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# ABSTRACTS OF SCIENTIFIC PAPERS

## PLATFORM AND POSTER PRESENTATIONS 1994 AALAS ANNUAL MEETING PITTSBURGH, PENNSYLVANIA

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### PLATFORM SESSIONS

#### PS01 Lesions Caused by *Clostridium perfringens* in Germ-Free Mice

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*Clostridium perfringens* is one of the most important animal pathogens, but few reports exist of disease in laboratory mice caused by this organism. Routine quality assurance monitoring detected *C. perfringens* contamination of positive pressure isolators housing a colony of germ-free Balb/C mice. Shortly after this discovery, diarrhea, hunched posture, and unkempt coat were observed in adult mice. Six multiparous females and two males were culled for diagnostic evaluation. Necropsy revealed the small and large intestines were very distended by dark fluid and large amounts of gas. Intestinal rupture and peritonitis were observed in two animals. The females had uterine lesions and enlargement of the left cardiac atria with thrombus formation. Histologic examination revealed inflammatory lesions of lung, uterus, and intestinal wall at all levels, with clostridia visible in many vessels. *Clostridium perfringens* serotypes B and D were isolated from the gastrointestinal tracts, and *C. perfringens* serotype B was isolated in pure culture from the left atria. The lesions caused by mono contamination with *C. perfringens* in laboratory mice have not been previously described.

#### PS02 Hyperkeratosis in Athymic Nude Mice Caused by a Coryneform Bacterium: Microbiology, Transmission, Clinical Signs, and Pathology

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The purpose of this study was to characterize a spontaneous disease condition causing hyperkeratosis in nude mice and to explore the etiologic role of a particular species of coryneform bacteria in this disease, colloquially known as scaly skin disease. The study was divided into two parts. In the first phase, a series of inoculation experiments were conducted by using a field isolate of the coryneform species in order to study the clinical and histopathologic development of the disease syndrome. Athymic nude mice (4 to 5 weeks of age) were inoculated onto the skin of the back with a suspension of a pure culture of the coryneform bacterium, isolated from a field case, using a sterile cotton swab and concentrations of organisms varying from  $6.1 \times 10^4$ /ml to  $5.0 \times 10^7$ /ml. All inoculated mice became persistently infected throughout the 33 days of the experiment. Clinically

evident hyperkeratosis in inoculated animals developed more frequently in mice housed in a micro-isolator cage than in a semi-rigid isolator, and more frequently in mice inoculated with higher numbers of organisms. In all animals in which hyperkeratosis developed, it was first noticed on the 7th day after inoculation.

The second series of studies were transmission experiments designed to determine the success of various housing methods in excluding the infection, mechanisms of transmission, the susceptibility of other stocks and strains of mice to the organism, and whether the other strains might serve as a source of the organism. Results of the study in various strains demonstrated that both immune-competent and immune-deficient mice, whether glabrous or hirsute, could be infected with the organism, but only glabrous animals developed hyperkeratosis. Infection was transmitted by direct body contact of uninfected mice with infected mice and through fomites (latex gloves). Culture of skin and buccal specimens were effective in demonstrating the organism. Histologic lesions were similar in spontaneous cases, in the inoculation studies, and in the transmission experiments. Histopathologic changes were characterized by marked acanthosis, moderate hyperkeratosis, and a scant mononuclear cell infiltrate. Gram staining demonstrated numerous gram-positive bacteria in the stratum corneum, and occasionally in dyskeratotic hair follicles. The acanthosis persisted after hyperkeratosis was no longer grossly evident. The bacteria were often arranged in palisading or irregularly branching angular arrays, similar to those of *Corynebacterium* spp. Analysis of the organism disclosed a biochemical and fatty acid profile consistent with the genus, *Corynebacterium*, but distinct from other *Corynebacterium* spp. The descriptive term Hyperkeratosis-Associated-Coryneform (HAC) is suggested for the organism, and Bacterial Hyperkeratosis of Mice is proposed as the name for this disease syndrome. Present studies confirm the etiologic role of HAC in Bacterial Hyperkeratosis of Mice, and suggest HAC may have a reservoir in the laboratory animal environment allowing infection of naive animals from asymptomatic carriers or by fomite transmission.

#### PS03 Pathogenicity of Cilia-Associated Respiratory Bacillus in Immunodeficient Mice

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Cilia-associated respiratory (CAR) bacillus is an important pathogenic agent causing respiratory disease in rodents. This study investigated the extent and severity of disease induced by CAR bacillus in several commonly used immunodeficient mouse strains. Twenty-five four-week-old mice of each of the following strains: athymic NCR-nu; CB.17 *scid*; CR:NIH-bg-nxid; and BALB/cAnNCR were inoculated intranasally with  $1.4 \times 10^5$  bacilli (mouse origin) in allantoic fluid, and housed in microisolator cages on a ventilated rack. All inoculated mice developed infections demonstrated

by positive postmortem tracheal washes and/or the development of respiratory lesions. Five additional mice per strain were inoculated with uninfected allantoic fluid as controls. These mice remained healthy and free of infection. Fifteen DBA/2NCR mice received bedding from inoculated mouse cages twice weekly, as sentinels, and did not become infected. Infected mice developed rough haircoats and had poor weight gain. Mice were necropsied at 2, 4, 8, and 16 weeks after inoculation, and tissue sections were evaluated histologically with H&E and Steiner's stains. Lesions including bronchopneumonia, bronchiectasis, and otitis media were most severe in the BALB/c mice, comparable in CB.17 *scid* and *nu/nu* mice, and least severe in bg-*nu*-*xid* mice. The results indicate that while immunocompromised mice develop important lesions due to CAR bacillus, a competent immune system contributes to the overall severity of disease.

#### **PS04 Antigenic Analysis of Cilia-Associated Respiratory Bacillus Isolates with Monoclonal Antibodies**

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To assess antigenic relationships among cilia-associated respiratory (CAR) bacillus isolates, mouse monoclonal antibodies (MoAbs) were developed to a rat isolate (R-3) of CAR bacillus. Evaluation of MoAbs by enzyme-linked immunosorbent assay (ELISA) against the R-3 isolate, mouse fibroblast cells, and various bacterial species indicated that 87 of 241 hybridomas secreted CAR bacillus-specific MoAbs. Fourteen of the MoAbs were selected for further characterization. The ELISA's indicated that the epitopes recognized by the MoAbs were not shared among most rat and rabbit isolates of CAR bacillus. A rat isolate of CAR bacillus (R-2) reacted with 12 of the 14 anti-R-3 MoAbs, whereas another rat isolate of CAR bacillus (R-1) and 5 rabbit isolates of CAR bacillus did not react with any of the 14 MoAbs.

MoAb 6B1 reacted only with isolate R-3 and identified a single peptide of approximately 42 kDa on Western blot (immunoblot) analyses. Peptides of 96, 57, 42, and 24 kDa were the predominant peptides identified by the other 13 MoAbs. Indirect fluorescent antibody analyses indicated that some of the MoAbs reacted with a diffuse cell surface component of isolate R-3 and may recognize the capsule-like material reported to be present on these organisms. Our results indicate that CAR bacillus isolates obtained from different or the same host species may represent antigenically diverse isolates.

