hour study. We have had a 92% success rate in the 12 completed terminal studies.

P40 Comparative Safety Evaluation of Two Orbital Bleeding Methods in Mice

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Collection of blood samples from laboratory mice is an important method for obtaining biological data. Methods which minimize tissue damage and stress are always preferred. This study compared the safety of collecting 100 ml of blood from the orbital venous sinus of mice using glass micropipet tips and plastic micropipette tips with micropipetter (open method) and plastic micropipette tips with micropipetter (closed method). These methods were selected because clinical observations indicated the glass micropipet tips caused a moderate incidence local tissue and ocular damage with repeat bleedings. By contrast, the micropipette method has been used for repeat bleedings in pharmacokinetic studies and produced a very low incidence of local tissues or ocular damage. Additionally, progressive lesions of the optic nerves and cerebrum have been associated with the capillary tube method (private communication). Sixty ICR mice, 30 males and 30 females, were bled weekly for 2 to 16 weeks and then killed one week after the final bleed. All mice were clinically evaluated weekly and at time of euthanasia. Twelve naïve mice, 6 males and 6 females, were used as controls. The eye, adnexa and brain were histologically examined. Hematocrit data were compiled for all test mice to determine if either bleeding method caused anemia. Preliminary data at eight weeks appear to confirm that clinically important progressive changes occur in orbital tissues of mice bled by the micropipette method. Data from mice bled using micropipetter have only infrequent, transient clinical observations, which appear to heal between bleedings. Both methods appear to stimulate hematopoiesis after just one bleeding. Histology results are pending.

P41 A Method for Collection of Blood from the Subclavian Vessels of the Degu (Octodon degus)

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The degu (Octodon degus) is a diurnal, hystricomorph rodent native to Chile. The popularity of the degu as a model in sleep biology, circadian rhythms, and jet-lag has increased considerably in recent years. Despite their increasing use, a concise description of standard biomethodologies has not been published. Many standard methods that would be useful in survival studies involving the degu, such as sequential blood withdrawal, intravenous injection, and anesthesia, have not been described in detail. The development and description of standardized techniques would greatly assist investigators wishing to utilize the degu in their studies. We developed a method for collection of 1–2 ml of blood from the subclavian vessels in anesthetized degus. The degu is induced (chamber induction) and maintained (mask delivery) with isoflurane and oxygen. It is placed in dorsal recumbency and the hair is shaved on both the right and left side in the region of the thoracic inlet. The head is extended so the ventral surface of the neck is fairly flat (horizontal) and the front leg on the side from which blood will be collected is directed laterally but not hyperextended. The clavicle should be palpated with the blunt tip of a hemostat to identify the necessary landmarks. The point of entry through the skin is 1 mm ventral to the clavicle and 1 cm lateral to the midline, immediately adjacent to the thorax. After swabbing the skin with alcohol, a ½ in., 22g needle attached to a 3 cc syringe is inserted into the skin. The needle is directed at a 45° angle relative to the midline and 10–20° from the horizontal plane of the table. The needle is advanced approximately 2 mm and gentle retraction of the plunger is applied while slowly withdrawing the needle. When a flash of blood is observed in the hub, the needle and syringe are stabilized and the desired volume is extracted, up to the maximum safe bleed. In a 150 gm degu, 1–2 ml of blood can readily be obtained by this method. Blood can be collected from either left or right subclavian vessels although we have been more successful with the right side. It has not been possible to select arterial vs. venous blood, and, in fact, arterial samples are more commonly obtained than venous samples.
P42 Use of Vascular Access Ports for Duodenal Access in Ferrets (Mustela putorius furo)

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A practical and reproducible method for intestinal access in the ferret was developed by direct catheterization of the duodenum. Gastric pH can potentially affect the stability and absorption of orally administered experimental compounds. Regionally targeted delivery of experimental compounds into the small intestine facilitates the evaluation of intestinal absorption by avoiding the effects of gastric pH. The use of intestinal catheterization to deliver experimental compounds into different locations of the gastrointestinal system has been reported in the dog and nonhuman primate.

Twelve male ferrets underwent a ventral mid-line laparotomy for exposure of the small intestine. The duodenum was isolated and catheterized through an enterotomy made just distal to the entrance of the common bile duct using a “burp” valve intragastric catheter (Model 5IGBS). The 5IGBS is a 5 French silastic catheter with a 7 mm diameter Dacron® mesh glued 0.5 cm from the distal catheter tip. The 5IGBS catheter was secured to the duodenum by monofilament nonabsorbable suture using an interrupted pattern. The catheter was then routed subcutaneously to the dorsolateral thoracic area and attached to a Vascular Access Port™ (Model SLA-AC).

Following catheter attachment to the Vascular Access Port™, the catheter was tested for substance administration into the duodenal lumen by bolus saline infusion. Both skin incisions were closed routinely and duodenal access was confirmed two weeks post-surgery by contrast radiography. Duodenal access persisted in all twelve ferrets throughout the 4-week study period without complications. Postoperative complications such as peritonitis or intussusception were nonexistent and maintenance was minimal. This suggests that duodenal access can serve as a method for the targeted delivery of novel compounds into the duodenum of ferrets.

P44 Validation of a Microport Model for Chronic Cerebrospinal Fluid Collection in the Rat

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The collection of cerebral spinal fluid (CSF) in pharmacokinetic studies provides critical data for evaluating compounds that cross the blood brain barrier. Manual puncture and external cannulation of the cisterna magna are techniques commonly used for CSF collection. The limitations of manual puncture include possible contamination of the CSF, potential spinal cord trauma, and limitations in the number of sample collection time points. The limitations of external cannulation include extensive invasive surgery, dislodgment of the device, and contamination with subsequent septicemia. We describe a method of surgical placement and maintenance of microports placed in the cisterna magna for chronic CSF collection in the rat. We compared the frequency and volume of collection of the microport model with an exteriorized cannulae model. In addition, histopathologic analysis on animals with repetitive needle puncture were performed. The pathology associated with the development of the microport, exteriorized cannulae, and repetitive needle puncture was determined. We found the microport compared favorably to previously published methods of CSF collection in both frequency and volume of fluid collects as well as minimal pathology. The microport also offers the advantage of ease of sample collection, purity of the sample and the ability to collect multiple samples over time. We conclude the use of microports has proven advantages for the collection of CSF in rats.

P43 A Novel Method for Continuous Lymph Collection in Conscious Dogs

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A novel method of lymph collection was developed for continuous sampling from beagle dogs that were instrumented with a chronic vascular access port (VAP). Lymph is often analyzed to detect hormone levels, inflammatory factors, cytokines, chemokines, and drug compound levels. A 3.5 Fr., 20 inch long heparin coated polyurethane catheter was placed into the jejunal trunk distal to the mesenteric lymph nodes. The catheter was attached to a VAP that was secured subcutaneously. The lymph fluid was collected by accessing the port via a 20 gauge right angle 0.5 inch tip Clear-view Huber infusion set connected to a six-inch extension set and 50 ml collection bag. This was secured to the animal’s abdomen using rolled gauze, porous tape and Vetwrap under a Lomir canine jacket. For instances in which the samples needed to be collected at a decreased temperature, a custom insulated pouch was designed to fit securely against the animal’s abdomen. This pouch holds reusable ice packs that keep the samples chilled without affecting the animal’s body temperature. The samples were collected between 15–18 degrees Celsius with the average at 16 degrees. Additional ice packs can be added if a lower collection temperature is needed. This system enabled lymph to be collected for up to six hours while the animal moved freely about its home cage (enclosed run). The collection bags and ice packs were changed hourly in order to maintain and analyze the lymph at a consistent temperature. The volume of lymph collected at each hourly interval varied from 1 ml to 14 mls per hour with the average total daily volume of approximately 7 mls. The subcutaneous VAP was accessed daily by prepping the site aseptically and kept patent with the use of heparinized saline flushes (1000 IU/ml). The advantage of this system is that it provides a minimally invasive approach to collecting lymph fluid continuously from a freely moving chronic beagle model.

P45 Comparison of Catheter Lock Solutions in Rats

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Rats with vascular catheters have been extensively used in pharmacokinetic studies. Once a catheter is implanted, patency life will be a concern for most investigators. One of the major factors which affects patency of a vascular catheter is the type of lock solution used. In this study the four most common heparinized lumen lock solution (in order of increasing viscosity), saline, 50% dextrose, glycerol and polyvinylpyrrolidone (PVP) were evaluated. Polyurethane catheters were implanted in the femoral veins of rats and locked with one of the four solutions. The patency of the catheters and lock solution status in the extravascular tubing were evaluated at 7, 14, 21, and 28 days post implantation. The patency was classified as 1) fully patent —successful withdrawal at first at-
CO₂ cylinder. The rack and control cabinet are connected by a control cabinet (20 in. wide x 20 in. deep x 40 in. high) and a steel rack (59 in. wide x 20 in. deep x 65 in. high), stainless steel. The fully assembled unit consists of a 20-cage capacity stainless cycle is completed to ensure that the animals are euthanized.

The animals to be observed by the animal caretaker after the euthanasia process. This novel CO₂ delivery device was designed to accommodate up to 20 polycarbonate cages of various sizes. Carbon dioxide is delivered to a rack of cages through a unit that controls timed cycles dictated by the size and species of animals to be euthanized. It was designed so that shoebox cages may be moved from an animal housing rack onto the CO₂ rack without moving the animals into another cage or chamber. This reduced animal stress and operator time. This method also allows the animals to be observed by the animal caretaker after the cycle is completed to ensure that the animals are euthanized. The fully assembled unit consists of a 20-cage capacity stainless steel rack (59 in. wide x 20 in. deep x 65 in. high), stainless steel control cabinet (20 in. wide x 20 in. deep x 40 in. high) and a CO₂ cylinder. The rack and control cabinet are connected by four flexible hoses connected to each tier of the rack. The CO₂ cylinder is also connected to the control cabinet by a flexible hose. The control unit contains all of the controls and indicators. The unit must be installed in a well ventilated area that does not use recirculated air. The unit must also be located near and easily accessible to a 110 VAC duplex outlet. The CO₂ rack can be washed in a rack washer or autoclaved. However, the control unit must be hand washed. This new system permits the simultaneous euthanasia of a large group of small animals, minimizes animal stress and significantly reduces operator time.

P46 A Simplified System for CO₂ Euthanasia of Small Animals

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A new, simple method to euthanize small animals with CO₂ was developed to replace an inefficient and cumbersome system. This novel CO₂ delivery device was designed to accommodate up to 20 polycarbonate cages of various sizes. Carbon dioxide is delivered to a rack of cages through a unit that controls timed cycles dictated by the size and species of animals to be euthanized. It was designed so that shoebox cages may be moved from an animal housing rack onto the CO₂ rack without moving the animals into another cage or chamber. This reduced animal stress and operator time. This method also allows the animals to be observed by the animal caretaker after the cycle is completed to ensure that the animals are euthanized. The fully assembled unit consists of a 20-cage capacity stainless steel rack (59 in. wide x 20 in. deep x 65 in. high), stainless steel control cabinet (20 in. wide x 20 in. deep x 40 in. high) and a CO₂ cylinder. The rack and control cabinet are connected by four flexible hoses connected to each tier of the rack. The CO₂ cylinder is also connected to the control cabinet by a flexible hose. The control unit contains all of the controls and indicators. The unit must be installed in a well ventilated area that does not use recirculated air. The unit must also be located near and easily accessible to a 110 VAC duplex outlet. The CO₂ rack can be washed in a rack washer or autoclaved. However, the control unit must be hand washed. This new system permits the simultaneous euthanasia of a large group of small animals, minimizes animal stress and significantly reduces operator time.

P47 Fixation and Histoprocessing Methods of Eyes in Dutch-Belted Rabbits

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Dutch-Belted rabbits are routinely used in ophthalmic toxicity studies for the development of age related macular degeneration treatments. Four protocols were evaluated for artifacts and morphologic detail. Perfusion fixation and immersion fixation were compared using 2% paraformaldehyde/2.5% glutaraldehyde in a 0.1M phosphate buffer (1/4 strength Karnovsky’s) or 1% formaldehyde/4% glutaraldehyde in a 0.1M phosphate buffer (McDowell-Trump). There were three animals per protocol that had their eyes enucleated and the lenses removed prior to immersion in fixative. The posterior segment of all eyes had two areas of retina and choroid trimmed, processed, and stained with Toulidine Blue. One section was embedded in glycol methacrylate and sectioned at 2 microns. The second section was embedded in epoxy resin and sectioned at 1 micron. The slides were evaluated for artifacts and morphologic detail. Although all slides were acceptable, the perfusion fixed tissue had greater morphological detail than the immersion fixed tissue. This study showed that for routine toxicology screens immersion fixation is adequate. In studies where the retina or choroid is a target, perfusion fixation with either McDowell Trump or 1/4 strength Karnovsky’s is the recommended method of fixation.

P48 A Comparison of Glutaraldehyde and Bouin’s Fixative when Examining Bladder Tissue by Light Microscopy, Immunohistochemistry, and Scanning Electron Microscopy

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It may be difficult in studies examining fixed tissue to choose a fixative that is optimal for each procedure. The number of animals could be increased so that the best fixative for each procedure could be utilized, however, this may not be practical when taking into consideration the 3 R’s in planning an animal experiment. In a short-term bioassay rat bladders and stomachs were collected for light microscopy, immunohistochemistry, and scanning electron microscopy (SEM). Half of the tissue from each group was fixed in Bouin’s fixative, and half was fixed in glutaraldehyde. Evaluation of sections by light microscopy stained with hematoxylin and eosin exhibited no differences. The problem occurs when attempting to perform immunohistochemistry and SEM on the same tissue. The bladder must be inflated with the fixative at the time of necropsy, which makes utilization of two fixatives impossible. Bouin’s fixative is preferred for immunohistochemical staining techniques because extensive pretreatment of the fixed tissue prior to staining is not required, and detection of labeled cells is obvious with few false negatives. In this study, all of the Bouin’s fixed stomachs, which serve as the positive control, were positive for BrdU immunohistochemistry. By SEM examination it was evident that increased cell proliferation had occurred in the treated bladders, however, the control group that was fixed in Bouin’s displayed definite signs of fixative effects, which included brittleness on gross observation, cracking of the bladder epithelium and cell separation. It is possible to evaluate Bouin’s fixed bladders by SEM as long as the effects are recognized prior to examination and a normal baseline has been established. Glutaraldehyde fixative is preferred for SEM because it penetrates the tissue more slowly without swelling and cracking of the cells. This allows examination of the bladder epithelium in normal tissue with less artifacts and tissue damage, however glutaraldehyde fixation makes immunohistochemical staining more difficult. In this study, both treated and control tissues that were fixed in glutaraldehyde had either weak or negative cell labeling. The staining technique was modified to increase trypsinization time and BrdU antibody concentration, but results were still poor. It appeared that the bladder tissue was separating; to increase trypsinization time further would cause more damage. More variations of the BrdU staining technique on glutaraldehyde fixed tissue need to be developed. The ultimate goal is to find a fixative that is compatible for all analyses to be performed.
P49 Elevated Glycemia and Local Inflammation following Marginal Ear Vein Injections of Pharmasolve® in Rabbits

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The objective of this pilot study was to evaluate glycemia in rabbits after different vehicles (drug solvents) used to dissolve poorly soluble drugs and to subsequently evaluate potential drugs for the treatment of diabetes. In the course of the study we found that one solvent was causing important negative side effects. Nine New Zealand White rabbits were used (Charles River, Canada). Different rabbits groups (n = 3) received intravenously: 1) saline 2) 2 IU/kg insulin (Humulin 100 U/mL) 3) 23–45% DMA/10% Cremaphor (cy) in water or 4) N-methyl-2-pyrrolidone (NMP) (Pharmasolve, ISP). Control rabbits receiving saline also received insulin (n = 2) and DMA (n = 1) with a 2 wk interval between injections. Blood (2 drops) was collected following a marginal ear vein puncture (22G needle) for the determination of blood glucose (Glucometer Elite) at pre-dose and at 0.25, 0.5, 1, 2, 4 and 24 h post-dose. Results show that glycemia following saline and DMA/Cremaphor injections remained stable and within normal (3.6–5.0 mmol/L) glycemia values (P = n.s.). Blood glucose decreased (1–2 mmol/L) as expected in insulin-injected rabbits (P < 0.001). Following NMP injections, glycemia was elevated (mean: 7.8 mmol/L 2 hrs post-dose) (P < 0.01) and only these animals were hyperactive upon injections. Injected ears gradually became bluish and 48 hrs following injections, they were edematous and necrotic. In conclusion, DMA/Cremaphor seems an acceptable vehicle for poorly soluble drugs, however NMP caused stress and local irritation upon injection and therefore cannot be recommended as a drug vehicle when injected intravenously in rabbits.