#### **PS05 Age-Related Susceptibility and Lesion Development in Experimentally Induced Hamster Proliferative Ileitis, Using a Cell Culture-Maintained Inoculum**

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Young hamsters have long been regarded as highly susceptible to the development of proliferative ileitis, implying a narrow "window" of susceptibility. The effect of age on susceptibility to proliferative ileitis was evaluated in this study by determination of enzyme-linked immunosorbent assay (ELISA) titer and lesion development following experimentally induced infection. Hamsters were experimentally infected by gavage with cell culture-maintained bacteria at 17 to 18 days of age (young test animals, n = 31), or 30 to 38 days of age (old test animals, n = 30). Age-matched controls were dosed with uninfected cell culture filtrates. The study duration was 24 days, with 6 sampling intervals for necropsy and blood collection. No significant differences in ELISA titer were seen between

the 2 age groups until the last sampling day, when the titers of the young test animals were higher than those of the old test animals. Less than 10% of test animals from either age group developed gross lesions of proliferative ileitis. Warthin-Starry staining revealed characteristic rod-shaped bacteria in the apical cytoplasm of fixed ileal sections from 10% of old test animals and 23% of young test animals. While these results suggest age relationship to infection, no correlation was seen between age and serologic titer.

#### **PS06 *Helicobacter bilis*-Associated Active, Chronic Hepatitis in Aged Inbred Strains of Mice**

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Twenty-four mice varying in age from 19 to 28 months (12 male, 12 female) consisting of four inbred strains, C57Bl, CBA/CA, DBA/2, and BALB/c, were housed in a barrier colony. Five strains of *Helicobacter bilis* were isolated from the livers or bile of 5 mice and an additional 11 strains and 18 strains from the ceca and colon, respectively. *Helicobacter bilis* has strong urease activity and was oxidase- and catalase-positive. Bacteria grew microaerophilically at 37°C and 42°C and also grew in 1% and 3% bile. Ninety-five percent of the total RNA sequence for the type strain *H. bilis* was analyzed. Comparison of the consensus sequence with other bacteria in our database indicated that the *Helicobacter bilis* sequence was most closely related to that of *Helicobacter rappini*. In C57Bl, DBA/CA and C57Bl/6 mice, multifocal hepatitis was characterized by multiple large and small foci of hepatic necrosis with inflammation, similar to that described for *H. hepaticus* hepatitis. In the BALB/c mice, liver lesions were minimal, but all 6 mice had multiple foci of lymphoid hyperplasia with lymphoid tissue displacing mucosal glands. Further studies are underway to characterize the pathogenic potential of *H. bilis* in mice.

#### **PS07 *Helicobacter hepaticus* in Mice: Isolation of the Bacterium from Multiple Tissues**

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In a series of 28 *scid* mice and 12 A/JCr mice from endemically infected colonies, *H. hepaticus* was isolated in 22 of 28 *scid* livers sampled versus 7 of 12 A/JCr livers with persistent hepatitis. In the same mice, *H. hepaticus* was isolated from the *scid* intestinal scrapings of 7/12 colon and 2/12 ceca, whereas in A/JCr mice, isolation was achieved from only 4/12 of both colons and ceca. However, by filtering the same samples through 0.45-mm filters, isolation rates were 7/12 and 8/10 in *scid* and 2/12 and 9/12 in A/JCr colon and ceca, respectively. The identity of the bacteria as *H. hepaticus* was confirmed in 20 strains by 16S ribosomal RNA-based polymerase chain reaction (PCR). Using similar techniques, intestinal *H. hepaticus* colonization was documented from inbred and outbred mice without liver lesions from commercial sources and research institutions (data not shown). In pups examined at 20 to 21 days of gestation from 6 pregnant *scid* and 4 A/JCr mice, *H. hepaticus* was recovered from 2 *scid* pups in utero, but not from the other 11 *scid* pups and 14 A/JCr pups examined. Eradication strategies must account for intestinal colonization of *H. hepaticus*, use of selective filtering and PCR techniques for accurate diagnosis, and the potential transplacental transfer of *H. hepaticus*.

**PS08 *Helicobacter hepaticus* IgG Antibody Titer and Alanine Transaminase (ALT) Liver Enzyme: Markers of Hepatitis in A/JCr Mice**

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*Helicobacter hepaticus* is known to cause a persistent chronic hepatitis in A/JCr mice. Two groups of viral antibody-free barrier-maintained A/JCr mice endemically infected with *H. hepaticus* were monitored 3 times at 6 to 10-week intervals over a 5-month period for serum alanine transaminase (ALT) concentrations and *H. hepaticus* IgG antibody titers with an enzyme-linked immunosorbent assay (ELISA), using a whole-cell bacterial sonicate as antigen. Twenty mice (10 male, 10 female) were 8 months old and another 20 mice (10 male, 10 female) were 8 weeks old when the study commenced. The older male mice had *H. hepaticus* IgG titers that increased from ~1:740 to 1:1200 to 1:1540 at the 3 time points, and the aged females had mean titers at these time points of ~1:400. The 8-week-old male and female mice had mean IgG *H. hepaticus* titers of ~1:20 at the first 2 time points, which increased to ~1:170 in the males and ~1:100 in the females at the last time point. In the older male mice, the mean ALT concentration was 228, compared with 153 for the older females; in the younger mice, the mean ALT concentration was 103 versus 77 in the males and females, respectively. Thus, ELISA titers and ALT appear to be age- and sex-dependent in *H. hepaticus*-infected mice, with the highest values in aged, male A/JCr mice, which also have the most severe hepatitis.

**PS09 Rodent Isolates of *Pasteurella pneumotropica*: Demonstration of O Polysaccharide Chains and Serologically Specific Lipopolysaccharide Antigens**

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*Pasteurella pneumotropica* infection is common in both conventional and barrier-maintained colonies of laboratory rodents. The organism is regarded as a weakly pathogenic, largely opportunistic secondary invader. Little is known about its serologic effects, including whether possible differences in virulence among various isolates might correlate with lipopolysaccharide antigens (LPS; also known as O antigens, somatic antigens, and endotoxin) as is found for several other genera of gram-negative bacteria. Lipopolysaccharide from five isolates of *P. pneumotropica*, 4 of mouse and 1 of rat origin, were prepared by proteinase K digestion. The digests were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis; some gels were silver stained for LPS and others, after Western blotting (immunoblotting), were reacted with homologous and heterologous sera from rats or mice naturally infected with *P. pneumotropica*. Silver-stained gels revealed 3 of 4 mouse isolates had LPS banding patterns typical of LPS from rough variants of *Enterobacteriaceae*, whereas the LPS of the other 2 *P. pneumotropica* isolates had ladder-like profiles characteristic of LPS from smooth variants of *Enterobacteriaceae*. Smooth LPS was antigenically distinct from rough LPS. In Western blots, sera from mice infected with rough LPS isolates reacted with all 3 isolates with rough LPS but not with smooth LPS isolates. Similarly, sera from rats or mice infected with smooth LPS isolates reacted strongly with the 2 smooth LPS isolates but not with rough LPS isolates. These results demonstrate LPS antigenic specificity among rodent isolates of *P. pneumotropica*, which could be used to serologically distinguish isolates of this widely distributed organism and to elucidate the role of LPS as a possible virulence factor influencing the pathogenesis of infection.

**PS10 The Efficacy of Various Therapeutic Regimens in Eliminating *Pasteurella pneumotropica* from the Mouse**

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*Pasteurella pneumotropica*, a gram-negative opportunistic pathogen, can be isolated from the oropharynx and the intestinal tract of normal mice and has been associated with various clinical syndromes including conjunctivitis, uterine infections, otitis, and subcutaneous abscess formation. Enrofloxacin, a fluoroquinolone antimicrobial, has been shown to be effective in eliminating *P. multocida* from rabbits. We sought to determine whether enrofloxacin would eliminate evidence of *P. pneumotropica* infection from oropharyngeal and gastrointestinal sites in mice. Forty male and forty female *P. pneumotropica*-positive (culture and immunofluorescence assay) C57BL/6N mice were randomly assigned to 3 treatment groups or a control group (10 males and 10 females/group). The treatment regimens consisted of the administration of either injectable enrofloxacin administered subcutaneously (8.5 mg/kg divided every 12 hours for 14 days), enrofloxacin in the drinking water (8.5 mg/kg/day for 14 days), or tetracycline in the drinking water (60 mg/kg/day for 14 days). All mice received reverse osmotic, deionized water or reverse osmotic, deionized treated water ad libitum. Antimicrobial efficacy was evaluated by attempts to recover *P. pneumotropica* from oropharyngeal and fecal culture samples. At treatment termination and 6 days after treatment, *P. pneumotropica* was not isolated from either the oropharynx or feces from 20 of 20 mice given the injectable enrofloxacin or from 20 of 20 mice receiving enrofloxacin via the drinking water. In contrast, 18 of 20 mice receiving tetracycline in the water were culture positive in at least one site at the termination of treatment. Nineteen of 20 mice in the control group were culture positive for *P. pneumotropica* in at least one site at the termination of treatment. It was concluded that both the injectable and oral formulation of enrofloxacin were effective in eliminating *P. pneumotropica* from the oropharyngeal and gastrointestinal tract of mice for at least 1 week. However, the oral route may be a more practical way to treat large numbers of mice. Studies are continuing to determine the length of time that these mice remain culture-negative for *P. pneumotropica* infection after treatment.

**PS11 Morphologic Changes in the Nasal Cavity Associated with Sialodacryoadenitis Virus Infection in the Wistar Rat**

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A sequential study of lesions of the nasal cavity associated with sialodacryoadenitis virus (SCAV) infection was made in the laboratory rat. Wistar rats were intranasally inoculated with approximately 10<sup>3</sup> TCID<sub>50</sub> of the coronavirus, SCAV. Transverse sections of four regions of the nasal cavity from inoculated and control animals were examined by light microscopy and immunohistochemical analysis on postinoculation (PI) days 2, 4, 6, 8, 10, and 14. Lesions were observed in the following regions of the upper respiratory tract: respiratory epithelium, transitional epithelium, olfactory epithelium, nasolacrimal duct, vomeronasal organ, and the submucosal glands of the nasal passages. Viral antigen was demonstrated by immunohistochemical analysis in all regions during the acute stages of the disease, with the exception of the vomeronasal organ. Of particular interest was the presence of lesions in the nasolacrimal duct, the olfactory epi-

thelium, and the vomeronasal organ, which were still detectable in these tissues at PI day 14. In view of these findings, it is evident that infections of the respiratory tract with viruses such as SCAV could have important effects on functions such as olfaction and chemoreception for up to two or more weeks after exposure in this species.

#### **PS12 Protein Profiles of Prototype and Wild-Type Rat Coronavirus Isolates Grown in the L2p.176 Subline of Mouse Fibroblasts**

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Rat coronaviruses (RCVs) are common agents causing both subclinical and clinically apparent infections in laboratory rats and potentially confounding research results. Recent advances in the in vitro growth of rat coronaviruses are permitting study of the basic and applied biology of RCVs and will lead to better understanding of their pathogenesis and epidemiologic factors. However, cell culture systems so far described have not permitted generation of plaque-cloned virus stocks, reliable isolation of RCVs from rat tissues, or growth of the high titered RCVs needed for protein analysis by immunoblot. Because only a proportion of L2 (Percy) cells were permissive to RCV infection, sublines of L2 (Percy) cells were produced and were selected to maximize growth of wild-type and prototype RCVs. Generation and screening of 238 sublines of L2 (Percy) cells yielded a subline, L2p.176, which is susceptible to all RCVs tested. L2p.176 cells have been used to isolate virus from natural outbreaks and to propagate individual RCV plaques into high titered stocks. Proteins from six RCV isolates grown in L2p.176 cells were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose and reacted with polyclonal rat and mouse antibodies to sialodacryoadenitis virus (SDAV-681) and polyclonal monospecific antibodies against the peplomer, nucleocapsid, and matrix proteins of mouse hepatitis virus (MHV). Although the exact sizes and ratios of protein forms varied among RCV isolates, proteins consistent with those of MHV were seen in the two prototype, one Japanese, and three wild type RCVs examined, confirming the similarity of RCV proteins to those of MHV.

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#### **PS13 An Enzyme-Linked Immunosorbent Assay for the Detection of Antibody to Lymphocytic Choriomeningitis Virus in Mouse Sera, Using Recombinant Nucleoprotein as Antigen**

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Lymphocytic choriomeningitis virus (LCMV) is a natural pathogen of the laboratory mouse. It is usually transmitted vertically and causes immune tolerance and persistent infection in affected animals. Because of the persistent viremia, these mice shed virus throughout their lives and usually die of renal failure due to the accumulation of antigen-antibody complexes in their kidneys. LCMV is a zoonotic agent. Apparent disease in humans is generally associated with flu-like symptoms, but encephalomeningitis and occasional deaths have been reported. LCMV is not a common pathogen in laboratory mice, but since it is used fairly often in immunology studies and because of its zoonotic nature, it is important to monitor laboratory mouse colonies regularly for the presence of this agent. In the past this has been done serologically by immunofluorescence assay (IFA) using infected cells as antigen. This method requires the handling of infectious virus in the laboratory, a potential hazard to human and animal health. To avoid this, we have developed an enzyme-linked immunosorbent assay (ELISA)

based on recombinant LCMV nucleoprotein (NP). The gene encoding the LCMV-NP has been cloned into the polyhedrin gene of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) baculovirus. This recombinant insect virus now expresses LCMV-NP in high quantities. Crude extracts of sf9 cells infected at high multiplicity of infection with the recombinant baculovirus were used as antigen for the ELISA. This assay detected antibody to LCMV in sera of naturally and experimentally infected mice. It is currently being validated by comparison with an established IFA. The new ELISA eliminates the need to work with a zoonotic agent in the lab while still allowing effective screening of laboratory mouse populations for LCMV.