P50 Assessment of Pain in Laboratory Animals

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The recognition, assessment and effective alleviation of pain in animals should be an important objective in research facilities. Any procedures that cause pain or distress in humans may be assumed to cause pain in animals; however, consistent evaluation of post-procedural pain is often not rigorously performed, especially when large groups of animals are involved. To address the problem of inconsistent and subjective pain assessment, our university IACUC has widely advocated the use of pain scoring to determine the need for analgesic therapy or euthanasia. This program was adopted based on recommendations made by a university ad hoc committee formed to examine recognition and alleviation of chronic pain in animals. Scoring systems are developed based upon expected changes in an animal’s species-specific normal behavior and appearance induced by pain. Some of these criteria include activity, appearance, temperament, vocalizations, feeding behavior, physiological changes, and appearance and or use of the surgery site. Each protocol and species typically requires its own pain scoring system, and the frequency of observations and training of the persons making evaluations is specified for each protocol. The IACUC evaluates the measures to be taken when certain scores are exceeded and may ask to participate in scoring or be notified of outcomes. Pain scores are recorded on animal records to assist the IACUC and veterinary staff in gauging the outcome of protocols. Refinement of the initial pain scoring is often made based on variation noted between observers, poor predictive value of scored variables, or when intervention scoring levels require revision. Pain assessment scores have been developed for sheep, horses, dogs, guinea pigs, rats and rabbits for a variety of surgical and nonsurgical procedures. This system of defining an objective measure of animal distress provides a more accurate form of pain assessment for each animal undergoing procedures, allowing pain to be managed with a greater accuracy and consistency.

P51 Clinical Management of Buphthalmia with Novel Ophthalmics in a New Zealand White Rabbit

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A New Zealand White rabbit was identified with typical clinical signs of buphthalmia (corneal clouding, megaglobus) after inclusion on a surgical research protocol. Due to the need to hold this animal for 3 months, treatment with two human glaucoma ophthalmics drops (Trusopt®, Cosopt®, Merck & Co., Inc) were initiated to assess their effectiveness in management of this disease. Both drugs were applied twice a day with Cosopt to the right eye and Trusopt to the left eye. Intraocular pressures were taken initially and at two months with a pencil style tonometer. The initial pressures were: OD-22, OS-17 in the buphthalmic rabbit, and 8–10 for 2 comparative normal rabbits of similar age. At 2 months the intraocular pressures were OS-33 and unobtainable in the right eye due to the degree of corneal edema. Pressures at necropsy (1 month later) were abandoned due to the degree of corneal edema in both eyes at that time. In addition to the tonometer measurements, visual assessment of both eyes over the 3 month period indicated a progression of the buphthalmia based on progressive corneal clouding and megaglobus. Histopathology revealed increased corneal diameter, corneal edema, filtration angle obliteration, and cupping of the optic disc in both eyes. Based on this limited trial, the use of the commercial human glaucoma ophthalmic drops, Cosopt and Trusopt, are not recommended for control of intraocular pressures for buphthalmic rabbits.

P52 Sudden Death in a Neonatal Puppy

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A female Brittany spaniel/beagle cross (Canis familiaris) was born during an uneventful parturition. Three days after birth, the puppy was nursing, but not gaining weight as fast as its littermates, and it was then supplemented with proprietary milk replacer. Ten days after birth, the puppy was found dead. The pelves of both kidneys were grossly enlarged and filled with urine. Both ureters were thin throughout their entire length, and urine could not be manually expressed from either kidney into its respective ureter. The bladder contained no urine and was firmly embedded in the umbilicus. Histologically, hydronephrosis and hypoplastic collecting tubules were evident in both kidneys. The diameter of the right (0.55 mm) and left (0.57 mm) ureters at the ureteropelvic junction were narrow compared to an age-matched control of the same breed (1.03 mm and 1.02 mm). Trichrome staining of the ureter illustrated excessive collagen and disorganized smooth muscle fibers compared to the control, which had predominantly circular smooth muscle fibers and...
less fibrous tissue. Although blood could not be obtained for serum biochemistry, it is suspected that this puppy died from uremia. This case of congenital hydronephrosis is similar to a form of uteropelvic obstruction in humans, which results from stenotic ureters.

P53 Surgical Repair of a Cleft Palate on a *Macaca nemestrina*

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Congenital Birth Defects, commonly seen in human medicine, have also been observed in nonhuman primates. In March 1999, a female *Macaca nemestrina* was born at the Washington Regional Primate Research Center with a Cleft Palate. A surgical team was assembled from the Division of Craniofacial, Plastic & Reconstructive Surgery at a nearby hospital. The surgical technique to correct this birth defect in human infants was applied to the nonhuman primate infant and the surgery was successful. A Two (2) Flap Palatoplasty was performed to correct the animal’s Cleft Palate; i.e., the palatine tissue was elevated from the Buccal edge to the cleft. Then the edge of the cleft tissue was freshened, and sutured to close the cleft. After a four-week postop period, the nonhuman primate infant’s weight and size increased comparable to other in its sex and age group.

P54 The Management of an Antebrachial Wound in a Juvenile Rhesus Macaque (*Macaca mulatta*)

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A recently weaned, one-year-old, 2.05 kg female rhesus monkey was reported to the veterinary staff for a swollen right arm and anorexia. On physical examination this monkey had a body temperature of 105°F and marked swelling and inflammation of the right shoulder, arm, and hand. Mucopurulent exudate in the subcutis of the forearm drained from a fistulous tract that exited between the second and third phalanges. The exudate was cultured, the fistulous tract was lavaged with 0.9% saline solution, and the forearm and hand were bandaged. The monkey was also treated with procaine penicillin G, 40,000 IU/kg intramuscularly once a day pending culture and sensitivity results. Passive drain tubing was placed subcutaneously in the forearm to remove exudate and foreign material from the abcess cavity. Within 24 hours a 6.5 x 3.4-cm area of full thickness skin superificial to the drain tubing sloughed. The resultant open wound was managed with wet-to-dry bandages for two days to aid in necrotic tissue debridement and exudate removal. The adherent wet-to-dry bandages successfully removed necrotic tissue, but also damaged viable tissue. On the third day a non-adherent porous woven acetate dressing containing bisabolol and glycerine ointment was added as the primary bandage layer. The non-adherent dressing was easier to change daily, and it provided a moist sterile environment that enhanced the growth of healthy granulation tissue. Culture and sensitivity results yielded a coagulase positive *Staphylococcus* sp. that was sensitive to cefalozin sodium. Antibiotic therapy was changed to cefalozin sodium, 100 mg/kg, IM, q12 hours, for 10 days. By the seventh day after initial observation a granulation bed had formed. At this time the proximal and distal wound edges were apposed with 2–3 simple interrupted sutures; this closure was continued every other day 2–3 sutures at a time. Management of the open wound in this manner allowed it to be closed completely in 13 days, and the wound then healed uneventfully. Large open wounds on the extremities of nonhuman primates have historically been difficult to manage. The primary objective in open wound management is to achieve wound closure as soon as possible. Use of a non-adherent bandage promoted wound healing, decreased trauma to healthy tissue, and enabled the surgical apposition of viable wound edges, which hastened recovery time.

P55 Hypotensive Infarction of the Spinal Cord in a Rhesus Monkey (*Macaca mulatta*)

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A 6.5-year-old male rhesus macaque was presented with acute onset paraplegia following a six-hour surgical procedure. Anesthesia had been induced with xylazine, ketamine and atropine and was maintained with isoflurane at 1.5–2%. During the procedure, he experienced an acute hypotensive episode which required three hours of supportive therapy (intravenous fluids, Solu deltacortef, Dopram, Yohimbine and dexamethasone) before hemodynamic stability was restored. After 72 hours of additional supportive therapy (cystocentesis, frequent turning, subcutaneous and intravenous fluids, antibiotics, steroids and analgesics) there was no improvement. Paralysis, loss of bowel and bladder control, and failure to respond to deep pain persisted so he was euthanized. At necropsy, severe hemorrhage and necrosis was present in the muscles of the right hind limb and back. Hemorrhage and myelomalacia involving the central regions of the spinal cord was present from T10 to the cauda equina. On histologic examination, spinal cord segments T10 – S3 had acute massive pan-medullary necrosis, which involved the majority of the central and mid spinal cord, sparing the peripheral white matter. Additional lesions included arterial border zone necrosis of the brain, centrilobar hepatic necrosis and proximal renal tubular necrosis. The histologic picture of central spinal cord necrosis with a preserved rim of white matter is consistent with obstruction or severely decreased flow of the anterior spinal artery. Such lesions can result from episodes of generalized hypoperfusion. Several contributing factors were present in this case. After six hours of surgery, the animal underwent a severe hypotensive episode which required three hours of supportive therapy before hemodynamic stability was re-established. In addition, his hind limbs were placed at a 90° angle to the rest of the body, which may have reduced lumboperfusion. The animal was not moved during the surgical procedure, and the presence of hemorrhage and necrosis of the dependent musculature supports a diagnosis of muscular ischemia followed by reperfusion injury. The addition of xylazine to ketamine and atropine in the preanesthetic medication may have contributed to hypotension. It is likely that monitoring and maintenance of blood pressure and repositioning during surgery could have prevented spinal cord necrosis.

86 CONTEMPORARY TOPICS © 2000 by the American Association for Laboratory Animal Science Volume 39, No. 4 / July 2000
Baboons (*Papio anubis*) are being utilized in deep venous thrombosis (DVT) research because of their analogous vasculature to humans, comparable coagulation system, and their upright physical posture. Our research focuses on how antithrombotic and anti-inflammatory drugs affect normal coagulation activities. After a 90-day quarantine period we evaluated baseline coagulation data from 36 wild-captured juvenile baboons weighing 5.0–8.3 kg. Mean values were total leukocyte count—6.8 $10^9$/cm^3^, hemoglobin—12.6 g/dL, activated partial thromboplastin time (aPTT)—30.7 seconds, thrombin clotting time (TCT)—15.0 seconds, fibrinogen—124 mg/dL, and platelet count—280 $10^9$/cm^3^. Plasma fibrin degradation products (FDP) were found to be less than 5 µg/mL, and D-dimer values were less than 0.25 µg/mL. Also, the bleeding time range was 1–3 minutes (n = 24). Past literature documents that the mechanisms of blood coagulation in baboons closely resembles that of humans. However, a literature search found no data since 1986, and no reference values for FDP, D-dimer, or bleeding time. Furthermore, there have been no citations of baseline coagulation data for wild-caught baboons. Our laboratory needed baseline coagulation data for this species and population in order to evaluate the effects of antithrombotic and anti-inflammatory drugs. Presently, it is difficult to obtain captive-bred juvenile baboons of uniform age, sex, and weight that are surgically and chemically naive. Therefore, investigators must increase their use of wild-caught animals to further research concentrating on coagulation abnormalities, DVT, or venous insufficiency studies.

**P56 Coagulation Reference Values for Wild-Caught Juvenile Baboons**

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Baboons (*Papio anubis*) are being utilized in deep venous thrombosis (DVT) research because of their analogous vasculature to humans, comparable coagulation system, and their upright physical posture. Our research focuses on how antithrombotic and anti-inflammatory drugs affect normal coagulation activities. After a 90-day quarantine period we evaluated baseline coagulation data from 36 wild-captured juvenile baboons weighing 5.0–8.3 kg. Mean values were total leukocyte count—6.8 $10^9$/cm^3^, hemoglobin—12.6 g/dL, activated partial thromboplastin time (aPTT)—30.7 seconds, thrombin clotting time (TCT)—15.0 seconds, fibrinogen—124 mg/dL, and platelet count—280 $10^9$/cm^3^. Plasma fibrin degradation products (FDP) were found to be less than 5 µg/mL, and D-dimer values were less than 0.25 µg/mL. Also, the bleeding time range was 1–3 minutes (n = 24). Past literature documents that the mechanisms of blood coagulation in baboons closely resembles that of humans. However, a literature search found no data since 1986, and no reference values for FDP, D-dimer, or bleeding time. Furthermore, there have been no citations of baseline coagulation data for wild-caught baboons. Our laboratory needed baseline coagulation data for this species and population in order to evaluate the effects of antithrombotic and anti-inflammatory drugs. Presently, it is difficult to obtain captive-bred juvenile baboons of uniform age, sex, and weight that are surgically and chemically naive. Therefore, investigators must increase their use of wild-caught animals to further research concentrating on coagulation abnormalities, DVT, or venous insufficiency studies.

**P57 Hepatocystosis in a Baboon (*Papio anubis*)**

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A 26 lb. adult female baboon was obtained from a commercial vendor 5 months after she was wild-captured in Kenya. The animal was healthy, and routine tests for parasites, pathogenic bacteria, and tuberculosis were negative throughout the 10-week duration of quarantine. However, routine radiographs performed at the end of the quarantine period detected multiple small radio-opaque nodules evenly dispersed throughout the liver. Differential diagnoses included tuberculosis and chronic hepatitis due to parasites or parasite migration. Due to concerns about possible contagious diseases, her entire quarantine group was retained in quarantine. A liver biopsy was taken during a subsequent ovarioectomy, and a diagnosis of granulomatous hepatitis was made based on histopathology. No cause was definitively identified, but *Hepatocystis* spp. infestation was considered a likely etiology. Serology taken at the time of surgery was positive for hepatitis A virus IgG antibody. However, since no live organisms were found in the liver biopsy, and the lesions were calcified, she was released from quarantine. The baboon was euthanized approximately one year after her arrival for unrelated reasons. Necropsy findings were limited to hepatic changes. The liver contained multiple (40–50) 1–3 mm diameter white foci, visible on its surface, as well as throughout the parenchyma. There was multifocal, moderate capsular fibrosis, with adhesions between the hepatic lobes and between the diaphragm and liver. On histologic examination, multiple degenerate merocysts were present. Remaining merocysts were seen as a 1–2 mm sphere, composed of an outer granular amphophilic hyaline wall (approximately 100 um thick), surrounding basophilic granular material. In all cases, the parasite was surrounded by a robust tissue reaction composed of layers of eosinophils, macrophages, giant cells and fewer lymphocytes. A diagnosis of *Hepatocystosis* was made. *H. kochi* and *H. simiae* are malarial-type non-pathogenic protozoa endemic to Old World non-human primates, including baboons. In this case, most parasitic lesions were residual in nature, and only one hypermature merocyst was identified in four liver sections examined. Typically, these animals which harbor mature merocysts have gametocytes detectable on blood smear; none were seen in blood smears from this animal, confirming that this was an old inactive infection. Although the infection is very persistent, it is non-pathogenic. Infected animals are asymptomatic and do not experience hemolysis. Transmission requires an insect vector, and consequently, *Hepatocystis* infection has minimal implications for colony health. There is no known danger of transmission to humans.

**P58 Pituitary Adenoma with Resulting Lactation in an Adult Male Cynomolgus Macaque**

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Endocrinopathies are relatively well documented in many animal species, however a recent case presented an interesting diagnostic challenge. An adult, male, wild-captured cynomolgus macaque (*Macaca fascicularis*) presented for cervical dermatitis. This particular animal had been fitted with an aluminum collar one month prior to presentation to allow chairing for behavioral assessments. Previous physical examinations had been unremarkable with the exception of a Grade 2 systolic murmur. At the time of presentation, the animal was involved in an IACUC-approved study during which he was subjected to water restriction. Intermittent assessments of hydration according to an approved SOP had been within normal limits. At presentation, the animal exhibited an ulcerative dermatitis primarily on the dorsal cervical area. During the assessment, urine was collected via cystocentesis for analysis. The findings were within normal limits, with the exception of the findings of glucosuria. Blood was drawn for a complete blood count and serum chemistries. A mild hyperglycemia was noted. At a follow-up examination one week later, a fasting glucose confirmed hyperglycemia. Also, a white, flaky material was noted surrounding the nipples and a white milky substance could be expressed from both nipples. Cytology confirmed lactation. Blood was drawn for a complete endocrine panel as well as a glycosylated hemoglobin. Elevated cortisol and prolactin levels were noted, indicative of a presumptive diagnosis of pituitary-dependent Cushings Syndrome. The glycosylated hemoglobin was suggestive of uncontrolled diabetes. Ultrasonic evaluation of the adrenals was unremarkable. In light of these findings, a decision was made to euthanize the monkey.