#### **PS14 Long-Term in Vivo Immune Dysfunction Occurs after Infection with Mouse Hepatitis Virus**

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We have previously reported that MHV strain A59 inoculated intranasally into adult BALB/c mice results in permanent immune dysfunction as demonstrated by decreases in several in vivo immune responses. Inoculation of C57BL/6 mice does not produce this altered state. Interestingly, whereas infectious virus could be recovered in the thymus and bone marrow of BALB/c mice during acute infection, no virus could be recovered from the bone marrow of C57BL/6 mice. This result prompted studies regarding the reconstitutive properties of bone marrow and thymus from previously infected BALB/c mice. By this protocol, mice were infected with a sublethal dose and held for 100 days. This is a time point known to reproducibly demonstrate in vivo dysfunction and is a point when no infectious virus is recoverable. Recipient mice were irradiated with 900 R and then injected with  $10 \times 10^6$  T cell depleted bone marrow cells intravenously. The experimental groups were control bone marrow into control mice, control bone marrow into MHV mice, MHV bone marrow into control mice, and MHV bone marrow into MHV mice. Recipient mice were monitored for reconstitution by flow cytometry, which indicated normal levels of lymphoid cells. At day 45, 4 animals were euthanized from each group and monitored for in vitro immune responsiveness. Six animals were given injections of T dependent and independent antigens and assayed by ELISPOT as a monitor of in vivo responsiveness. Significant decreases were observed in proliferative responses to concanavalin A and anti-CD3mAb in control recipients of MHV bone marrow as well as in MHV recipients of control bone marrow. In vivo responsiveness was also significantly decreased in both groups. Throughout the experiment, no infectious virus could be detected, and no seroconversion occurred in control recipients of MHV bone marrow excluding the possibility of virus transfer. These data indicate that critical changes occur in the thymus and bone marrow of MHV-infected mice resulting in dysfunction, which can be reproduced with the transfer of either tissue to prevent complete reconstitution of in vitro and in vivo immune responsiveness. Furthermore, the results suggest that long-term immune dysfunction in MHV-infected mice may be due, in part, to changes in the primary compartment.

#### **PS15 Detection of Mouse Hepatitis Virus Shedding in the Feces of Mice by the Reverse Transcriptase Polymerase Chain Reaction**

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Current control methods and disease elimination strategies for mouse hepatitis virus (MHV)-infected colonies are based on physically stopping the fecal/oral/aerosolization routes of transmission with barrier containment,

or stopping horizontal transmission by rederivation of lines or cessation of colony breeding for a defined period of time. Recent application of reverse transcription polymerase chain reaction (RT-PCR) in the detection of MHV in murine somatic tissues has created a sensitive assay of tumor contamination and postmortem confirmation of disease of this infectious disease. We have extended the use of RT-PCR to detect viral shedding in feces. Female four-week-old BALB/c mice were injected once with the JHM strain of MHV by intraperitoneal (I.P.) injection. A control group received a single I.P. saline injection. All mice were singly housed in microisolator cages within an Illinois cubicle. All cage materials, including bedding, food, and water, were autoclaved prior to use. One cohort of mice, containing both MHV-injected and saline controls, was assigned a study duration period of 6 days, the remainder a duration of 11 days. Serum was obtained on the day of arrival, the day of injection (day 0), and on the last day of the study for that particular cohort. Fecal specimens were obtained daily from each mouse directly from the anus. All mice on their respective final day of study were euthanized under humane conditions with charged carbon dioxide and subsequently necropsied. Tissues sampled for histologic examination included liver, spleen, brain, duodenum, jejunum, cecum, and colon. Virus excretion in feces of MHV-inoculated mice was detected on the second day following inoculation and thereafter, using a modified guanidine isothiocyanate extraction procedure that eliminates the need for chloroform/phenol purification of RNA. A modified RT-PCR procedure on a crude nucleic acid preparation was performed with commercially supplied reagents, using primers previously reported by other investigators to be solely targeted against MHV mRNA 1-6. No virus was detected from the control mice. Clinical signs of disease were evident on day 3 in MHV-inoculated mice. Histologic lesions found in the day-6 MHV-injected group were consistent with MHV infection. No mice had seroconverted by the end of the study. This preliminary study suggests that detection of individuals shedding MHV can be accomplished at an early stage of infection prior to serologic detection. This detection offers the ability to selectively cull or isolate infectious individuals, thus providing an additional strategy in the management and eradication of this disease.

#### **PS16 Detection of Orphan Parvoviruses by Polymerase Chain Reaction**

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Diagnosis of rodent orphan parvoviruses currently relies on evaluation of sera in multiple immunologic assays using conventional rodent parvoviruses as antigens. Sera that test positive by indirect fluorescent antibody or enzyme-linked immunosorbent assay and negative by hemagglutination inhibition are tentatively identified as positive for orphan parvovirus. A single definitive assay to identify infected animals is critically needed. In this project a polymerase chain reaction (PCR) assay specific for orphan parvoviruses was developed. The DNA sequence of the capsid region was obtained from three recently isolated orphan parvoviruses, two of mouse origin and one of hamster origin. Sequence data were compared with sequence data for the characterized rodent parvovirus; regions unique to the orphan parvoviruses were identified; and, orphan parvovirus-specific primers were designed. These primers amplified only the three orphan parvoviruses isolated when tested against DNA from a panel of murine viral pathogens, including all known rodent parvoviruses. The PCR assay was also able to detect orphan parvovirus DNA in tissues from rodents experimentally infected with orphan parvovirus. This PCR assay provides an extremely sensitive and specific test to detect orphan parvovirus infection in animals and will enhance diagnosis and aid in epidemiologic studies of these viruses.

#### **PS17 Development of an Murine Animal Model of Replicative *Legionella pneumophila* Lung Infection**

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Legionnaires' disease, characterized most frequently by severe pneumonia in humans, is caused by *Legionella pneumophila*, a facultative intracellular bacteria of mononuclear phagocytic cells (MPCs). Susceptibility to *L. pneumophila* is mediated by permissiveness of MPCs to growth of the bacteria and by cellular immunity involving the production of cytokines including gamma interferon (IFN- $\gamma$ ). Replication of *L. pneumophila* in MPCs is dependent on the presence of iron. IFN- $\gamma$  limits growth of the bacteria by decreasing the concentration of intracellular iron. The most frequently used animal model of Legionnaires' disease is the guinea pig. This species is highly susceptible to *L. pneumophila*-induced infections, due to both permissiveness of guinea pig MPCs to growth of the bacteria and to the lack of production of IFN- $\gamma$  in response to *L. pneumophila*. However, studies investigating the pathogenesis of *L. pneumophila* infections by using this species are severely hindered by the current lack of guinea pig-specific reagents. The purpose of this study was to develop a murine animal model of replicative *L. pneumophila* lung infection to facilitate future studies investigating the pathogenesis of *L. pneumophila* lung infection in vivo. While MPCs from most mouse strains are not permissive to growth of *L. pneumophila*, results of previous studies indicate that peritoneal MPCs from A/J mice support the growth of the bacteria in vitro. We hypothesized that alveolar MPCs from A/J mice would support growth of *L. pneumophila*, resulting in replicative *L. pneumophila* lung infections in these mice following intratracheal (I.T.) inoculation of bacteria in vivo. To test this hypothesis, A/J mice were inoculated I.T. with virulent *L. pneumophila* (serogroup 1, strain AA100,  $10^6$  organisms/mouse). At specific times thereafter (0 to 72 hours), the mice were euthanized and the lungs were excised and homogenized. *Legionella pneumophila* in the lungs was subsequently quantitated by culture of lung homogenates on BCYE agar. *Legionella pneumophila* replicated in the lungs of A/J mice over the first 48 hours following I.T. inoculation of the bacteria, resulting in an approximate 10-fold increase in the number of bacteria in lungs of mice 48 hours after I.T. inoculation. Over the next 24 hours, the number of bacteria in the lung declined significantly. Because IFN- $\gamma$  has previously been implicated in the resolution of *L. pneumophila* infections, effects of I.T. *L. pneumophila* on plasma levels of IFN- $\gamma$  was assessed by an enzyme-linked immunosorbent assay specific for murine IFN- $\gamma$ . Results of these studies indicated that IFN- $\gamma$  is induced in A/J mice within 9 hours following I.T. inoculation of *L. pneumophila*, reaching peak concentrations 24 hours after induction of lung injury. These results indicate that production of IFN- $\gamma$  precedes clearance of *L. pneumophila* from the lungs, suggesting that this cytokine may mediate resolution of *L. pneumophila* lung infections in A/J mice. In conclusion, these results indicate that A/J mice, inoculated I.T. with *L. pneumophila*, may provide a murine model of replicative *L. pneumophila* lung infection, facilitating future studies regarding the pathogenesis of *L. pneumophila* lung infection in vivo.

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**PS18 *Helicobacter*-Associated Hypergastrinemia: The Ferret (*Mustela putorius furo*) as an Animal Model**

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*Helicobacter pylori*-related hypergastrinemia has been well documented in humans. However, the mechanism by which *H. pylori* causes hypergastrinemia is unclear. We examined plasma gastrin levels in 21 *H. mustelae*-infected ferrets and 10 ferrets specific pathogen-free (SPF) for *H. mustelae* to determine whether *H. mustelae* was associated with hypergastrinemia. Withholding of food followed by 30 and 60 min post-meal stimulated plasma samples were obtained, and gastrin was measured by radioimmunoassay. The results for the *H. mustelae* group in pg/ml (mean  $\pm$  SEM) were as follows: food withheld (54.4  $\pm$  2.56); 30 min (94.5  $\pm$  6.05); and 60 min (82.6  $\pm$  5.73). The SPF group results were: food withheld (55.8  $\pm$  7.35); 30 min (80.8  $\pm$  5.77); and 60 min (59.7  $\pm$  4.95). There was a significant difference at the 60-min time point between the two groups of animals. Although there was not statistical significance at the 30 min time point, the *H. mustelae* group had a 17% higher mean gastrin level. Future studies should provide additional data on the peak rise in meal-stimulated plasma gastrin in the ferret. Thus, the ferret appears to be useful as an animal model to study *Helicobacter*-associated hypergastrinemia. Supported in part by NIH grants RR01046 and RR07036.

**PS19 Prior *Helicobacter mustelae* Infection Does Not Confer Protective Immunity Against Experimental Reinfection in Ferrets**

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*Helicobacter pylori* infections in people have been associated with gastritis, ulcer formation, and gastric adenocarcinoma. It is important to know whether prior infection and treatment for *H. pylori* results in protective immunity. This question was investigated by using *H. mustelae* in ferrets, which is an accepted model for human *H. pylori* infection. Two groups of ferrets naturally infected with *H. mustelae* were treated by using a therapeutic regimen known to eradicate *H. mustelae* (amoxicillin, metronidazole, and bismuth subsalicylate). Results of repeated culturing of endoscopic gastric mucosal biopsy specimens were negative for *H. mustelae* for 6 months (4 ferrets) and 17 months (5 ferrets) after eradication. The ferrets were then reinfected by using an oral dose of  $4.5 \times 10^7$  colony-forming units of a strain of *H. mustelae* with a distinctive DNA restriction enzyme pattern. Culture of gastric biopsy specimens performed at 4 to 5 weeks and 9 to 11 weeks after reinfection revealed infection with the new strain. These findings indicate that eradication of *H. mustelae* from naturally infected ferrets does not protect against experimental reinfection. A study is currently underway to determine whether the natural fecal-oral route of transmission is affected by prior exposure to *H. mustelae*. Supported in part by NIH grants RR01046 and RR07036.

**PS20 The Role of *Helicobacter mustelae* Homolog *cagA* Gene in Gastritis and Ulcer Disease in the Ferret**

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*Helicobacter pylori* is the predominant cause of chronic gastritis and peptic ulcer disease in humans. The importance of the putative virulence factors of *H. pylori* is being actively studied. Approximately 60% of *H. pylori* isolates produce a vacuolating cytotoxin in vitro and express a 120 to 128-kDa protein recently termed the CagA protein. Studies of *H. pylori* infection indicate that infection with cytotoxin and CagA-positive strains is associated with a higher incidence of ulcer disease. Because the *H. mustelae* ferret model of *Helicobacter* infection is the only animal model that develops gastric and duodenal ulcer disease, we are investigating the presence of the *cagA* gene in *H. mustelae* isolates. Colony blot hybridizations were performed in which various *H. mustelae* isolates were hybridized with a radiolabeled oligonucleotide probe (pMC3) to the *cagA* gene of *H. pylori*. The dot-blots of *H. mustelae* exhibited heterogeneity for the presence of the *cagA* gene similar to that of *H. pylori*. Five *H. mustelae* isolates from ferrets with ulcers and severe gastritis were positive for the *cagA* gene while 3 nonpathogenic isolates were negative for the *cagA* gene. Therefore, the CagA protein may represent an important virulence factor for the development of gastritis and ulcer disease in *H. mustelae*-infected ferrets as hypothesized in human *H. pylori* infection. Further characterization of the homolog of the *cagA* gene and the CagA protein of *H. mustelae* and its relationship to disease in the ferret is currently underway.

**PS21 Evaluation of Cecal Ligation as a Model of Mucoïd Enteropathy in SPF Rabbits**

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Mucoïd enteropathy is a serious disease of rabbits, for which the cause is unknown. Toofanian and Targowski found that ligation of the cecum caused a mucoïd enteropathy-like syndrome in 70% of rabbits. The goal of this study was to evaluate this model of mucoïd enteropathy in *Pasteurella*-free, coccidia-free rabbits. Five rabbits served as nonsurgically treated controls (group 1). Eight rabbits underwent ligation of the cecum, sparing large vessels and nerves (group 2). In six rabbits, the distal branch of the ileoceocolic vessels and nerve were incorporated into the cecal ligature (group 3). Necropsy 3 to 5 days following surgery revealed copious amounts of clear gelatinous mucus in the colon of all group-3 rabbits. Only one group-2 rabbit had grossly evident mucus hypersecretion, while none of the group-1 rabbits did. Group-3 rabbits had areas of necrosis in the cecum; this was not seen in group-1 or group-2 rabbits. Both group-2 and group-3 rabbits had inflammation of the distal portion of the colon. In SPF rabbits, cecal ligation alone does not reliably stimulate mucus hypersecretion. Cecal ligation including vessels provides a reproducible model of mucoïd enteropathy; however, the severe cecal necrosis seen is not consistent with the naturally occurring disease.

## PS22 Mucus Secretagogue Activity in Cecal Contents of Rabbits with Experimentally Induced Mucoïd Enteropathy

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Cecal filtrates from rabbits with cecal ligation-induced mucoïd enteropathy have been reported to cause goblet cell hyperplasia in intestinal explants. This study was performed to see whether such filtrates would stimulate mucus secretion from intestinal explants. Filtrates were prepared from cecal contents of 5 control rabbits (group 1), 5 rabbits that had undergone cecal ligation (group 2), and 5 rabbits in which the distal branch of the ileocecolic vessels and nerve were incorporated into the cecal ligature (group 3). Following incubation of each with ileal and colonic explants from 5 healthy rabbits, the amount of mucus in the media/filtrate solution was measured by using an enzyme-linked lectin assay. Significantly more mucus was secreted by colonic explants in the presence of cecal filtrates from either group 2 or group 3, compared with group 1. Using pooled filtrates from each group, these results were confirmed by measuring secretion of radiolabelled mucus from explants that had been preincubated with <sup>3</sup>H-glucosamine. This work supports the hypothesis that cecal contents of rabbits with cecal ligation-induced mucoïd enteropathy stimulate mucus secretion from colonic explants.