Gross necropsy findings included white, liquid fluid seeping from the tissues overlying the pectoralis muscle. The heart was enlarged and lacking in normal muscular tone. The pituitary was visibly enlarged, but no discrete mass was visible. On histopath, the mammary glands showed mild hyperplasia of glandular elements, lined by hypertrophied epithelial cells,
and containing scant proteinaceous droplets in the lumen of the glands and ducts. The pancreatic islets were expanded by large accumulations of pale eosinophilic material, consistent with amyloid deposition. The normal architecture of the pituitary was focally expanded by a poorly circumscribed mass of cuboidal to polygonal cells, arranged in irregular sheels and nests and separated by fine fibrovascular stroma. Cells were moderately pleomorphic, and had modest amounts of paley eosinophilic cytoplasm. Immunocytochemical stains of the sections of the pituitary mass showed positive cytoplasmic staining of the cells within the mass by antibodies to prolactin, but not ACTH, GH, LH, FSH or TSH. These findings were consistent with a prolactin secreting pituitary adenoma.

Reports of diabetes in nonhuman primates are relatively common. Cushings Syndrome is less commonly reported. A recent report documented a similar case in which Cushings Syndrome was present in conjunction with diabetes mellitus. However, this is the first report of a pituitary-dependent Cushings Syndrome resulting in hyperprolactinemia in a male macaque. The unique features of this case and its similarities to reported human cases will add to the body of knowledge of endocrinopathies in nonhuman primates.

P59 Mammary Ductal Adenocarcinoma in an Elderly Rhesus Macaque

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Examination of a 23-year-old female rhesus macaque revealed a lobulated mass 2.5 cm in diameter under a right accessory nipple. Diagnostic plans included chest radiographs, blood screening, and needle aspirate cytology. Chest radiographs were within normal limits. Blood screens showed minimal chemistry variations and normal hematology. Cytology was highly suspicious of neoplasia. Surgical excision and biopsy of the mass were performed. Grossly the mass was well circumscribed, firm, homogeneous tan, with glandular appearance. Differential diagnoses included hyperplasia, carcinoma in situ, and interstitial or ductular adenocarcinoma. Histopathologic diagnosis was mammary ductular hyperplasia, carcinoma in situ, and interstitial or ductular adenocarcinoma. Two weeks later there was swelling in the region of the right axillary lymph nodes. Cytology showed tumor cells and few lymphoid cells, interpreted as re-growth or lymphatic spread. Chest radiographs two and six weeks post-surgery showed no evidence of pulmonary metastasis. Mammary neoplasia is a major concern in human medicine, which is rarely reported in nonhuman primates, only ten cases have been reported in rhesus macaques. This case is important in characterizing and reporting another mammary tumor in a rhesus macaque, and monitoring clinical response of the animal.

P60 Intracranial Lymphomas in Simian Retrovirus-Positive Cynomolgus Monkeys

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Primary brain lymphoma is the most common Central Nervous System (CNS) neoplasm in immunosuppressed human patients. In humans, the majority of these tumors are of B cell origin and the neoplasm appears to contain Epstein-Barr virus (EBV) genomic material within the transformed B cells. This neoplasm has rarely been reported in immunocompetent nonhuman primates. The observed increase in frequency of malignant lymphomas in Simian Immunodeficiency Virus (SIV)-infected monkeys as well as Human Immunodeficiency Virus (HIV)-infected patients indicates an important pathogenic role of virally-induced immunosuppression for lymphoma development. Although Simian Retroviruses (SRV) are not related to lentiviruses such as HIV and SIV, the disease syndrome induced by SRV in macaques resembles AIDS in humans. Presented are two male Macaca fascicularis of Philippine origin. Animal #1 was 7 years old, and presented with unilateral ptosis, mydriasis, and ventrolateral strabismus of the right eye. Based on neurologic and ophthalmal examinations, oculomotor nerve [Cranial Nerve (CN) III] paresis was diagnosed. Differential diagnoses included neoplasia, inflammation, infarction, and hemorrhage, with the lesion localized to the right CN III. Five weeks after the eye lesions were observed, the animal developed left hemiparesis and circling to the right. Due to a poor response to treatment and apparent extention of the CN III lesion to involve the right midbrain and cerebral cortex, the animal was euthanized. Animal #2 was 6 years old, and presented with moderate to severe piosis and swelling of the right eyelid following intradermal administration of Mammalian Old Tuberculin in the same eyelid. The animal was euthanized due to a suspected Mycobacterium sp. infection, subsequently shown to be M. avium. Antemortem serology revealed both monkeys were seropositive for EBV Viral Capture Antibody and measles, and seronegative for antibodies to Simian T-cell Leukemia Virus type 1 (STLV-1), SRV and SIV. Virus isolation from both monkeys was positive for SRV and negative for SIV (whole blood samples). At necropsy of both animals, there were intracranial single masses attached to the ventral surface of the right midbrain, surrounding the right CN III. Histologically, each mass was composed of neoplastic lymphoid cells, and a diagnosis of lymphoma was made. Immunohistochemistry was done using the following antibodies, to rule out other intracranial round-cell neoplasms: Neuron Specific Enolase, Vimentin, and Glial Fibrillary Acidic Protein Antibodies. Immunostaining using B- (BLA 36) and T- (CD3) cell markers revealed a predominantly B-cell population in one neoplasm (Animal #2), and a mixed B- and T-cell pattern in the other (Animal #1). Based on histopathologic examination and immunohistochemical staining, lymphoma was confirmed. Concurrent viral infection with EBV and SRV may have contributed to the development of these neoplasms. This association of viral infection and lymphoma parallels that seen in HIV-infected human patients.
Gastrointestinal Helicobacter Infection in a Rhesus Macaque

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Helicobacters are important pathogens in animals and humans. A four-year-old male rhesus monkey (Macaca mulatta) presented with sudden onset of severe diarrhea, inappetence, inactivity and slow responsiveness. Previous tests for B-virus, SIV, STLV-1 and tuberculosis had been negative. Unilateral nephrectomy had been performed sixteen months previously for donor purposes in a renal transplant study. Subsequently, the animal was clinically normal except for occasional bouts of mild diarrhea and one episode of emesis. Repeated diagnostic tests (CBCs, fecal cultures for aerobic bacterial pathogens, fecal exams for parasites, abdominal radiographs, tuberculin tests) had given negative results. Tests performed at time of the acute illness revealed: marked leukocytosis, neutrophilia, lymphopenia and radiographic evidence of gas distended intestines. On exploratory laparotomy fibrous adhesions were found occluding the mesenteric blood supply to the small bowel which was extremely congested, distended with gas and leaking intestinal contents into the peritoneal cavity. Euthanasia and necropsy were performed. Histopathologic findings included: severe congestion, edema and hemorrhage of jejunum and ileum compatible with early infarction; severe lymphoplasmacytic and proliferative gastritis with glands containing an abundance of silver positive curved rods consistent with Helicobacter pylori (PCR tests were positive for H. pylori, negative for Helicobacter bizzozeroni); and moderate, diffuse lymphoplasmacytic, proliferative cecoccolitis with massive numbers of silver positive spirillum rods closely associated with glandular and luminal epithelium (identified as Helicobacter sp. by PCR). We concluded that abdominal distension in this animal was most likely due to bowel ischemia secondary to vascular obstruction. However, severe Helicobacter infection of both the stomach and large intestine could have been contributing factors.

P62 Gastrointestinal Helicobacter Infection in a Rhesus Macaque

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A 12-14-year-old intact female rhesus macaque was presented for necropsy evaluation after dying unexpectedly overnight. The only ante mortem signs were inappetence and the onset of menses noted the prior afternoon by the subject’s caretaker. The subject had been part of a diuresis study since 1994 and had no prior history of any medical problems. She had been receiving an experimental kappa opioid antagonist IM once per week for the duration of the study. Differential diagnosis included a cerebrovascular accident and cardiac arrhythmia. On gross examination the subject had good body condition, moderate dental tartar and gingivitis, and mild bruising on the ventral abdomen. Upon opening the abdominal cavity a strong fetid odor was noted. The peritoneal cavity contained 40–50 ml of a white exudate, and the visceral and parietal peritoneal surfaces were covered with a white sticky substance that loosely adhered the viscera together. The serosal surface of most of the abdominal organs was also covered with multifocal coalescing red spots. Multifocal, red, fluctuant, bosselated masses with a smooth, irregular surface, ranging in size from 1 to 5 cm, were located throughout the omentum. In several areas round, smooth, brown, fluid filled masses 0.5–1.0 cm were seen in the wall of the small intestine and uterus. An area where the intestinal loops were tightly adhered to one another in the distal third of the small intestine was also noted. In this area, a 1.0 cm rent in the intestine surrounded by necrotic tissue was observed on the antimesenteric border. Several of the cystic masses were also noted in the intestinal wall near the rent. No foreign bodies were found in the intestines. The cause of death was determined to be septic peritonitis secondary to a small bowel rupture. The bosselated and cystic masses were identified as endometrial cysts by histopathology. We believe that the rupture of an endometrial cyst was responsible for the tear in the bowel wall. The mechanism of action may have been either a direct perforation or the formation of an adhesion which later dissected the intestinal wall. The unique features of this report are the lack of ante mortem signs and the tearing of the bowel wall secondary to endometriosis. Schaerdel (1986) had previously reported death in a rhesus macaque from colonic infarction due to endometriosis. To our knowledge this is the first report of a bowel rupture precipitated by this disease.
P65 Study on Physiological Profiles between Obesity-Susceptible and Obesity-Resistant in Sprague Dawley Rats

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The primary goal in the present study is to define peripheral and central physiological differences between dietary obesity-susceptible (DOS) and obesity-resistant (DOR) Sprague Dawley (SD) rats. In order to compare several physiological parameters between two types of SD rats when given a high fat diet (50% of calories from fat), we examined body gain, feed and energy intake, weight of fat mass, serum biochemical components, expression of leptin receptors and neuropeptide Y (NPY) in the hypothalamus, and expression of adipogenic factors in the liver and adipocytes. After feeding a high fat diet for 8 weeks (a total of 40 rats), 15 rats per group were designated as obesity susceptible (the upper body weight) and resistant (lower weight) by pair, respectively. The DOS-SD rats consumed significantly more feed and energy than the DOR-SD rats. The DOS-SD rats had higher epididymal and retroperitoneal fat pads compared with the DOR-SD rats. However, we did not observe any differences in triglyceride, cholesterol, glucose, and insulin levels in serum. The next stage was to determine whether leptin levels in serum and expression of leptin receptors and neuropeptide Y (NPY) in the hypothalamus were affected by the genetic basis of trait. No difference in serum leptin concentrations were found between the two groups. Also, the expression of leptin receptors and NPY in the arcuate, supraoptic and paraventricular nuclei of the hypothalamus by mRNA semiquantification and immunohistochemical assay were similar between the DOS-SD and DOR-SD rats. In order to examine de novo fatty acid synthesis in the liver and adipocytes, we tested acetyl CoA synthase (AC3) mRNA expression and fatty acid transcriptional factor (peroxisomal proliferated activated factory, PPARy). The DOR-SD rats had dramatically reduced expression of ACC in the liver and adipocytes, whereas expression of PPARy was significantly enhanced compared with the DOS-SD rats. We conclude that increased de novo fatty acid synthesis in the peripheral system was an important factor in the development of dietary obesity in the DOS-SD rats.

P66 Age- and Sex-Related Changes in Plasma Copper and Zinc Levels in Mongolian Gerbil

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Zinc (Zn) and copper (Cu) are two oligoelements that participate in a wide variety of enzymes. There is little information on plasma oligoelements in Mongolian gerbil. In this work we have studied the age and sex-related differences in Zn and Cu plasma values in healthy Meriones unguiculatus. Male (n = 30) and female (n = 30) gerbils were allocated in three groups of age: group A (90 days old), group B (180 days old), and group C (360 days old), with 10 males and 10 females in each age group. The animals were kept in polycarbonate type III cages under standard environmental conditions (temperature: 22 ± 1°C, higrometry: 55 ± 10%, 12 air renovation/hour, 12:12 h light/dark cycle). Gerbils were given free access to pelleted rodent maintenance diet and water. The concentration of Zn in water was undetectable and Cu was less than 0.45 mg/l. In the diet, levels of Zn and Cu were 95 mg/kg and 30 mg/kg, respectively. Blood samples were taken through cardiac puncture as a terminal procedure under anesthesia (60 mg/kg pentobarbital IP). Plasma was obtained by centrifugation and frozen (-20°C) until analysis. Cu and Zn analyses were performed, after dilution in Millipore MilliQ deionized water, by flame atomic absorption spectrophotometry in air/acetylene flame, with a Smith-Hieftje 11, and all measurements were carried out in triplicate. With regard to Cu plasma values, we have obtained higher values in females (2.03 ± 0.59 μg/ml) than in males (1.30 ± 0.31 μg/ml), with high statistical significance (P < 0.0001). In males the lower values were shown in the group B (180 days old), nevertheless, in females the lower values obtained in group A (90 days old). In relation with Zn plasma values, we have not shown differences between sexes (males: 2.96 ± 0.47 μg/ml; females: 2.96 ± 0.50 μg/ml) neither between age groups. In general, plasma Cu and Zn values are higher in gerbils than in rats. We concluded that gerbils could be an interesting model for the study of these oligoelements.
P67 Postoperative Mortality in a Guinea Pig Ototoxicity Model

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Postoperative mortality occurred in Hartley Guinea pigs after a stereotaxic procedure was performed to measure auditory brainstem responses. The guinea pigs were induced with and maintained by mask on isoflurane. Electrodes were placed in the occipital and mastoid regions and into the tympanic bulla. Buprenorphine was given postoperatively. Guinea pigs recovered from anesthesia but died within 7 to 24 hours after recovery. One Guinea pig was treated with furosemide and dexamethasone for mild respiratory distress, died 48 hours later. There was yellow-green dried material around the nares and the lungs were diffusely firm and red. On histologic examination, a fibrinopurulent pneumonia with small refractive pale yellow to green material was observed. Histologic examination of lungs from a second Guinea pig that died two days postoperatively revealed diffuse fibrinopurulent pneumonia with refractive pale yellow material in the exudate. Examination of lungs with polarized light helped define plant-like architecture of the refractive material. The diagnosis was acute foreign body pneumonia with adult respiratory distress syndrome (ARDS), a syndrome in which death results from decreased perfusion and hypoxia from extravasation of fluid and cellular necrosis of the alveolar-capillary unit.

P68 Phylogenetic Relationship of CAR Bacillus Isolates from Several Species

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The cilia-associated respiratory (CAR) bacillus is an unclassified bacterium that has been associated with chronic respiratory disease in laboratory rodents. The bacterium’s name refers to its interdigitation among the cilia of the respiratory epithelium. Based on 16S rRNA gene analysis, rat isolates of CAR bacillus are most closely related to Bacteroides-Cytophaga-Flexibacter group of bacteria. Morphologically similar bacteria have been reported in mice, rabbits, pigs, cows, and goats, however, the genetic relatedness of these isolates has not been established. In this report, complete sequences of 16S rRNA genes from six rat isolates, one mouse isolate, one rabbit isolate, four pig isolates, and two cow isolates were obtained and compared. Sequences from rat and mouse isolates were 100% identical. Isolates from pigs and cows were closely related to each other (95–99% sequence similarity), and to rodent isolates (85–87% sequence similarity). The rabbit isolate was distantly related as evidenced by less than 70% sequence similarity to rodent and livestock isolates. These findings suggest that isolates of CAR bacillus from livestock and rodents are phylogenetically related and likely represent different species of the same genera. Rabbit CAR bacillus, however, may belong to a separate genera.