## PS23 Characterization of Gastric Ulcers in TGF $\beta$ 1-Deficient 129/CF1 Mice

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Transforming growth factor beta-1 (TGF $\beta$ 1) is a polypeptide homodimer with a molecular mass of 25 kDa that is a member of a larger family of growth and differentiation regulatory peptides including TGF $\beta$ 2 and TGF $\beta$ 3. Mice deficient for TGF $\beta$ 1 were obtained through disruption of the endogenous TGF $\beta$ 1 allele in murine embryonic stem (ES) cells via homologous recombination. In homozygous TGF $\beta$ 1-deficient 129/CF1 mice (mutants), there were numerous inflammatory lesions associated with mortality between the ages of 15 and 35 days. One of the more severe lesions found in 48% of the mutant mice was severe stomach ulceration. A serial study of neonatal mice was performed to determine the onset and progression of the stomach ulcers. Ten neonatal and ten control mice were examined when they were 5, 7, 10, and 14 days old. In addition, 50 moribund mutant mice and 30 control mice were examined ranging in age from 15 to 35 days. Initial lesion development was characterized by mild lymphocytic and neutrophilic infiltration of the nonglandular submucosa in 4 14-day-old mice. In mice over 14 days old, mild lesions were characterized by mild hyperplasia of the nonglandular epithelium, mild submucosal edema, and mild inflammation. In the moribund animals, the lesions progressed in severity. Lesions in the moribund mice included submucosal edema (27/50), hyperplasia of the nonglandular epithelium (30/50), hyperkeratosis (36/50), increased inflammatory cell infiltrate (40/50), and ulcers (24/50). All of the severe pathologic changes were confined to the nonglandular region and glandular/nonglandular junction of the stomach. Mild lymphocytic infiltration into the submucosa of the glandular stomach was observed in 4 mutant mice. No lesions were observed in control mice or in mice less than 14 days old. The results of this study support the possibility of a role of TGF $\beta$ 1 in the prevention of gastric ulcers. Several possible mechanisms for the role of TGF $\beta$ 1 in ulcer prevention are presented.

## PS24 Glutathione and Immune Status in a Mouse Model for Human Aging

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Deficiencies in glutathione (GSH) status and immunocompetence are well known factors in the aging process, and both are associated with increased morbidity and mortality in the elderly. Recently, a number of important correlations between GSH status and lymphocyte function have been demonstrated by modulation of cellular GSH, but there has been only one limited investigation in relation to aging. Our hypothesis is that GSH status and immune function are closely linked and have causal roles in aging. To establish this relationship, we began to systematically determine GSH and immune status in a mouse model throughout its life span to document changes associated with growth, maturity, and senescence. GSH and immune status were initially determined in 3 groups of 6 C57BL/6 mice: one group at 6 to 8 weeks of age, one group at one year of age, and one group at two + years of age. State of the art flow cytometry and mouse monoclonal antibodies were used to obtain total T, B, T helper, and T suppressor lymphocytes, and lymphocyte response to mitogen stimulation was determined by radiometric technique. We also determined GSH status by measuring the concentrations of reduced and oxidized GSH and cyst(e)ine with key high-pressure liquid chromatography-dual electrochemical analysis that simultaneously and specifically measures these metabolites. Correlation of life span profiles of immune and GSH status was accomplished by using correlation coefficients, coefficients of variation, and Student's *t* test to emphasize the onset, rate, and extent of changes. Decreased GSH and its metabolites in adult and aged animals corresponded with decreased numbers of lymphocytes as well as decreased lymphocyte response to mitogen stimulation. The most significant correlation was observed in T lymphocytes, especially T helper cells. Our research provides objective and meaningful indices of immunocompetence and GSH status in a mouse model for human aging, which may provide the basis to help determine the mechanisms of senescence and to develop methods to delay, prevent, or reverse these changes.

## PS25 mac-Peripheral Blood Leukocytes-*scid* Mice: A Small Animal Model for Studying Interactions of SIVsmmPBj14 with Pig-Tailed Macaque Lymphocytes

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Severe combined immunodeficient (*scid*) mice engrafted with adult human peripheral blood leukocytes (PBL) have been used to study the interactions of human immunodeficiency viruses with T lymphocytes. To facilitate analysis of interactions of an atypical simian immunodeficiency virus, SIVsmmPBj 14 (SIV-PBj 14), with pig-tailed macaque (*Macaca nemestrina*) lymphocytes, we developed an analogous *scid* mouse model by using PBL from adult pig-tailed macaques. Various protocols, all using intraperitoneal inoculation of 2 to 5 x 10<sup>7</sup> macaque PBL into young adult C.B-17 *scid* mice, were attempted; these included inoculation of resting or phytohemagglutinin-stimulated macaque PBL, followed at 3 weeks by inoculation of cell-free SIV-PBj14 or SIV-PBj14-infected lymphocytes, and in vitro SIV-PBj14 infection of activated PBL prior to inoculation of *scid* mice. Although macaque IgG was detected in serum samples from some mice at 3 weeks after cell inoculation, the presence of serum IgG was not always predictive of T-cell engraftment since SIV-infected cells were recovered from spleens and peritoneal washes of some IgG-negative

mice 3 weeks after virus injection (6 weeks after PBL inoculation). Using recovery of SIV-infected cells as a measure of engraftment, 86% of mice that received PHA-stimulated PBL, compared with only 33% that received resting PBL, were successfully reconstituted (mac-PBL-*scid*). Because high viral load, immune activation and cytokines, such as tumor necrosis factor- $\alpha$  and interleukin-6, appear to be important in SIV-PBj14-induced rapid death of pig-tailed macaques, we are currently evaluating these factors in serum samples from the infected mice. The mac-PBL-*scid* model may be valuable for defining the role of cytokines and identifying specific biologic properties that are predictive of acute SIV-PBj14 disease. Furthermore, this model may be useful for screening molecular chimeras derived from SIV-PBj14 before selection of those clones most likely to provide maximum information for testing in pig-tailed macaques.

## PS26 Cage Height Preferences of Laboratory Rats

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Laboratory animal scientists and the public at large are becoming increasingly concerned about the welfare and well-being of animals used in teaching, testing, and research. There has been a long history of laboratory animal cage size increases in an effort to improve their well-being. However, the cage changes were based on anthropomorphic assumptions since little objective information exists regarding what cage features are considered desirable from the animal's perspective. In the present study, 350 to 400-g male CRL:SD rats were housed in standard (64 x 25 x 17.8 cm), stainless-steel, hanging-wire cages divided into two equal-sized chambers by a Plexiglas wall. The rats had free access to both sides of the cage via a cut-away door; food and water were available ad libitum in both chambers. Each animal's position within the apparatus was determined through analysis of video images recorded in 42-hour-long segments equally distributed in each 24-h period. Each animal's position was determined by videotape playback with locations noted every 10 min. Following initial acclimation to the cage in which both chambers have 17.8 cm cage heights, the following cage height comparisons were made: 17.8 cm vs. 12.7 cm and 17.8 cm vs. 7.6 cm. After each trial, the cage heights were returned to 17.8 cm, and the rats were allowed to reacclimate. Baseline data indicating side preferences were determined, and downward adjustments to the cage height were made on the nonpreferred side. All of the rats demonstrated an immediate and reliable preference for the 12.7 cm height. This change was particularly evident during the rats' inactive periods, but was apparent at all time points. The preference increased over each of 5 days, and the chamber preference persisted following return of the height to 17.8 cm. Short-cage preferences were also seen for 7.6-cm cages; however, the effects displayed a strong circadian cycle, with 7.6 cm being preferred during the inactive period and 17.8 cm and 7.6 cm being equally preferred during the active period. The chamber preference did not persist when the cage height was returned to 17.8 cm. This study describes an experimental method useful in determining rat preferences for housing features. It also indicates that laboratory rats prefer a cage height lower than the currently accepted standard.