P69 Pathology and Clinical Pathology Interpretation of Genetically Altered Mice

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The use of genetically altered mouse models has become an important tool in many areas of medical research. Since many human diseases are the result of multiple genetic and environmental factors, the development of mouse modeling technology that allows for targeted genetic manipulations to create mice that are homologous to genetically based human diseases is a logical approach. The interpretation of pathology and clinical pathology is critical not only during the development of the genetically altered mouse model but also in the use of that genetically altered mouse in various types of medical research, especially carcinogenesis testing. Early in the development of each mouse model, characterization should include a fairly complete battery of tests including hematology, clinical chemistry, urinalysis, complete gross necropsy, organ weights, collection of tissues and histopathologic evaluation of tissues collected. Using the information derived from this initial examination, it may be necessary to pursue special histopathology, molecular pathology, bone marrow evaluation, or immunoprofiling by flow cytometry in order to completely characterize the new mouse model. It is also critical to have a good understanding of these same parameters for the background strain of the new mouse model. Genetic background modulates expression of the mutant phenotype through modifier loci and determines mutation-independent phenotypes. Currently under investigation for use in short term carcinogenesis bioassays are Tg.AC and p53 +/- mice. These are tumor models which provide a relatively straightforward example of a gene background effect. Although unusual and unique neoplasms may arise that are specific for the genetic alteration, the overall spectrum of tumor types that occur during the testing period tend to follow that of the background strain. Tumor modifier loci, such as those in the p53 +/- and Tg.AC mice, can also affect aspects of tumor phenotype such as multiplicity, size and vascularization. Background strain effects can also produce variability in phenotypes other than neoplasia such as time to death as seen with the epidermal growth factor receptor knockout mice. Therefore, an understanding of the background mouse strain assists the pathologist in the evaluation of the reaction of the test system to the test material. Clearly, clinical pathology and pathology interpretation of studies using genetically altered mouse models require a complete characterization and understanding of the mouse model as well as the background strain of the genetically altered mouse.

P70 Clinical Chemistry and Hematology Reference Values of Selected Transgenic Mice

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CETP, ApoB100, sPLA2 and TNFalpha are transgenic mouse lines carrying microinjected human transgenes, (cholesterol ester transfer protein, apolipoprotein b100, Group 2 Phospholipase A2, and TNF alpha, respectively), on C57BL/6, (or C57BL/6 x SJL), backgrounds. Each of these transgenic lines have well-defined target effects on the host blood chemistry. To further define these models and investigate possible secondary effects of the transgenic manipulation, whole blood and serum samples from animals of each of these lines were analyzed for thirteen hematologic parameters and twenty-five clinical chemis-

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try panels. The results from these panels were tabulated and examined. It can then be seen that the transgenic lines all have decreased levels of certain parameters. Namely, these are alkaline phosphatase, phosphorous, potassium, lipase, and GGTP. Other items of note are an increased level of cholesterol in the ApoB100 and a decreased level of tri-glycerides in the splA2 models. The full results of these analyses are presented and compared with non-transgenic controls, to serve as reference values for further investigation and comparison of these lines.

P71 Hepatic Lesions in Guinea Pigs Infected with Helicobacter cinaedi

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A high incidence of hepatic lesions were found in guinea pigs of breeding colonies and research colonies in Japan. Grossly, the livers had multiple pale to yellow foci 0.5 to 2 mm in diameter. Histologically, multifocal coagulative necrosis of hepatocytes with minimal inflammatory cell infiltration was observed. The organisms were isolated from the large intestines of these guinea pigs and found to be spiral-shaped bacteria with bipolar sheathed flagella by electron microscopy. On the basis of biochemical and 16S rRNA gene sequence analysis, the organism was identified as Helicobacter cinaedi. The hepatic lesion was reproduced in Helicobacter-free guinea pigs and scid mice that were experimentally inoculated with the isolate of H. cinaedi. Infected scid mice also developed moderate to severe proliferative typhlocolitis. The novel Helicobacter species was detected by PCR in the feces of all experimentally infected guinea pigs and scid mice. H. cinaedi was suspected to be a potential pathogen in guinea pigs and scid mice. Several rodent helicobacters have been associated with hepatitis and inflammatory bowel disease. This is the first observation that H. cinaedi can induce hepatitis in guinea pigs and scid mice.

P72 A Novel Urease Negative Helicobacter Species of Mice Persistently Colonizes Sprague Dawley Rats

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A novel urease-negative Helicobacter species has been shown to cause severe cholangiohepatitis within 7 weeks of experimental inoculation in mice. However, fibrosis is not a prominent feature of the lesion in mice. There are numerous experimental models of hepatic fibrosis in the rat. The objective of this study was to determine if this novel urease-negative Helicobacter species could cross the species barrier and colonize rats, in search of a better model for hepatitis with hepatic fibrosis. Four 4-week-old, male Sprague-Dawley rats were dosed intraperitoneally and orally with 10^9 colony forming units of a broth culture of the novel urease-negative Helicobacter species on days 1, 3, and 7. One rat was sham-dosed with saline. The sham-dosed rat was housed separately until 3 days after the final inoculation, when it was co-housed with the other rats to determine if natural transmission of the novel urease-negative Helicobacter species could occur. Fecal samples were collected weekly for culture and PCR analysis. Rats were necropsied at 8 weeks post-infection (WPI). The novel Helicobacter species was detected by PCR in the feces of the sham-dosed rat from 1 WPI onwards, although it was not detected by culture at any time point. The novel Helicobacter species was detected by both PCR (2 WPI onwards) and culture (3 WPI onwards) in the pooled feces of the experimentally infected rats. At necropsy, the Helicobacter species was cultured from the liver of one experimentally infected rat and the ceca of two experimentally infected rats. It was detected by PCR in the feces of all of the experimentally infected rats. Histopathology revealed mild cholangiohepatitis, characterized by a mixed inflammatory cell infiltrate, in the livers of the experimentally infected rats, the novel Helicobacter species in 3 of the 4 experimentally infected rats, demonstrating that it could experimentally colonize this species. Detection of Helicobacter in the feces of the sham-dosed cage contact rat was consistent with natural fecal-oral transmission. Although the experimentally infected rats became persistently colonized, the liver lesions they developed were milder than those reported in mice at the same time point, suggesting that they would not make a superior model to mice.

P73 Gastric Zygomycosis in an Ifl1 Knockout Mouse

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An adult female mouse with a targeted mutation of the interferon regulatory factor 1 (Ifl1) gene was submitted to the University of Missouri Research Animal Diagnostic and Investigative Laboratory. Upon physical examination, mild bilateral conjunctivitis was noted. At necropsy, there was multinodular enlargement of the stomach with adhesions to the liver and abdominal body wall. The gallbladder was markedly dilated, as a result of bile duct obstruction by the enlarged stomach. On histologic examination, there was severe multinodular pyogranulomatous gastritis with giant cells, programuloma formation and intralesional fungal hyphae. On silver-stained sections, thin-walled, non-septate, non-dichotomous branched fungal hyphae with non-parallel walls were identified. These features are consistent with zygomycosis, a mycotic infection caused by 11 morphologically similar species of zygomycetes that are classified into 8 genera. Zygomycosis has been reported in several species and is especially common in immunosuppressed individuals. The lack of the Ifl1 gene may have rendered this mouse more susceptible to fungal infection. This conclusion is based on the fact that mice lacking Ifl1 do not efficiently mount type 1 immune responses which are critical in protection against fungal infections. To the authors’ knowledge, this is the first reported case of gastric zygomycosis in mice.

P74 Detection of Rodent Parvoviruses by Fluorogenic 5’ Nuclease Assays

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PCR assays have proven useful for the detection of rodent parvoviruses in animals and contaminated biomaterials, however PCR assays are relatively labor-intensive and prone to carry-over contamination. Fluorogenic 5’ nuclease assays (FFPNA) combine PCR with an internal fluorogenic hybridization probe, thereby enhancing specificity and eliminating post-PCR processing. Consequently, three FFPNA assays were developed, one to detect all rodent parvoviruses, one specific for minute virus of mice (MVM), and one specific for mouse parvovirus-1 (MPV). Primer and probe sequences were selected from an alignment of all known rodent parvovirus sequences. Each assay was then tested against 7 different rodent parvovirus isolates and other rodent DNA viruses. The genus-specific assay detected only the 7 rodent parvovirus isolates, while the MVM- and MPV-specific assays detected only MVM or MPV isolates,
respectively. All FFPNA assays met or exceeded the sensitivities displayed by PCR assays and mouse antibody production tests currently used to detect MVM and MPV. Each FFPNA assay was also able to detect the targeted parvoviral DNA in tissues and feces obtained from mice infected with MPV or MVM. These studies indicate that FFPNA assays should provide a high-throughput, PCR-based method to detect rodent parvoviruses in both infected mice and contaminated biomaterials.

P75 Development and Validation of a Fluorogenic 5' Nuclease Assay Specific for Rodent Coronaviruses
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Reverse transcriptase-polymerase chain reaction (RT-PCR) assays have proven useful for the detection of mouse hepatitis virus (MHV) and rat coronavirus (RCV) in acutely infected animals and contaminated biomaterials, however RT-PCR assays are relatively labor-intensive. Fluorogenic 5’ nuclease assays (FFPNA) combine RT-PCR with an internal fluorogenic hybridization probe, thereby eliminating post-PCR processing. Consequently, an FFPNA assay specific for rodent coronaviruses was developed. Primer and probe sequences were selected from an alignment of rodent coronavirus M gene sequences available through GenBank. The FFPNA assay detected all strains of MHV and RCV that were evaluated (total of 6), but did not detect other RNA viruses that naturally infect rodents. The assay detected less than 1 tissue culture infectious dose 50 for multiple strains of MHV, and when compared directly was more sensitive at detecting MHV than mouse antibody production tests. Finally, the assay detected coronaviral RNA in tissues and feces obtained from mice experimentally infected with MHV, and also in tissues and cage swipes obtained from rats naturally infected with RCV approximately 1 week prior to seroconversion. These studies indicate that this FFPNA assay should provide a high-throughput, PCR-based method to detect rodent coronaviruses in both infected rodents and contaminated biomaterials.

P76 High-Throughput Diagnostic Assays for the Detection of Helicobacter hepaticus and H. bilis in Laboratory Rodents
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PCR is commonly used to detect Helicobacter spp. infections in laboratory rodents, however PCR is relatively labor intensive and false-positive/negative results are problematic. Fluorogenic 5’ nuclease assays (FFPNA) combine PCR with an internal fluorogenic hybridization probe, thereby enhancing specificity and eliminating post-PCR processing. Therefore, FFPNA assays specific for Helicobacter hepaticus and H. bilis were developed to provide high-throughput diagnostic assays for these pathogenic bacteria. Primer and probe sequences for each assay were selected from an alignment of 16S rRNA gene sequences for all Helicobacter species listed in the GenBank database. The sensitivity of each assay was comparable to that displayed by current PCR assays, and each assay was shown to be specific for the targeted Helicobacter species through evaluation of DNA extracted from numerous Helicobacter, Campylobacter, and other enteric bacterial species. Target DNA was detected by each FFPNA assay in colon tissues and feces both in naturally infected mice and in mice experimentally infected with either H. hepaticus or H. bilis. These studies indicate that FFPNA assays should provide sensitive and specific high-throughput diagnostic assays for the detection of H. hepaticus and H. bilis in laboratory rodents. In addition, these assays are quantitative and may be useful for future pathogenesis studies.

P77 PCR Testing of Cryopreserved Mouse Embryos and Sperm for the Presence of Infectious Agents
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With the generation of considerable numbers of mutant mice around the world cryopreservation of mouse lines is becoming increasingly important. Both embryos and sperm can be cryopreserved. However, technology for successful and reliable preservations and rederivation of mouse lines utilizing sperm, while highly desirable, is relatively new. Infectious agents are transmitted via sperm in many species (e.g., Human Immunodeficiency Virus, Equine Arteritis Virus, Bovine Viral Diarrhea Virus) yet there is little published information indicating what agents may be transmitted with mouse. Infectious agents may potentially be transmitted with embryos as well. We are cryopreserving several hundred mouse lines in preparation for rederivation via embryos or sperm into a new facility. We report here results of PCR analysis of cryopreserved embryo and sperm specimens for the presence of infectious agents. Embryos and sperm were collected from a colony of mice endemic for Mouse Parvovirus (MPV), Minute Virus of Mouse, Mouse Adenovirus 2, Polyoma, Epizootic Diarrhea of Infant Mice (Rotavirus), GD VII (Mouse encephalomyelitis), Mouse Hepatitis Virus, Citrobacter rodentium, and Pasteurella pneumotropica. Both embryos and sperm were cryopreserved in heat sealed straws utilizing Glycerol and Raffinose/Skim Milk non-equilibrium techniques respectively. Specimens for analysis were randomly selected and submitted to the University of Missouri Research Animal and Investigative Diagnostic Laboratory for PCR testing. Twenty sperm specimens were submitted and five embryo specimens for standard panels which did not include Citrobacter rodentium or Pasteurella pneumotropica. In addition, 5 embryo and 5 sperm specimens were submitted for testing of all the pathogens for which the colony is known to be infected (these results are pending). In all but one case there was no PCR evidence of any infectious agents being present in the cryopreserved specimens. One sperm specimen was positive by PCR for MPV. Based on a small sample of cryopreserved specimens the risk of disease transmission was 0% for embryos and 0–4% for sperm. These results suggest that the likelihood of transmitting infectious agents via cryopreserved mouse embryos or sperm is small, even when a number of agents may be present in donor animals. Mouse antibody production testing of the single positive specimen is also indicated to determine if the DNA detected represents viable virus. The risk of disease transmission, although relatively small in the specimens evaluated, reinforces the importance of testing recipient animals and offspring prior to release from isolation to ensure successful disease agent elimination.
P78 Critical Amino Acid Changes at the N-terminus of Feline Leukemia Virus (FeLV) Envelope Protein Are Linked to T-cell Pathogenesis

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Feline leukemia virus (FeLV) is a Type C oncoretrovirus that is a major cause of morbidity and mortality in pet cats. FeLV is divided into four known subgroups (A, B, C, and T) which exhibit different pathogenic phenotypes. The pathogenic properties of the subgroups are related to sequence changes in the envelope surface (SU) protein that affect interactions with viral receptor proteins expressed by varying feline cell types. For example, subtle changes in SU have been shown to convert a non-cytopathic subgroup A virus (FeLV-A) into a cytopathic subgroup T (FeLV-T) variant. To more precisely define the sequence changes required to convert FeLV-A into FeLV-T, the infectivity of several FeLV clones encoding chimeric SU proteins was analyzed by in vitro infection of a feline T cell line. Cultures were analyzed every 2 to 3 days to assess viral cytopathic effects (CPE), including syncytia formation. The production and spread of viruses in cell culture were monitored by reverse transcriptase activity and by an Enzyme Linked Immunosorbent Assay (ELISA) that detects p27gag in the supernatant. When compared to the SU protein of a transmissible non-cytopathic FeLV-A isolate (61E), a viral chimera containing an N-terminal amino acid change at position 7 and a C-terminal insertion at position 352 was cytopathic, syncytia-inducing and had altered cell tropism. The significance of the N-terminal amino acid change will be further examined in the context of specific receptor interactions.

P79 Report on Activities of the Genetics Division, ICLAS Monitoring Center

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Genetic monitoring has been developed as a testing system to assure the genetic quality of laboratory animals and to discover genetic contamination in the early stage by checking various gene loci. Our center, designated by ICLAS in 1979, has been undertaking genetic monitoring of mice and rats at the request of animal facilities. Nineteen gene loci are routinely checked by biochemical and immunological marker genes. Based on this monitoring, some cases of genetic contamination were detected over the period from 1990 to 1999. Cases 1 and 3 were found in mouse inbred strains in 1990 and 1997 respectively; case 2 was a mouse F1 hybrid strain reproduced from an embryo bank in 1999 and case 4 was a rat inbred strain in 1999. All strains showed different genotypes at more than 2 out of 19 loci. The importance of monitoring in the quality control of laboratory animals has been reinforced. Our other activities include: (1) gene mapping by fluorescence in situ hybridization (FISH), (2) genetic monitoring of closed colonies and (3) genetic monitoring of cell lines derived from laboratory animals using microsatellite markers. These activities and future prospects of the genetic monitoring system in our center are introduced.