## PS27 Determination of End Points for Euthanasia of Immunosuppressed Rats with Natural *Pneumocystis carinii* Infection

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*Pneumocystis carinii* pneumonia is a leading cause of morbidity and mortality in patients with AIDS and other immunocompromised hosts. Research on *P. carinii* has been dependent on animal models because there is

no continuous in vitro cultivation system. Immunosuppressed, viral antibody-positive rats, which develop *P. carinii* pneumonia with histologic features identical to those in the human form of the disease, have been the most widely used experimental model. In order to maximize the yield of organisms from an individual animal, the rats are allowed to progress to a moribund state and are euthanized. We investigated possible end points to determine a potential objective time point for euthanasia of the immunosuppressed rats before they are moribund. Two hundred Sprague-Dawley, 60 Long Evans, and 60 Lewis rats were given weekly subcutaneous injections of 4 mg of methylprednisolone acetate, starting at 6 weeks of age (150 to 175g). Rats typically develop *P. carinii* pneumonia within 6 to 12 weeks of initiation of immunosuppression. Data on four variables—end weight, weight loss, food intake, and length of immunosuppression—were collected weekly and at euthanasia. Results were compared with the severity of infection in the rat and the amount of recoverable *P. carinii*. The Spearman rank correlation coefficient assuming nonparametric statistics was used to examine the data. There was no correlation between the four objective variables nor any combination of the four variables and severity of disease. Thus, these four variables are not adequate measures of organism burden. The only means currently available for determining organism burden remains to be subjective—that is, listlessness, no interest in food or water, and severe lethargy.

## PS28 Humane and Practical Implications of Rodent Anesthesia and Euthanasia Using Different Mixtures of Carbon Dioxide and Oxygen

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A study was undertaken to determine whether, and under what circumstances, CO<sub>2</sub> can be used humanely for rodent euthanasia and anesthesia. Based on PHS Policy, which states that, unless the contrary is established, investigators should consider that procedures that cause pain or distress in humans may cause pain or distress in animals, the sensory qualities of CO<sub>2</sub> were studied in humans. Volunteers (N=8) were asked to breathe different mixtures of CO<sub>2</sub> and oxygen and rate the resulting sensations from 1 to 10 (1 = not at all unpleasant; 3 = mildly unpleasant; 5 = mildly uncomfortable; 7 = mildly painful; 10 = extremely painful). Each mixture was presented twice in random order. One hundred percent and 80% CO<sub>2</sub> were rated as painful (mean scores = 8.44  $\pm$  0.37 and 7.8  $\pm$  0.34, respectively) and 70%, 60%, and 50% CO<sub>2</sub> were rated as uncomfortable but not painful (mean scores = 6.58  $\pm$  0.36, 5.77  $\pm$  0.48, and 5.54  $\pm$  0.36, respectively). In a parallel study, CO<sub>2</sub> (60 to 100%) was used to anesthetize adult male Sprague-Dawley rats (N = 16) and, 3 weeks later, to euthanize these rats. The 50% concentration was not tested because, in a pilot study, time to anesthesia was significantly longer with 50% versus 100% CO<sub>2</sub>, and seizures or hemorrhaging from nose occurred in 3/4 animals anesthetized with 50% CO<sub>2</sub>. In the present study, half of the rats were placed in an enclosed 18-liter chamber that had been precharged with the gas mixture, and half were placed in the chamber prior to the introduction of the gas. Total gas flow for both conditions was 10 liters/min. Time to anesthesia or death was recorded, and data were analyzed by analysis of variance and the Tukey HSD Multiple Comparisons test. With 100% CO<sub>2</sub>, time to anesthesia (unresponsive to periorbital bleeding) was 1.71  $\pm$  0.36 min and time to death was 3.87  $\pm$  3.13 min. Times to both anesthesia and death were increased with lower concentrations of CO<sub>2</sub> but the increases were significant only with 60% CO<sub>2</sub>. Precharging the chamber had no significant effect on time to anesthesia or death. It was concluded that animals exposed to CO<sub>2</sub> in concentrations 80 to 100% probably experience considerable pain and distress. It is likely that anesthesia or euthanasia with 60 to 70% CO<sub>2</sub> is more humane, 70% CO<sub>2</sub> being the most practical alternative to the higher concentrations.

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## **PS29 Increasing Awareness of Animal and Human Relationships Among Elementary School Students**

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Early in 1993, the Michigan Society for Medical Research (MISMR) was approached by a local elementary school to participate in an 8-week educational enrichment program for children. MISMR contacted members of the Unit for Laboratory Animal Medicine at our institution to participate in the project. During organizational meetings, it was decided that the goal of the program would be to increase the awareness of students about the many areas of human endeavor that involve animals and how these interactions influence everyday human life. Specific topics for presentation were identified, including Pets, Farm Animals, Working Dogs, Wild Animals, Zoo Animals, Laboratory Animals, and Careers with Animals. Staff members volunteered to present specific topics and formulated lesson plans for each topic. These lesson plans were directed at two different grade groupings, grades 1 and 2 and grades 3 to 5. The lesson plans were key tools that addressed the program's overall goals as well as the specific learning objectives, teaching methods, evaluation techniques, and resources required for each topic. In preparation for the enrichment classes, each staff member performed a mock presentation to the other members of the group for general feedback. Presentations to the students were made once a week for 8 consecutive weeks. They were designed to actively involve students and maintain their attention during the entire session. Specific activities included storytelling, discussion groups, question and answer sessions while viewing slides, origami techniques, interviews, demonstrations with live animals, and handling of various animal husbandry supplies and equipment. Success in reaching learning objectives was measured at the conclusion of each weekly session by using crossword and word search puzzles, word-matching games, information retention from previous presentations, and slide identification exercises. The program was presented at two different schools in 1993, and both requested the program again in 1994. The value of this program is that it has enabled our institution to become involved in the process of educating elementary students in the ways that both animals and humans benefit from interaction. In addition, it has provided us with an opportunity to be proactive in presenting biomedical research and its participants positively to the community.

## **PS30 The St. Louis Consortium for Animal Welfare Education**

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The St. Louis Consortium for Animal Welfare Education was formed in 1993. Member institutions are Monsanto, Washington University, St. Louis University, Little Creek Farms, Inc., and Mallinckrodt Medical, Inc. The goals are to promote a high standard of animal care and use that is shared among its members. The principal tool is to combine training resources within the membership. Among the 3 major institutions there are 14 veterinarians to provide teaching services. One objective is to arrange seminars and workshops for investigator training at each of its member sites. These are developed to meet the needs of each institution based on the training objectives of the USDA regulations. In addition, animal technician needs are met with formal AALAS certification courses and continuing education seminars at Washington University. A second objective includes developing a central database for resource materials of all members. Consortium members are research institutions that join as either resource or contributing members. Resource members Washington University and St. Louis University provide much of the teaching staff. Contributing members

Monsanto, Little Creek Farms, Inc., and Mallinckrodt Medical, Inc., provide financial support in a formula based on staff size and on-site training. Contracts extend a 3-year period and specify the services of a multi-center training coordinator and member participation in an advisory committee. The organization and activities of the consortium will be discussed.