P80 Genetic Monitoring of an In-house Mouse Breeding Colony

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Stanford University has maintained a colony of several unique inbred mouse substrains for several decades. Although there were functional indications that the genetic integrity of these substrains had been preserved (coat color, acceptance of syngeneic cell lines, etc.) it was determined that a more definitive quality control program should be established. With the advent of sensitive molecular techniques to examine genetic contamination and drift, we chose to undertake restriction fragment length polymorphic analysis using multi-locus DNA probes to examine genomic DNA from our mice and compare them with genomic DNA obtained from substrains of commercial vendors considered to be closely related to the our colonies. Vendor strains used for comparisons were chosen based on several factors, with the intent of identifying substrains closely related to the original Stanford breeding nucleus. Specifically, we compared C57BL/6J (Jackson Laboratory), BALB/c (Stanford) to BALB/cN (N.I.H.), and C3H/Ka (Jackson) to C3H/HeJ (Jackson Laboratory). Three test mice (Stanford) were used for each strain comparison. In all cases the test samples were genetically highly similar to the reference (vendor) substrains. The results demonstrate that the breeding strategies and management of the in-house colony appear to have maintained the genetic integrity of the mice. In addition, the data obtained can be used as baseline information to prospectively monitor the integrity of the substrains in the future.

P81 Helicobacter and PCR Testing: What Do the Results Really Mean?

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Helicobacter spp. was undetected as a pathogen in mouse research colonies until 1992. Polymerase Chain Reaction (PCR) is considered the most useful method of detection for this organism. Our facility routinely tests for Helicobacter before shipping animals to another facility. On two separate occasions that facility received reports that animals from our facility tested positive for Helicobacter spp. In an attempt to understand why there were conflicting results we decided to do further testing. Fresh fecal pellets were collected using separate sterile forceps for each sample from mice housed individually in static microisolator units. The fecal pellets were placed into sterile 1.5mL Eppendorf tubes and shipped to the testing laboratories over an eight-week period. Samples from the same mouse were sent to two different laboratories on four separate occasions. Results from these tests showed an agreement level of only 45%. Testing was then expanded to three laboratories, each receiving individual samples from these same 10 mice. The level of agreement on the presence of Helicobacter spp. between Lab 1 and 2 was 40%; between Lab 2 and 3, 70% and between Lab 1 and 3 only 30%. To evaluate within-laboratory differences, two samples from each mouse were sent to each of three laboratories. Results indicated “within laboratory” agreement was between 70% and 90%, however there was complete agreement on only 2 animals.

These results suggest individual animals may intermittently shed Helicobacter spp. and that animals found to be positive are probably positive, but repeated testing may be required to assure animals found negative really are negative.
Selective breeding has been carried out over 50 generations using the Japanese quail (Coturnix japonica) to produce an avian experimental model animal, which will be used to save the distinct wild bird. And we succeeded to produce two avian models, one is a distinct, the other is survival type in Japanese quail. By the way, we are exploiting a new method to analyze the trend of inbreeding depression in egg shape by image processing.

The egg shape analysis by image processing is a epoch-making method to distinguish the strain and species difference as follows: The egg size is standardized by same dimension (1) and only the shape is extracted by our original method, which is quantified into binary images with egg photograph. This time, we applied this method to the eggs of Japanese quail and Bobwhite quail for diagnosing the species difference and inbreeding depression.

As a result, we found that the egg shape analysis by image processing is an effective diagnostic method to distinguish the species and strain differences, and also to grasp the trend of inbreeding depression.

P83 A Program to Evaluate the Health Surveillance of Commercial Rodent Suppliers to Facilitate Management of In-house Rodent Colonies

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Expanding research efforts and transgenic initiatives have required our facility to acquire rodents with more variable health statuses, making rodent health monitoring more complicated and time-consuming. We developed a program to facilitate monitoring of health surveillance at commercial rodent vendors and improve the management of the variable health statuses of our rodent populations. Our goals were to: 1) ensure assessment of the health status of rodent vendors, 2) provide current information to utilize in animal procurement, 3) prevent transmission of pathogenic or opportunistic organisms within our facility, 4) streamline our ordering process and 5) maintain information regarding the most current diagnostic procedures for rodent pathogens and opportunistic organisms. To accomplish these goals we created an information management database. A list of all salient mouse and rat viruses, bacteria, parasites and protozoa was compiled and identified as primary, opportunistic or nonpathogenic. A notation for acceptable and/or preferred testing methods for each organism was made. Vendor barriers were categorized based on health status as: approved (can be placed anywhere within our facility), conditionally approved (restrictions as to placement within our facility), quarantine (required to be under special procedures within our facility) and unapproved (unacceptable to order). We also compiled a list of all current mouse and rat strains and listed approved vendors for each that has facilitated our ordering process. Lists are reviewed and updated on a quarterly basis. We have been able to expedite procurement of rodents, identify rodent colonies with unwanted opportunistic and known pathogens in a more timely manner, and more effectively prevent transmission of unwanted pathogens within our facility. This program has enhanced our surveillance of commercial rodent vendors, facilitated communication within our facility and improved ordering efficiency of rodents.

P84 Diagnostic Testing in Rodent Health Surveillance Programs

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The importance of disease surveillance and diagnosis in rodent animal facilities is well documented and clearly stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council). Clinical and subclinical infections occur in conventional and barrier-type facilities and obviously can have a devastating impact on the health of animals in the entire facility as well as on the conduct and interpretation of results from studies using these animals. Diagnostic testing in support of rodent health surveillance programs can include many different laboratory services and methods. These can include a clinical examination, complete gross necropsy, clinical pathology, microbiology and serology of the mice themselves as well as bacterial testing of the environment. New diagnostic technologies and methods are being developed rapidly for newly-emerging and conventionally known health threats. Making decisions on the type(s) of and time frames for diagnostic testing in rodent facilities can be a daunting task. A successful program used in our rodent facility includes a clinical examination, complete gross necropsy with collection (into 10% neutral buffered formalin) of gross lesions for histopathologic examination, oropharyngeal and fecal bacterial cultures for pathogens (i.e., Corynebacterium kutscheri, Yersinia pseudotuberculosis, Salmonella spp., Citrobacter freundii 42880), skin/hair examination for ectoparasites (i.e., Polyplax serrata, Polyplax spinulosa, Myobia musculi), examination of the rat urinary bladder for Trichomonoides crassicauda, examination of the feces for helminths (i.e., Syphacia obvelata, Syphacia muris, Aspicularis tetrapetera), microscopic examination of ileal and cecal contents for protozoa (i.e., Giardia muris, Spirurinae muris, Trichimonas muris, Eimeria falciformis, Cryptosporidium muris), and serology for a general agent profile. In mice, serologic testing for the most commonly encountered infectious agents includes EDIM, GDVII, MHV, Mycoplasma pulmonis, MPV, MVM, PVM and Sendai virus. A complete mouse serologic profile would also include CAR Bacillus, Ectromelia, LCM, MCMV, Polyoma virus and Reovirus. In rats, serologic testing for the most commonly encountered infectious agents includes CAR Bacillus, H-1 virus, KRV, Mycoplasma pulmonis, Parovirus, Mouse Adenovirus, PMV, RCV/cDA and Sendai virus. A complete rat serologic profile would also include LCM, Reovirus and Orphan Parovirus. For guinea pigs and hamsters, a good serologic profile includes LCM, PVM, Reovirus, Sendai virus and SV5. In general, serologic testing uses the enzyme-linked immunosorbent assay (ELISA) for screening with positive results confirmed by more definitive testing methods such as immunofluorescent assay (IFA) and polymerase chain reaction (PCR). Rodent bacterial testing focuses on bacterial pathogens of which there are only a few. These include Salmonella spp., Mycoplasma pulmonis, Corynebacterium kutscheri, Citrobacter freundii and Helicobacter sp. Histopathologic examination of selected tissue sections particularly using special stains can also be an important adjunct to bacterial and serologic testing. In facilities with specific needs such as facilities designed for the production of immunosuppressed and/or aging rodents, a more
extensive panel of testing and/or the development and use of new, focused and specific testing methods and technologies should be performed. This poster presents an overview of a testing plan for rodent facilities along with commonly used methods for detection.

P85 Using an Orientation Manual for New Employees
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A well thought out and constructed orientation manual for new employees takes time to create, but the investment is worth the effort in the clarity and continuity it brings to the orientation process. The new employee manual currently in use by the husbandry management team at our institution is constructed so that it clearly illustrates what information will be discussed with the new employee and by whom. The manual is contained in a large three-ring binder. The first item in the binder is a zipper pocket containing pens, Post-it® notes, a pad of paper, and a highlighter, to help the employee get through the first furious note-taking days on the new job. Following the zipper pocket is an index of all documents, Standard Operating Procedures, brochures, and memoranda contained within the manual. The manual is sectioned according to the administrator who will be discussing the information with the new employee. At our institution, the administrators could include the manager, assistant manager, administrative associate, administrative assistant, supervisor, or information technologist. Each administrator’s section contains a checklist of what will be discussed with the new employee and a pocket folder containing all the documents to be discussed. Topics contained in the manual range from attendance and vacation policies to parking permits, organizational benefits, and city maps. After the first administrator completes his/her section, he/she schedules appointments with the next people to meet with the new employee. The employee’s direct supervisor sets up additional meetings as needed, and the last administrator to meet with the new employee collects all the checklists. This collection of checklists is brought to a husbandry management meeting when the employee has reached his/her 30-day anniversary to determine what topics might need to be revisited. This tool gives everyone who works with the new employee collects all the topics to be discussed. Topics contained in the manual range from attendance and vacation policies to parking permits, organizational benefits, and city maps. After the first administrator completes his/her section, he/she schedules appointments with the next people to meet with the new employee. The employee’s direct supervisor sets up additional meetings as needed, and the last administrator to meet with the new employee collects all the checklists. This collection of checklists is brought to a husbandry management meeting when the employee has reached his/her 30-day anniversary to determine what topics might need to be revisited. This tool gives everyone who works with the new employee a chance to keep track of their own training and information flow while providing the new employee with a tangible, solid information base. It also gives the new employee the sense that he/she is valued, and that the management team, through its one-on-one contact and efforts to support and inform, is invested in the employee’s success.

P86 Design of a Simple Auto-Tutorial Program for Laboratory Animal Personnel
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Custom-designed training materials using interactive CD-ROM or internet-based training programs can cost several thousand dollars or more, depending on the complexity of the technology required. In our experience, development time for these materials, from initial design to delivery, has taken up to eighteen months. To meet emerging training requirements, a more rapid, cost-effective method for developing training materials was identified. This poster describes how standard presentation software was used to create an auto-tutorial on a new animal health reporting program. The training program was posted on a departmental web site and listserv, which allowed it to be viewed by individuals at their convenience. Although the use of standard presentation software for training is not new, this poster will demonstrate how laboratory animal personnel, who have little or no programming experience, can quickly and easily develop low-cost, high-quality, customized training materials. This effective, easy-to-use training program has enabled us to instruct a large number of people in a short period of time, while allowing them the flexibility to learn at their own pace. Scheduling of training sessions and conference rooms was eliminated and time-sensitive research project work was not disrupted. This training method facilitated the rapid implementation of a new health reporting program.

P87 Web Page Promotes Teamwork
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Communicating information between animal care staff, veterinarians, and investigative staff is vital to the operation of any Animal Research Facility. Web-based technology can afford an effective and very appealing mode of delivering various types of information. The use of a web page as a communication tool has proven to be extremely beneficial in our experience. A variety of materials are well suited for this venue. A newsletter, which was updated quarterly, contained relevant facts on facility-specific topics that were concerning to the investigative staff. This included issues such as emerging rodent infectious disease, pathogen status of rooms within the facility, and explanation for changes in housing materials. Work schedules detailing daily static work assignments and weekend duty schedules were linked to introductions of animal care staff including photos and relevant information such as professional achievements and AALAS certification. Relaying this type of information caused an improvement in employee morale as a result of increased employee recognition by the investigative staff. Interactive links to accepted vendors were listed on the home page for easy access to product and service information. Didactical information on per diem rates and service fees were available as well as a special request form routed to a designated staff E-mail recipient. The purpose of the web page was to provide a mechanism for communicating topical issues and presentation of services available. In addition to meeting this goal, the web page had the unexpected result of improving employees’ attitudes and promoting team-building between the investigative staff and the animal care staff. While there is no substitute for direct face-to-face communication, especially in time-sensitive issues, a web page can provide a convenient, broad-based mode of transmitting information and can easily be customized to the needs of the facility.

P88 Bridging the Gap Between Husbandry and Research
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In an effort to create a better understanding of how husbandry work impacts the research process, Lab Animal Technicians at the University of Pennsylvania have implemented a program that brings Investigator and Technician together on common ground. This program was created to foster the exchange of information
and resources, and to reduce conflict and eliminate perceived differences. By using various methods including questionnaires, briefing sheets and discussion groups, the lines of communication are opened to both the Technician and Investigator to create a better understanding and mutual respect of the part each plays in the research process. Prior to this program, the Investigators and Technicians found themselves on “different sides of the stream” on many issues. Now these people are interacting with each other. The bridge has been created and the Lab Animal Technicians have extended the invitation for the Investigators to cross. Developed upon the biosafety level briefing sheets, the Lab Animal Technicians developed a series of questions to ask the Investigators in order to better understand them and their studies. The questions range from a personal level to a humanitarian level. For example, “Why did you personally choose to research this particular subject? What may this mean for humanity? What are your special needs from husbandry for this study?” This questionnaire is then completed by the Investigator and discussed with the Lab Animal Technicians in a relaxed environment where questions and concerns may be addressed. The questionnaires are then kept in a notebook that is readily available to the Lab Animal Technicians for future reference. With their department’s backing, this program has helped open the lines of communication between Lab Animal Technicians and the Investigators they work with to create a more harmonious work environment.

P89 The Stress Test: Management Tool to Improve Morale

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Stress levels for animal caretakers at our VA Medical Center has risen in direct proportion to low unemployment and government reinvention. Changes are occurring faster than we can hire people to implement them. As a way to refocus our goals, “vent” our gripes and improve communication, we developed the Veterinary Medical Unit Stress Test. Sample tests and positive results will be included in the poster. The test consists of ten to twenty True/False and multiple choice questions. Twice a year, we replace our routine monthly staff meeting with the test. There is no mandate to participate and no penalty for not doing so. Employees use a standard #2 pencil and circle the answers they feel best represent their feelings on a variety of topics (i.e., division of duties, work hours, personal conflicts, problems with superiors). No names are written on the test. There are no grades. Tests do not have to be turned in to the supervisor unless the employee agrees to do so. The purpose of this test is to increase awareness. As caretakers, are we following our mission to care for research animal models in the best way possible? As managers, are we dynamic and approachable? Sample questions: “Things would be better in the animal facility if: a. We ever had a full staff, b. We hired a person just to work weekends, c. I didn’t have to work more than everyone else, d. Communication between workers was better.” “T or F: There are too many interruptions in my day to work effectively.” In five years no one has refused to take the test. Some employees who are reluctant to approach the supervisor about a perceived problem waste no time writing it down on paper. As the facility manager and veterinarian go over the responses, they learn much about themselves and how their management styles are viewed by others. Adjustments can then be made as necessary. The Stress Test has had a positive impact on morale for the animal technicians. It has made management more keenly aware of staff needs and forced us to find novel solutions to work problems in a timely manner.

P90 A Policy to Reduce Absenteeism among Animal Facility Staff

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The Case Western Reserve University Policies and Procedures Manual defines excessive absenteeism as repeated occurrences (more than six in six months) of tardiness and/or unscheduled absence. This excludes approved time for vacation or leave of absence. Our department was experiencing excessive absenteeism. During meetings with Human Resources, it was determined that the department needed a written Attendance Policy to use in conjunction with the University’s manual. Several members of the Animal Resource Center (ARC) Management Group met with the University’s Assistant Director for Employee Relations to draft an attendance policy. Since the University policy is based on “occurrences” we needed to define an occurrence. We decided to use a point system where 2 points equal 1 occurrence. We then needed to define how the points would be assessed. Incidents that minimally affect the daily operations of the department are assessed 2 points. These incidents include failure to punch out either for the day or whenever leaving their work stations for non-ARC business, failure to punch in, and nonscheduled absence of one or more consecutive whole days. Incidents that moderately affect the daily operations of the department are assessed 4 points. This is limited to failure to call in if absent. We realized that a written attendance policy might be viewed as punitive by some of our employees. In order to emphasize a positive aspect of the policy, we decided to reward employees for “Perfect Attendance.” If an employee has perfect attendance and a perfect work record for a three-month period, they receive a day off. This “free day” can be used within the quarter as long as it is prescheduled with their Team Leader and Manager. Some of our employees attempt to work when ill in order to avoid being absent. To reward this dedication, no points are assessed to any employee that works more than 50% of their scheduled day before leaving ill. The first couple of months after the policy was implemented were hectic. Some of the employees who had chronic attendance problems left the department. Overall, since the implementation of the attendance policy three years ago, attendance has greatly improved. When the Assistant Director for Employee Relations speaks to other departments concerning instituting an attendance policy, our policy is given as an example.

P91 An Alternative Training Method for Reinforcing Good Hygiene Practices

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We observed an increase in the number of research staff who were not correctly following the standard operating procedures used within the core animal housing facility. Specifically measures which were implemented to prevent the spread of diseases or contaminants were being disregarded or were not being properly followed. Since our standard training methods were proving to be inadequate, we decided to try a new approach that would
be interesting, fun, and effective. We took a series of posed photographs demonstrating how easily improper use of protective measures could spread contaminants throughout the facility by using bright red tape in the shape of an X to indicate the contaminant. This new approach differed greatly from our standard written and verbal presentation formats because we enjoyed presenting the material numerous times and because the audience enjoyed the more humorous presentation. Given the positive response this new presentation format has received we hope to use this style for several of our general training sessions.

P92 An Effective Method for Tracking Lab Animal Technician On-the-Job Training

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One of the major challenges to a lab animal training program is the ability to keep track of new technicians’ daily activities. These daily activities may also be referred to as on-the-job training. Many tasks that are performed on a daily basis are also skills that technicians need to be signed off in the training records. In order to sign technicians off in the training records, a technician must be deemed proficient in a given skill or skills. There are two main questions that need to be addressed when deciding on proficiency: 1) what tasks the technicians have performed and/or observed; and 2) how often these tasks have been performed and/or observed. In order to answer these questions, training cards and a training track record was produced. The training cards consist of the following information: name, date, subject, trainer, and four codes (OB = observed, PF = performed procedure, PP = previously performed, SO = signed off). These cards should be used any time a technician observes or performs any procedure. The information from these cards is recorded on a training track record. The track record contains all the skills listed from the training records with a date and a code column. This track record provides an effective method for tracking the progress of new technicians’ training. There are many factors for determining the competency of a technician, this method aids in that determination, i.e., tracks the procedures performed and how many times it has been performed.

P93 An Innovative Computer-Based Training Program for Laboratory Animal Personnel at St. Jude Children’s Research Hospital, Memphis, TN

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One of the most challenging tasks faced by institutions in the field of laboratory animal care is training. Utilizing computer-based technology here at St. Jude Children’s Hospital, we have developed a training program that not only benefits the staff of the Animal Resources Center, but our research and investigative staff as well. In our continuing efforts to provide the highest standard of care for our research animals by highly skilled and well-trained technicians, we used existing computer resources to enhance our training program. As with many institutions, St. Jude has an Intranet. We developed training modules for many of our standard operating procedures as well as various technical procedures we regularly employ during the course of our operations. These included routine animal husbandry, operation of various types of equipment, routine biological sampling, injection sites and bone marrow harvest. In addition, we add specific procedures tailored to fit the needs of our investigators, under the approval of our Institutional Animal Care and Use Committee. Step-by-step photographs were taken of each procedure using an Olympus digital camera. These images were incorporated into training modules and then uploaded onto our web site on the St. Jude Intranet. An optional vocal narrative describing each step of the process was added to each module. These modules were then identified by category and can be accessed by means of a menu under the heading of ARC Training. Since all of our personnel, as well as all investigative staff have access to our web site, each individual can access these modules and go through each procedure at their own pace. Providing these modules serves a multiple purpose. The ease with which the technician is able to access the training modules allows them to augment their initial hands-on training in a procedure whenever they wish to review it. Additionally, it allows our researchers and investigative staff to access our training procedures and review procedures they would like us to perform. It gives our investigators an “inventory” of our available services and provides a format for requesting additional techniques to be incorporated in our repertoire. This enables us to continually add techniques and services through interaction with the ever-growing needs of our researchers. Our expanded level of training, along with our expanded services has been very well received. We are now able to provide continuous individual training without the necessity of having to schedule time with our training staff. This has, by no means, replaced our one-on-one, hands-on training. However, by allowing the technicians to review each module in a self-paced environment, we have been able to ensure a higher level of understanding of the procedure. The modules also enable the technicians to preview procedures they may not have received hands-on training in as yet. The modules act as incentive to inexperienced personnel to seek out additional training in order to become proficient in some of the more complicated procedures available. The modules encourage technicians to become AALAS Certified, and proficiency quizzes are available to technicians on a volunteer basis that can be used for continuing education credits for the AALAS Certification Registry.

P94 Neurotoxicity Training Program with Positive Control Testing

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Examining rodents for neurotoxicological effects with a battery of tests is an exciting challenge. This testing is very complex and requires extreme concentration. An intensive training program is essential to the success and accuracy of neurotoxicity training. Technicians must be able to identify a normal rodent and understand the wide range of normal behaviors for that species so that subtleties can be determined. A list of acceptable terminology and working definitions is necessary for each laboratory so the technician can concisely and accurately describe the behavior or condition of an abnormal rodent. Consistency can only be accomplished through handling multiple rodents and repeatedly completing the battery of neurotoxicity tests.

Clients often assess the neurotoxicity training program prior to placement of studies in the laboratory and are looking for positive control testing as part of the training package. The use of positive controls allows the trainer to ensure the trainees are able to correctly identify an abnormal condition or behavior. Each drug used should provide examples of different conditions or behaviors. Clients also look for the ability to produce inter-observer reliability among certified technicians. This is important when multiple technicians must record observations on the same animals.

Developing a complete training program has proved very beneficial to the technicians, study direction team, and company.
P95 Components to Necropsy Training in a GLP Environment

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Toxicity evaluations in animals typically conclude with a comprehensive necropsy, which is defined as a macroscopic examination of the body surfaces, orifices, cavities and their contents. Thorough detection and reporting of necropsy findings by proficient animal technicians is pivotal to the accurate assessment of toxicity. In order to ensure a high level of comprehension of the necropsy technique, a multi-component training program, which includes appropriate GLP documentation of necropsy skills, can be implemented. A necropsy training program designed to meet these requirements is composed of the following components: 1) an orientation to necropsy procedures and SOPs; 2) anatomic terminology and topographical location; 3) basics of pathology; 4) prosection techniques; 5) tissue alterations; and 6) tissue collection. Each training component is conducted by a prosector proficient in the procedure and is performed under the supervision of a veterinary pathologist. Components 1, 2, and 3 are conducted in the classroom followed by either an oral or written exam, while components 4, 5, and 6 are conducted in the laboratory followed by a hands-on demonstration of skill proficiency using naive animals excluded from other studies. After the completion of each training component, GLP documentation is added to the technician’s training record reflecting proficiency in the component. Only after completion of training and the appropriate documentation is the technician considered qualified to conduct unsupervised necropsies as a prosector. This multi-component necropsy training program is one which has proven effective for training and qualifying skilled technicians capable of accurately assessing and documenting necropsy findings in a GLP environment.

P96 Biodecontamination of Animal Rooms and Heat-sensitive Equipment with Vaporized Hydrogen Peroxide

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Process was monitored during five independent trials using aeration was developed to be effective. The biodecontamination cycle consisting of dehumification, conditioning, sterilization and peroxide generator to any animal room using the ventilation of our air-conditioning system allows to connect the hydrogen tested fumigation with vaporous hydrogen peroxide. The design hazard for staff members and environment. Therefore, we areas are hard to standardize, labor-intensive and potentially damaging after now more than ten fumigation cycles. Workload and potential health risk for staff members and environment is insignificant.

P97 Applications and Benefits of a Robotic Ultrasonic Cleaning System

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Ultrasonic energy in a liquid produces a powerful scrubbing action that is effective in cleaning objects immersed in the liquid. Ultrasonic cleaning can be used in an animal facility to improve sanitation of difficult-to-clean equipment such as sipper tubes and automatic watering system drinking valves. A robotic ultrasonic cleaning system has been installed in Pfizer Inc’s newest vivarium. The robotic element is a transport system that moves baskets containing equipment to be cleaned from a loading section, through one heated wash tank and three rinse tanks and onto a gravity roller section of a bottle washer. This system is designed to provide consistent sanitation from load to load by having programmable cycle times for the wash and rinse tanks. The robotic element transports the baskets precisely at the set times, reducing cycle variation between loads. This system is also very efficient, being able to process multiple loads simultaneously while freeing up the operator to perform other duties. It is an ergonomic process, minimizing lifting of full baskets by the operator. Using the robotic system, fully loaded baskets weighing approximately 25 pounds are only handled one time when lifting them onto the load end. Without the robotics, the operator would handle the baskets five times when loading them into the wash tank and transferring them to the rinse tanks and onto the roller section. This robotic ultrasonic cleaning system joins other robotic systems in automating the modern laboratory animal cagewash facility.

P98 An Improved Method for Biological Waste Disposal

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The previous method of biological waste disposal at Merck Research Laboratories (MRL) involved the extensive handling of individual corrugated boxes lined with a plastic bag. These boxes contained up to 40 lbs. of waste and had to be transported from the animal room to the incinerator located on the research site. Besides the cost of the biological waste box itself, the labor-intensive process involved seven separate handling steps for each box. During the past year, a system of portable waste containers was implemented in MRL. These plastic 48 in. L x 32 in. W x 49 in. H containers can hold up to ten waste bags and are easily rolled within the animal facility. The containers are eventually moved to a device which lifts and dumps the contents of the cart into the incinerator. An adjacent cart washer sanitizes the containers for return to the animal facility. The portable waste container system has greatly streamlined the handling of biological waste and protected employees against allergen exposure and ergonomic injuries. In addition, MRL will obtain a savings of over $430,000 by the elimination of the corrugated boxes and related labor savings. The authors have concluded that significant cost and occupational health benefits can result from the elimination of corrugated boxes as a primary means of waste disposal.
P99 Evaluation of a Bacterial Enzyme Indicator System for Providing Immediate Autoclave Quality Assurance

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Our facility sought to find a reliable, immediate method of determining that items entering the BSL III and SPF barrier, or exiting the BSL III facility were sterile. Immediate, confirmed results are ideal whereas biological indicator methods (Bacillus stearothermophilus) require 48 hours incubation and chemical test strips are sometimes unreliable. A new indicator based upon B. stearothermophilus enzyme activity/viability allegedly provides reliable sterility assurance with immediate results and with the added benefit of reducing lab costs and biohazard potential. To evaluate the efficacy and reliability claims of the new indicator system, test sterilization cycles were designed under various conditions representative of our facility. Gravity, pulse-vac, and liquid cycles were evaluated. In all trials, both indicators were run simultaneously. A purposeful “fail” cycle was also conducted. An additional trial was run to determine if delayed incubation of B. stearothermophilus or delayed interpretation of the enzyme indicator affected results. We determined that the new, bacterial enzyme indicator 1) is reliable, and results correlate with currently accepted biological indicators under both “pass” and “fail” sterilization conditions, 2) results are available immediately following a simple <2 min lab procedure, and 3) is reliable even if interpretation is delayed 24–48 hours. The advantage of the enzyme indicator system is that results are immediately available whereas the B. stearothermophilus requires 48 hours incubation. Also, the enzymatic method does not require immediate incubation, but can be processed up to 48 hours later in cases where immediate results are not critical. The enzymatic method we investigated, for determining autoclave load sterility, is superior to currently employed methods; providing consistent reliability and decreasing both the time interval for obtaining results, and labor expenses.

P100 Use of a Small Down-Draft Table with Custom Head Restraint for Middle Cerebral Artery Occlusion Procedures using a Volatile Anesthetic

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When using volatile anesthetics one must be aware of excess anesthetic being released near the surgeon. There are several ways of safely utilizing inhalant anesthetics in rodent surgery and many commercial systems are on the market today. One may even intubate the rat in order to attach it to the anesthetic machine. What becomes a challenge is adapting the delivery system to the type of surgery being performed. If surgery involves the head area (particularly the frontal face region), a coaxial breathing system is not always convenient. To address this, we have created an economical portable down-draft table which, along with a custom-designed head restraint/anesthetic apparatus, permits us to perform middle cerebral artery occlusion (MCAO) procedures safely and successfully using isoflurane in an open circuit system. The table is a plastic hollow box with six 6-mm holes drilled into the work surface directly below the head restraint. It measures 16 cm wide by 25 cm long by 5 cm in height, with extensions for added convenience to the surgeon. Attached to one extension is the custom-made head restraint/anesthetic apparatus, and below is attached a negative air source to create a vacuum inside the box. This design permits air around the animal’s neck region to be brought down into the table and away from the surgeon’s face area reducing anesthesia exposure. The oxygen flow rate was set at 1 LPM with isoflurane concentration set at 2.5%. Measurements were made roughly 6 inches above the rat, in what was considered the surgeon’s face area to evaluate anticipated exposure levels during surgery. Initial measurements ranged from roughly 0.0 PPM–4 PPM, but remained principally below 1 PPM. Measurements were performed with a direct reading infrared photometer (SaphiR®). The setup provided an appropriate level of worker protection (levels below 2 PPM were maintained according to The National Institute of Occupational Safety and Health (NIOSH). The use of this system has permitted us to perform MCAO procedures safely and successfully using isoflurane.

P101 Evaluation of a Back Draft and Portable Down Draft Table for Scavenging of Anesthetic Waste Gases

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In practice, anesthetic gases may leak into the laboratory environment from around the nose cone used by operators during rodent anesthesia. Traditional active and passive devices to scavenge these vapors may not always be sufficient to prevent instances of exposure of the operator to levels above the established exposure limits. As a result, we evaluated a custom-made back draft table and a commercial portable down draft table for the ability to exhaust anesthetic waste gas. Potential operator and animal exposure to noise, consistency of air flow, depth of anesthesia and ergonomic risks associated with each system were also evaluated. Twelve rats were induced and maintained with isoflurane for periods lasting up to 6 hours. Isoflurane exposure was assessed with organic vapor monitors. The depth of anesthesia in the animals was determined by monitoring temperature, heart rate, respiratory rate and toe pinch withdrawal, respectively. Potential ergonomic risks associated with the use of each system were evaluated. The results showed that with the use of either system, anesthetic waste gas exposure was well below the National Institute of Occupational Safety and Health limits of 2 parts per million. Exposure levels were lower in the down draft system compared to the back draft system. Each system compared favorably for noise levels, consistency of air flow and ease of induction and maintenance of anesthesia. Ergonomically, there was no advantage of using one system over the other.

P102 Use of Electronic Media in the Project Planning Process of a Major New Research Animal Facility

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Program and design development for new animal facilities typically requires the development, collection, collation, and distribution of significant amounts of data. Distribution and review of these data by the project team, users, and support groups commonly involves large volumes of paper documents and communication processes that are difficult to manage. A new approach to data management was employed in the project planning of a large (195,000 SF) research animal facility, reducing the volume of paper documents used by the project team by over 90%. Program development took place in real time, with data entered directly into a large multi-page Microsoft Excel™ work-
book during the project team meetings. The real time entries, viewed via a video projector, were easily modified to test various scenarios. Team meeting minutes, specifications, project schedules, white papers, and drawings were accessible via Documentum™, a state-of-the-art document management system on a document server for review and modification by team members. The use of on-line data management systems significantly reduced the volume of paper documents, reduced document review time, increased data accuracy, and facilitated the compression of the design development period. Electronic data management systems will significantly improve communication and shorten schedules for any major facility project.

**P103 An Innovative IACUC Protocol System**

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Animal use protocol forms evolved from paper to electronic templates but committee reviews, investigator revisions and filing of approved protocols were still completed on paper. The objectives of this project were to improve the process for the investigators and the IACUC and to make the approved protocols available on-line. The resulting web site/database directs the investigator through step-by-step protocol completion and submission. Electronic cues guide the investigator in completing the protocol accurately. An innovative navigation system keeps track of completed sections for the investigator. The protocols are automatically routed to the IACUC, veterinarian and others for review and comments. Protocols are stored in the database and are searchable by key fields. This innovative system eases the burden on investigators and the IACUC, expedites review and approval and creates an on-line database resource that benefits the research community. It also has the unexpected benefit of stimulating more committee discussion on-line than was typical in committee meetings. The system utilizes commonly available software for the database and active server pages for the web site programming.

**P104 An Integrated Database for Managing Animal Study Proposals and Animal Inventory for the Small Animal Facility**

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Our institutes needed an efficient way to track Animal Study Proposals (ASP’s), renewals, animal numbers, investigators assigned to an ASP, investigator training, and enrollment in the health surveillance program. Due to the size of our facility, we could not justify purchasing a commercially available database system. We created a custom designed database to manage our ASP’s and related information using a commercially available database software program. Each ASP has a master page which links to an investigator database that lists each investigator and their assigned ASP’s. It also links to the Training/Health Surveillance database used to verify completed training and health surveillance. In addition, the master page links to a database tracking animals ordered or born under an ASP. This database provides the annual USDA report, data for the Semimannual ACUC report, and total animal numbers used per protocol. Finally, we use this database to print cage cards on continuous feed index cards. In conclusion, we are able to track and manage 78 ASP’s and all related information, provide required reports to our ACUC, approve animal orders and print cage cards using this database created and designed using a commercially available database program.

**P105 A Comprehensive System for the Management of Animal Information in a Research Facility**

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An on-going management challenge in a large animal research facility has been the ability to maintain long-term data on individual animals and groups of animals for administrative and scientific reporting. At Merck Research Laboratories, the innovative use of bar code technology was developed to replace the previous system which could only provide a periodic update with minimal information. The use of the SIRIUS™ system was expanded to provide real-time census information and enhanced animal data retrieval. The implementation of the new system involved the conversion of cage identification cards for over 35,000 animals to a bar-coded format. Linkages to separate animal ordering and protocol systems were also created to electronically update the system. The system validates protocols and generates cage cards for incoming animals. Animal usage and movements are tracked with a portable scanner to keep a perpetual census, and an inactive file is updated after euthanasia. On-demand requests provide immediate animal usage information that can be made by any combination of species, strain, investigator, protocol, building/room, receipt date or age/weight. Based on our experience, the authors have concluded that bar code technology is the most effective method of maintaining a large database of animal usage for colony management and to serve as a planning tool.

**P106 An Effective Program to Identify and Assess the Use of Hazardous Agents in Laboratory Animals**

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There are inherent risks associated with the use of hazardous agents in animal-related research. Protocols involving the in vivo use of biological, chemical or radiological hazards must ensure the safety of personnel, the community at large, and the management of hazardous waste. Animal welfare regulations require the Institutional Animal Care and Use Committee (IACUC) to approve animal related research protocols prior to the initiation of research involving animals. However, a reliable mechanism is necessary for hazardous agent identification, risk assessment, safety protocol formulation and implementation. Through a collaborative approach among the IACUC, Principle Investigator, Animal Resources Center, Employee Health Department and the Office of Environmental Health and Safety, we have developed a comprehensive program that addresses the in vivo use of hazardous agents. The process is initiated by the identification of research protocols involving hazardous agents. This initiates a series of meetings with all relevant research, health and safety and animal resources personnel to assess and minimize the risks. Concurrently, institutional safety officials review the protocols and contact the corresponding research staff to ensure that the hazardous agent has been licensed and registered with institutional, local, state and federal authorities. Once approved, a safety protocol is developed collaboratively. These procedures have been used for the past five years to oversee 180 protocols associated with the use of hazardous agents in animals. It has been our experience that this is an effective mechanism to
ensure the safety of all personnel and safe handling of animals and wastes associated with the use of chemical, radiological or infectious agents.

P107 A Novel Approach for Documentation and Evaluation of Environmental Enrichment Programs

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In 1985, federal regulations provided for captive primates to be maintained in physical environments adequate to promote psychological well-being. Documenting and evaluating the appropriateness of environmental enrichment has posed a challenge to many institutions, in light of increasingly stringent guidelines. Eleven Aotus (Night Owl) monkeys were videotaped using infrared sensitive cameras for a five-hour period on six nights. An ethological analysis software program was used to score the videotapes to determine the frequency and duration of 17 activities, including interaction with enrichment items; passive behaviors such as perch-and-floor-walking, sitting, sleeping, eating and drinking; and active behaviors such as pacing, circling, climbing, and somersaulting. Time activity budgets were calculated from the data. There were individual preferences for enrichment items which included nest boxes, shredded paper, ropes, polyvinyl chloride pipes, plastic and paper spoons. Foraging for food in drop pans was demonstrated. A pattern of more active behavior in the early evening, followed by passive behavior for the remainder of the five-hour videotaping session was established by this method. Videotaping and computer analysis is a convenient and labor-saving method for documenting and objectively evaluating environmental enrichment programs for captive primates and other species.

P108 Developing an Intranet Web Page of Cage Specifications for Laboratory Animal Personnel

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A new intranet web page of cage specifications was developed to provide a quick reference for laboratory animal personnel to determine the caging available for each species and the maximum number of animals allowed in each cage. The introduction to the web page states the following: “These cage specifications represent the minimum space required by federal regulations and Merck policy. Exceptions that fail to meet these requirements must be approved by the Institutional Animal Care and Use Committee. The final determination of space requirements is subject to the professional judgment of the Comparative Medicine veterinary staff. Considerations include research requirements, health, sex, age, reproductive status, behavior, temperament and social needs of the animal(s). Group-housed animals must be compatible. If you have any questions concerning animal housing requirements, contact Laboratory Animal Resources.” The user can select one of 45 different cage types, listed by species, from a pop-up menu. Once a cage type is selected, the web page displays a photo of the cage, the dimensions of the cage and, where applicable, the rack, and the number of animals allowed to be housed per cage based on the size of the animals. The benefit of this web page is that investigators can look up all cage types available plus the information needed for the proper conduct of their studies. No phone calls or consulting a space manual is needed. This information is available at any time and can be easily revised.

P109 Novel Approach for Reporting Animal Health Observations

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Computer technology is becoming an increasingly useful tool in assuring the humane care and use of laboratory animals. This poster introduces an innovative approach for reporting non-emergency clinical observations by using electronic media and bar code technology to facilitate timely and accurate reporting of animal health observations. Animal care technicians record daily clinical observations, animal identification and IACUC number via a bar code, which is then downloaded onto a desktop PC. An electronic message is then automatically sent to the veterinary and research personnel informing them that a clinical record needs their attention. This data is easily available for importation into the animal’s permanent electronic medical record. Implementation of this system resulted in faster reporting, treatment and documentation of animal health problems for all species in the laboratory animal colony. This system eliminated the “telephone tag” commonly experienced by Supervisors which sometimes lengthened the time treatment was initiated and the user friendly interface simultaneously creates a written record for the animal and allows the Laboratory Animal Supervisor to track the status of the case without accessing multiple files.

P110 A Novel Approach to Pay Tribute To Research Animals

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An innovative, secular program was developed to pay tribute to the contributions that laboratory animals have made to the advancement of biomedical research and the quality of human and animal lives. It also acknowledged the bond that exists between research animals and the research team. The program was based on a carefully scripted videotape that incorporated still and moving images of the multiple species utilized in pre-clinical pharmaceutical research. It included the many benefits derived from animal models and illustrated the caring relationship between the animals and animal caregivers, veterinary technicians, veterinarians, supervisors, managers, and research scientists. This original video tribute was presented to the laboratory animal resources staff and members of our institution’s scientific community to coincide with “Thank You Research Month” in New Jersey. As a permanent reminder of the animals’ contributions, a painting was commissioned and unveiled after the video premiere. This artwork was subsequently displayed in the entry to our largest animal facility, with a plaque inscribed as follows: In tribute to research animals whose contributions have saved millions of human and animal lives and reduced suffering worldwide. Responses to the video and artwork were extremely positive. Appreciation for the research animals and the care and consideration provided by the various members of the research team were enhanced by this program.
P111 Housing of Athymic Nude Mice Using Convention Housing

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Common thinking has it that Athymic Nude mice cannot be maintained in a traditional animal room without the risk of contamination because of their vulnerability to pathogenic and opportunistic microorganisms. However, it can be demonstrated that with careful planning and following strict sanitation procedures, nude mice can indeed be housed using conventional cages instead of the more costly microisolator systems. The following techniques can be used to minimize contamination. The mice are housed in conventional cages that have been prefixed with bedding, sealed and autoclaved prior to use. Autoclavable feed with higher nutrient contents will be required since the autoclave process will ultimately result in degradation of the feed nutrients. Water source will be provided using water bottles and hypochlorinated water. Without using a laminar flow hood for technical manipulations, the technical staff can easily handle the animals by being equipped with sterile gloves or gloves treated with a disinfectant/sterilant solution and surgical masks. To further maintain a contaminant-free system, a positive pressure laminar flow BioClean tent needs to be used to house the conventional rack. With these established procedures, nude mice can be housed economically without major detrimental effects.

P112 The Problem with Nestlets and Athymic Nudes

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A colony of Hsd:Athymic Nude-nu mice was found to have an increased prevalence of conjunctivitis. Diagnostic techniques performed included conjunctival swabs for microbiology and microscopic examination of exudate found on the conjunctiva and palpebra. Microbiology identified coagulase negative staphylococcus as the predominant bacterial species. Microscopic examination of the exudate revealed fibers, which were considered to be from the nestlets.

It was theorized, because Athymic Nude mice lack the normal fur, i.e., guard hairs, and eye lashes, that fibers from nestlets can easily become embedded in the conjunctiva and periorbital tissues of the eye. The fibers may cause chronic irritation and inflammation of the tissues, predisposing the eyes to secondary bacterial infection of opportunistic pathogens.

To test this theory the nestlets were removed from 3 of the 6 boxes and the conjunctivitis was treated. Treatment included cleansing the eyes of exudate and applying a steriodal antibiotic ophthalmic ointment twice a day for 2 days. After treatment, conjunctivitis resolved in the mice house without nestlets, but improved only slightly for the mice housed in boxes with nestlets present.

As a result of these finding, Athymic Nude mice are now given paper towels as cage enrichment instead of nestlets.

P113 Effective Methods to Reduce Mouse Allergen Levels

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We hypothesized that four changes could reduce airborne mouse allergen: (1) convert from conventional caging to ventilated caging, (2) maintain pressure so cages were negative to the room; (3) change cages on a ventilated table; and (4) increase frequency of cleaning surfaces. Ambient air sampling used a 2 um PTFE filter (20 L/min) and filters were assayed for Mus m1 allergen by ELISA. In a mouse room using conventional caging and changing table, we compared allergen levels during 4 weeks of standard operating procedures with 4 weeks of increased cleaning; increased cleaning had no effect. The caging system was converted to ventilated caging with positive pressure; PIV caging did not reduce allergen levels. Conversion of PIV racks to negative pressure decreased allergen 81% (from 1.54 + 0.17 SEM to 0.25 + 0.10 ng/m3; P < 0.05). Using a ventilated table for changing rather than a conventional table reduced allergen another 34% (from 0.31 + 0.08 to 0.14 + 0.04 ng/m3; P < 0.05). High allergen levels in the caretaker’s breathing zone indicate the need to wear respirators and goggles while changing boxes. We also demonstrated that immunodeficient Scid mice maintained for 9 months in negative PIV caging systems and changed on a ventilated table do not develop Pneumocystis, Helicobacter or Pasteurella infections from known shedders housed within the same rack. Negatively pressured PIV caging and ventilated tables can reduce allergen levels without compromising animal health.

P114 Breeding Colony Management for Genetically-Altered Mouse Models of Colon Carcinogenesis

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Large breeding colonies of multiple intestinal neoplasia (Min) mice and p53 knockout mice have been maintained at our institution for several years to generate mice for colon carcinogenesis experiments. Generation of Min+/p53 homozygote knockout genotypes was found to be lower than predicted by Mendelian genetics. We have therefore used several approaches to optimize the reproductive efficiency of each colony and also to coordinate routine husbandry, breeding, and PCR genotyping procedures to maximize time- and cost-efficiency. We have found harem mating with separation of pregnant females during routine cage changing coupled with animal identification and tail-tip collection at weaning is optimal for both time- and cost-efficiency. In addition, the reduction of handling has decreased litter loss due to cannibalization or maternal neglect among Min mice, a strain that is particularly sensitive to environmental stimuli. Finally, p53 heterozygote breeding pairs were found preferable to p53 heterozygote/homozygote knockout breeding pairs for the generation of p53 null mice on the basis of reproductive indices for this genotype and breeder replacement frequency. The breeding colony management procedures developed for these colonies have maximized production while maintaining low costs and have the added benefit of fostering positive interactions among husbandry and research staff.
P115 Growth and Reproductive Performance of Degus (Octodon degus) on Commercial Rodent Diets

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The degu (Octodon degus) is an hystricomorph, diurnal rodent distantly related to guinea pigs and chinchillas. The use of the degu in research has increased substantially in recent years. They are currently utilized as models in studying sleep biology, circadian rhythms, jet-lag, and Alzheimer’s disease. Unfortunately, little has been published on proper methods for maintenance of degus in captivity. For example, several diets are mentioned in publications where degus have been utilized, including rabbit alfalfa pellets alone, rodent pellets alone, rodent pellets supplemented with vegetables and fruit, and rodent pellets in combination with cereal seeds, sunflower seeds and hay. However, no systematic comparison of the adequacy of these diets has been conducted to determine which optimally supports maintenance of adult animals, growth of offspring, and successful reproductive activity. The overall objective of these studies was to identify an adequate laboratory diet for degus. Our initial approach was to compare their growth and reproductive performance on existing commercial diets prepared for other small herbivores. Four groups of animals consisting of five breeding pairs each were established. Each group was fed free choice a different commercial diet (rodent diet, chinchilla diet, guinea pig diet, or a 1:1 mixture of chinchilla and guinea pig diet). Pups were weaned at 37–43 days of age. Reproductive performance (mean litter size, number born alive, mean birth weights, mean number weaned, weaning weights), mean weekly body weight, and weaning survival at 10 weeks were compared. The largest number of pups per litter (8.2) was obtained from parents fed the 1:1 guinea pig/chinchilla diet. Neonatal mortality was highest in pups born to parents fed the rodent diet (27%). In addition, 6/16 (37.5%) litters born to parents fed chinchilla diet were born prematurely. Premature births did not occur in animals fed any of the other diets. The mean number of pups weaned was significantly greater in the groups fed 1:1 guinea pig/chinchilla diet (6.7). In all groups, additional pup deaths occurred at 30–50 days of age. Survival at 10 weeks of age was greatest in the guinea pig diet group (64%) and the 1:1 guinea pig/chinchilla diet group (60%). Mean birth weights of pups born in the chinchilla diet group were significantly less than those born in the other diet groups. Weaning weights (at 5 weeks) were roughly equal in groups fed the four diets but weanlings fed the guinea pig diet were significantly smaller at 10 weeks of age. To date, our studies indicate that a 1:1 mixture of commercial chinchilla and guinea pig diets is most appropriate for reproduction of degus in captivity compared to either diet alone or to a rodent diet. Growth of pups in the post-weaning period was poorest on the guinea pig diet while all other diets produced equivalent growth of surviving pups. Our findings are consistent with field observations on the feeding behavior and natural food-stuffs of the degu. The degu is a folivore, subsisting on a diet of grass, shrub and forb foliage, flowers, and seeds from several plants native to the Chilean matorral. The loss of young degus during the neonatal and weaning periods in our colony may be due to the inadequacy of the diet for growth during these critical periods or to the presence of metabolic or infectious diseases. Studies are underway to evaluate the causes of mortality in nursing and weanling pups.

P116 Use of a Feeder Insert to Reduce Obesity in Rats

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Obesity in rats offered pelleted feed in a hopper-style feeder has become an issue in chronic studies. By inserting a modified stainless steel plate into the feeder, area of exposed food is reduced, and may result in increased exploratory activity, which may lead to decreases in body weights and food consumption values (all while continuing to provide ad libitum access to food). Animals were randomized into two groups (3 animals/sex/group); control animals were given a standard feeder, while the treatment group received a feeder with an insert. Animals were singly housed in suspended stainless steel cages. Environmental enrichment was limited to the foraging activity provided by the feeder inserts. Body weights, food consumption, and clinical observations were assessed weekly. Although body weights and food consumption were not significantly different for weeks 1–9, weeks 10–14 showed a trend towards lower body weights and food consumption in the treated groups. The study will be continued until either significant differences are seen, or it can be proven that no significant difference exists. Inserts covering a larger percentage of the feeder may cause greater differences in body weights and food consumption; further studies may be run to investigate this.

P117 A Light-Tight Isolation Chamber for the Housing of Hamsters in Circadian Rhythm Studies

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In order to facilitate a controlled environment for hamsters used in circadian rhythm studies, a light-tight Hamster Isolation Chamber (HIC) was designed. The studies required hamsters to be housed in total darkness for up to 14 days. The HIC holds four 19 x10 1/2 x 8 in. polycarbonate cages equipped with wheel-running hardware. The wheels have electromagnetic counters to keep track of subject-induced revolutions during a twenty-four hour period. The HIC contains compact fluorescent lamps controlled by seven-day programmable timers. To make the HIC light tight, seals surrounding the doors contact the case and effectively block all ambient room illumination. Air is supplied directly to the inside of the HIC by an attached fan. Air supply and exhaust areas are made light tight by use of either shrouding panels or other design elements. One advantage of the direct supplied air is that contact bedding in the cage stays dramatically drier, making cage changeouts much less frequent. When the HIC must be opened in total darkness during a study in order to perform routine husbandry procedures, night vision goggles are employed. In order to make work done in the dark easier, the HIC has a pullout shelf onto which the technician can slide the complete cage, aided by guides built in to the HIC. A commercially available revolving darkroom door on the animal room that contains the HIC prevents light from reaching the animals when they are outside of the HIC during a dark cycle. The door can be removed entirely at the end of studies in order to facilitate moving equipment in and out of the room. Ultimately the problem of reliable, secure and light tight hamster housing for use in circadian rhythm studies has been resolved by the input of many into the design and operation of the HIC.
P118 The Reduction of Wing Trauma in Food-Deprived Pigeons

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Pigeons in our facility were housed in standard pigeon cages with feed and water cups mounted on the outside. The birds were on limited feed as they were used for neurobehavioral studies and tested in Skinner boxes. During feeding time, they would extend their wings through the horizontal bars of the cages causing broken and bloody feathers. Careful examination of the pigeons found that over half of the birds had damaged either one or both of their wings. The investigator agreed to alternative housing for the birds as long as there were no changes in their training behavior. The pigeons were moved into stainless steel rabbit cages, which allowed additional space for the birds to fully extend their wings. The level of trauma was further reduced by the rabbit food cup, which extended into the cage. Changing to the larger cages has had several benefits. It significantly reduced wing bleeding and trauma. Because of the larger cage area, the number of weekly cage changes was reduced along with the level of odor in the room. The larger cages were also seen as a source of environmental enrichment. These changes have not appeared to have any impact on the pigeon’s training behavior. Since birds on a limited feeding schedule become excited at mealtime, they frequently damage their wings on the bars of the cages. To prevent injury, larger cages without horizontal bars appear to be a safer choice of housing.

P119 Spontaneous Outbreak of Hip Dysplasia in a Colony of Dutch-Belted Rabbits

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Hip dysplasia in rabbits presents clinically as inability to adduct the hind limbs and is known colloquially as splay leg. This condition is thought to have a genetic basis with probable involvement of environmental factors. Confirmation of the disease in rabbit pups can be made at the age of 3–4 weeks. Normal background incidence of splay leg in a colony of Dutch-Belted rabbits involved in a reproductive toxicology study at our facility was 1%. After changing the nest box materials from paper to metal with plexiglass flooring, the incidence rose to 18%. The condition was first noted at 3 weeks of age. Subsequently, roughened plastic strips were placed on the nest box floor to improve traction during the postnatal period, and the incidence of splay leg returned to 1%. Sixteen juvenile male Dutch-belted rabbits were studied, four normal and twelve affected. A physical examination including complete blood count, clinical chemistry analysis and pelvic radiographs was performed before euthanasia with sodium pentobarbital barbiturate (80 mg/kg) for necropsy at twelve weeks of age.

All animals were similar in size and weight. Hematologic and clinical values were similar between rabbits with or without splay leg. Neurologic/reflex examination revealed no abnormalities, and nerve conduction times were similar between the two groups. Gross lesions included spaying of one (usually right) or both hind limbs. This involved subluxation of the hip, valgus deformity and patellar luxation. Affected pups displayed varying degrees of lesion development. In one rabbit the right front and hind legs were affected. Histologically, affected animals showed evidence of mild hypoplasia and regeneration in the adductor muscles. Histologic examination of the proximal femur revealed marked thickening of the joint capsule with fibrocartilage formation. Femoral heads were often flattened and decreased in size. There was trabecular bone loss as well as bony sclerosis. This report indicates that traction during the postnatal period is an important environmental factor in the development of splay leg in rabbits. Our findings illustrate that this defect is associated with significant skeletal and secondary muscular changes but not abnormal neurological development.

P120 Enrichment Toy Trauma in a New Zealand White Rabbit

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We report an unexpected problem with a whiffle ball type toy (a perforated ball made of hard plastic commercially available as a rabbit toy) that caused us to remove this toy from the rabbit enrichment program. Manipulata and food treats form the basis of our rabbit enrichment program. All singly-housed rabbits are given toys such as balls, chains, wood blocks, PVC tubing, nylabones, and corrugated plastic tunnels. All potential enrichment devices are reviewed for safety, and veterinary problems such as intestinal obstruction from swallowing enrichment toys had not been seen. The whiffle ball toy had previously been considered safe because it was made of hard non-toxic plastic, had no sharp edges, was too large to be swallowed or inhaled, and was judged too sturdy to be broken by the rabbits. However, the ball became lodged in the incisors of a singly-housed adult female New Zealand White rabbit, preventing her from eating or drinking for 12 hours, and causing significant damage to the gums. Removal of the ball necessitated anesthetizing the rabbit and using bone cutters to cut away the hard plastic ball. Ideally, environmental enrichment should increase species-specific normal behavior, and minimize stereotypies and self- and conspecific-directed abusive behavior. This case illustrates that the safety assessments for an enrichment device must include both the inherent properties of the device (e.g. the toxicity of the materials) and the risks if the toy is misused or damaged by an animal. Considerations for safety assessment are discussed.

P121 A Comprehensive Program for Dental Prophylaxis in a Research Colony of Cats and Dogs

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A comprehensive program of dental prophylaxis, utilizing state-of-the-art technology, was implemented as part of our ongoing veterinary preventive medicine program for dogs and cats. All colony animals received a thorough annual physical examination, including an oral evaluation. The presence of dental calculus, and the severity of gingivitis and periodontal disease were rated on a scale of mild, moderate or severe to prioritize animal enrollment in the dental prophylaxis program. Manual or ultrasonic scaling, followed by polishing of the teeth, were routinely performed. Ultrasonic scaling was more efficient and less traumatic to sensitive gingival tissue than manual scaling. The ultrasound technology was also very effective for scaling subgingival and/or supragingival buccal, mesial, lingual, palatal, and distal tooth surfaces. Additional assessment of some animals utilized dental radiography.
unit was more cost- and time-efficient than the traditional dental radiographic technique and substantially reduced the radiation exposure of personnel. The introduction of different diets and/or chew toys to some animals was also used to enhance their oral health. The dental prophylaxis program decreased the potential for septic bacterial infections and reduced the incidence of gingivitis, halitosis, dental caries and tooth loss in our cat and dog colonies. This trend is demonstrated by a reduced incidence of plaque formation and gingivitis by 2.3-fold and 4.4-fold respectively for years 1996–1999 inclusive.

P122 Conversion of Canine Runs to Group Social Housing for Juvenile Baboons

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Within the last decade laboratory animal research programs have experienced a dramatic reduction in the use of dogs used in training and in biomedical research. This reduction has left many laboratory animal facilities with excess numbers of rooms equipped with runs or pens. Recent emphasis on socialization and environmental enhancement for nonhuman primates has prompted laboratory animal units to look for means of economically meeting these new requirements. Our Division recently converted two rooms equipped with 10 stainless steel, elevated floor canine runs into rooms providing social housing for young baboons. The detachable walls were removed to create larger primary enclosures and tops were fitted with stainless steel panels to provide complete containment. Enrichment devices were provided by constructing sturdy climbing structures and swings fabricated from PVC pipe and connections in combination with link chain. Juvenile baboons from 6 months to two years of age have been introduced into these primary enclosures. We have introduced juvenile baboons that were previously housed singularly, in pairs, or in social groups. This group housing provides the opportunity for young baboons to participate in normal socialization and the species typical play activities prevalent among juveniles. Our group has trained the juvenile baboons to enter squeeze cages through guillotine openings available in the front door of the primary enclosures. The conversion of these canine pens has provided the socially enriched and environmentally enhanced environment necessary to fulfill both regulatory requirements and foster psychological well-being.

P123 The Effect of Mother-Infant Separation in Captive Baboons on Time Intervals to First Postpartum Estrus, Confirmed Pregnancy and Subsequent Parturition

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It was generally thought that early forced-weaning of infants would reduce the time from parturition to the first fertile postpartum estrus thus maximizing reproductive efficiency. This study evaluates that concept and based on findings offers an alternative conclusion. From a survey of 23 animal records we determined the following values for mothers of both forced and naturally weaned infants: days to first postpartum cycle and days from first postpartum cycle to confirmed pregnancy. Mothers of naturally-weaned animals (those with infants) first cycled at 174 ± 31 days and were confirmed pregnant 26 ± 13 days after this. Conversely, mothers of force-weaned infants (weaning at 180 ± 16 days) first cycled at 187 ± 8 days and were confirmed pregnant 55 ± 26 days later. From these observations we suggest that both groups (mothers of force-weaned versus naturally-weaned infants) require similar times to exhibit their first postpartum estrus, but mothers of naturally-weaned infants appear to breed back more quickly (approximately one cycle) than those of force-weaned infants.

P124 Promoting the Psychological Well-Being of Restrained NHPs through an Environmental Enrichment Program

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The use of NHPs in medical research often requires both short and long-term restraint. Physical restraint of NHPs is an attractive and viable option for many studies allowing a variety of manipulations in conscious animals. A commonly used type of physical restraint is the pole and collar system which consists of a metal or plastic collar, a spring-loaded pole and a restraint chair. In an effort to promote the psychological well-being of our NHPs, we developed an enrichment program to reduce stress during restraint. This program involves a variety of techniques designed to promote positive interactions between the animal and technician including modifications of environmental factors. Some components of the program include: manipulation of room, visual and auditory stimuli in the room, interaction with humans, execution of a positive reinforcement plan, supplementation with food treats, and provisions for periodic unrestrained activity. Although animal behavior varies between individuals, our NHPs generally exhibit a more cooperative nature during restraint. Their posture is more relaxed, they accept food and fluids from technicians, they are more calm during manipulations of limbs during sampling, they remain calm for extended periods of time (>4 hours), and they are more receptive to visual stimuli (i.e., television).

Application of these enrichment techniques provide our restrained NHPs an experimental environment designed to reduce animal stress, thereby benefiting the animal, increasing staff safety, and generating data with minimal variables.

P125 A Novel Approach To Transporting Anesthetized Nonhuman Primates

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The ability to transport nonhuman primates safely and discretely between animal facilities and core services, such as surgery and imaging, is a critical function for geographically decentralized animal facilities. In many instances, travel through public corridors is required to reach core services or other animal facilities for rearrangement of housing. We have developed an inexpensive and safe transport system for this purpose. Using a commercially available 35 x 25 x 10.5 in. poultry coop, anesthetized nonhuman primates up to 18 kg can be positioned in lateral recumbency to allow for unobstructed respiration. The coop is constructed of high-density polyethylene, which can be easily sanitized in a rackwasher and is lightweight and stackable. Prior to introducing a nonhuman primate in the coop, the coop is placed on a cart and secured with bungee cords. Plastic backed paper is placed on the floor of the poultry coop for absorbency and as protection from the hard interior surface. The lower torso...
of the animal is placed in a plastic bag or diaper which serves two purposes: it catches urine and feces and it emits a crackling noise in response to the animal’s movement which alerts the transporter about the status of sedation. The animal is positioned within the coop, the door is secured and the coop is covered with an opaque cloth to allow adequate passive ventilation while preventing aerosol transmission and visualization. Perforations in the coop are large enough to facilitate intramuscular redosing of anesthetic agents without opening the door, thus making it safe for personnel. We have transported over 300 nonhuman primates using this technique without complication.

P126 Housing, Handling and Care of a Caiman (*Caiman crocodilus*)

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Despite the steady decrease in the number of animals used in research the number of reptiles and amphibians used in research has remained constant. In some cases nontraditional reptile species have entered the research arena bringing unique concerns regarding their housing, handling and care. This reptile measured 41 inches when received. It was missing teeth and had a large abrasion on its hind limb from being housed in too small an enclosure. Bacteriological cultures taken from the injuries were positive for *E. coli*, *Streptococcus* species and *Pseudomonas aeruginosa*. This poster describes the use of a murine fiberglass tank (usually used for housing fish) to provide adequate space for movement. Moreover, the poster will outline the use of heating lamps, slabs of slate and a half of a cat carrier to provide areas for basking and hiding. The use of a hand pump siphon device is described to facilitate the weekly cleaning of the animal’s tank. The animal is fed euthanized retired Balb/c mice from a maximum isolation room. The animal is handled using heavy leather gloves and is restrained by personnel by holding the mouth closed and restricting movement of the tail. While the reptile was housed in the system the lesion healed and all of the teeth grew back. The physical condition of the animal and data evaluating its weight and length will be cited. The focus of this discussion is to outline how one institution developed a housing and husbandry system to meet the creature comforts of *Caiman crocodilus*. In addition, this presentation will describe safe handling and restrain of this reptile species. The contents of this poster should provide information for others faced with housing larger reptile species.

P127 The Effect of High Temperature on Pulmonary Antibacterial Defense in Mice

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It has been reported that the immune system of experimental animals were damaged by high-temperature exposure. To determine the effects of high temperature on pulmonary antibacterial defense, 8-week-old male BALB/cA mice were placed in animal chambers (1,650 W x 700 D x 1,830 H mm) at 23°C, 32°C, and 35.5°C for 14 days. The chambers were adjusted at 50 ± 5% relative humidity, ventilation at 10 times per hour, an air velocity of 10 cm per second, and a 12 hr light-dark cycle. Commercial chow (CE-2, CLEA Japan Inc., Tokyo) and fresh sterilized distilled water were provided ad libitum. Rectal temperature averaged 36.9°C at 23°C, 37.6°C at 32°C, and 38.9°C at 35.5°C from days 1 to 14. In comparison to the 23°C group pulmonary bacterial activity against *Staphylococcus aureus* and *Proteus mirabilis* was significantly suppressed on day 14 in the 35.5°C group but not in the 32°C group. The number of alveolar macrophage (AM) in the Bronchoalveolar lavage fluid of the non bacterially challenged mice decreased significantly after exposure to 35.5°C, but the number of polymorphonuclear neutrophils (PMNs) did not change. It is well known that killing of *S. aureus* in the lung depends on resident alveolar Mφ, and the killing of *P. mirabilis* depends on both AM and PMNs which migrate into the alveoli. Pulmonary bactericidal activity against *S. aureus* and *P. mirabilis* was suppressed at 35.5°C, which was associated with changes in the phagocyte system including AM and PMNs. This fact suggests a depressed ability of the host to defend against respiratory infection.