## **PS31 Animal Alternatives Recognition Award**

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The Merck Research Laboratories Institutional Animal Care and Use Committee established an Alternatives Subcommittee (ALTSC) to help ensure that alternatives are satisfactorily addressed prior to initiating animal research. The ALTSC subsequently developed an Animal Alternatives Recognition Award to recognize employees who develop and publish novel animal alternatives in the category of Replacement, Refinement, or Reduction. The Merit Award for Animal Replacement Technology recognizes the development of methods that replace animals with nonanimal procedures. The Merit Award for Animal Refinement Technology rewards the development of animal research methods that eliminate or minimize animal pain or distress. The Merit Award for Animal Reduction Technology is awarded for the development of methods that reduce the number of animals used. In all cases, the methodology developed must have a quantitative and/or qualitative measurable impact on animal research activities and must be "in press" or published. In keeping with the Merck Worldwide Policy on Animal Care and Use, which states that Merck "supports research for scientifically valid alternative methodologies," the Animal Alternatives Recognition Award is designed to help educate investigators in the concept of animal alternatives, to encourage the development and use of animal alternatives, to recognize and reward individuals successful in the development of animal alternatives, to improve documentation of animal alternative developments, and to serve as evidence of the company's commitment to the development of animal alternatives.

## **PS32 The Northridge Quake of 1994 and Disaster Preparedness Program Planning for Laboratory Animal Care Facilities**

KA Overhulse

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On January 18th, 1994, at 4:30 a.m., an earthquake of magnitude 6.8 on the Richter scale struck in Northridge, Calif. This earthquake had major impact on the structure of the California State University Northridge campus. Due to the severity of the damages and the recurrent after shocks, the campus was shut down to all personnel. The research animals on campus were trapped in the buildings for 12 days before emergency personnel could rescue them. On the basis of that scenario, think through the typical hazards faced in your area. Develop an emergency response team: 1. Make officials on the emergency response team aware of the plan needed. 2. Have animal care staff member(s) assigned to your institution's emergency response team. 3. Train staff in emergency procedures and hazardous materials handling. Procure supplies: 1. For temporary losses: store water and get a backup generator or battery-operated lights and space heaters or fans. 2. Keep a store of emergency filter bonnets to cover cages in transit. 3. Set aside an emergency euthanasia kit. Develop a disaster response plan: 1. If the building is restricted to access for a term longer than 1 week, the animals should be removed to another facility or euthanized to prevent starvation, dehydration, or animal suffering. 2. If possible, arrange for another, off-site facility to provide backup housing. Have a representative of this facility knowledgeable of your emergency response team members, evacu-

ation plan, and contacts. Have your Animal Care and Use Committee give this person authority to take over animal care and when necessary, euthanasia. Prepare an evacuation plan: 1. Prioritize your animals (replaceable, irreplaceable). 2. Prepare an emergency contact system of home phone numbers to be kept by the facility manager and the head of the animal care and use committee and someone at the emergency relief facility (be sure to include USDA numbers). 3. Prepare for an emergency transport vehicle preferably from the safe area. 4. Have a monthly review of your emergency list and emergency supplies. 5. Develop scenarios, for emergency, partial destruction, total destruction, loss of utilities, toxic spills, biological hazards, etc....In the case of California State University Northridge, they were fortunate enough to have had a member of the emergency response team aware of the animals being housed on campus. The animals received food and water for two weeks following the earthquake. Because of the danger of remaining in the buildings for extended periods, cage changing was not performed. Only 5 of over 200 animals died. Unfortunately there was no disaster plan relating to the animals, no one from the animal care program could be contacted. There was no evacuation plan for the animals. But even with this barrier to overcome, the administrative officials responded to the animals' needs as soon as their attention was drawn to the situation. Therefore, it is important to have outside contacts, persons who will have a copy of the evacuation plans and a census of animals and their locations. Although a disaster preparedness program will not prevent interruption of research projects, it may save valuable animal resources. An example of this is the colony of captive bred Deer Mice maintained at the California State University Campus for over 20 years. With the current problem with Hantavirus, these animals could prove most valuable to researchers across the state.

### PS33 *Giardia* Vaccination in Puppies and Kittens

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Giardiasis is a common protozoan infection of humans and domestic animals and is considered to be an important zoonosis. We examined the potential of vaccination to prevent the disease in animals exposed to this parasite. *Giardia*-free puppies (n = 20) and kittens (n = 20) were immunized subcutaneously on days 0 and 21 with a vaccine prepared from the trophozoites of *Giardia lamblia* (S2 strain). Control animals (10 puppies and 10 kittens) received saline. On day 35, all animals were challenge exposed by an intraduodenal inoculation of 106 trophozoites. Clinical signs (diarrhea, anorexia, lethargy), weight gain, fecal cysts, and serum immunoglobulins were monitored following the challenge exposure. On day 56, animals were euthanized, and duodenal, jejunal, and ileal samples were collected for trophozoite enumeration, mucosal antibody determinations, and pathologic examination (light and electron microscopy). Control animals had an impaired growth rate and demonstrated a higher incidence of clinical signs when compared with vaccinated animals. Fecal cysts were passed in lower numbers and in fewer animals in the vaccinated group. On day 56, 5% of the vaccinated kittens and 15% of the vaccinated puppies had intestinal trophozoites, while 60% of the control kittens and 100% of the control puppies had intestinal trophozoites. Vaccinated puppies and kittens had an elevated serum IgG titer, compared with control animals. Vaccinated kittens had an elevated serum and mucosal IgA titre compared with control kittens. Vaccination of kittens and puppies may allow control of giardiasis in pet populations and minimize transmission from animals to humans.

### PS34 A Retrospective Examination of Dystocia in a Squirrel Monkey Breeding Colony

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Historically, squirrel monkey breeding colonies have had poor reproductive performance when compared with other nonhuman primates. The reproductive records of three subspecies of *Saimiri* housed at the Primate Research Laboratory from 1981 through 1993 were reviewed to determine the prevalence of dystocia. Dystocia occurred in 12% of all pregnancies (121/1033) during the 13-year period. Dystocia occurred significantly more frequently in pregnancies of colony-born monkeys (16%, 37/232) than wild-caught monkeys (11%, 84/801). Forty-nine percent of dystocias in colony-born monkeys occurred in primigravida monkeys. Peruvian squirrel monkeys were more likely to experience dystocia during delivery (28%, 18/65 pregnancies) than Bolivian (10%, 92/885) or Guyanese (13%, 11/82) squirrel monkeys. There was a significant relationship between dystocia and abortion, stillbirth, and intrauterine fetal death. Dystocia could not be attributed to large fetal mass; in most cases the fetus weighed less than 100 g. The mean weight for a term live infant was 104.6 ± 2.2 g. Twenty-eight of 30 caesarian sections were performed because of dystocia, either for malpresentation (7/30) or failure to deliver within 2 hours after the initiation of labor (21/30). Peruvian squirrel monkeys required caesarian sections (8%, 5/65) more frequently than Bolivian (3%, 23/885) or Guyanese (2%, 2/82) squirrel monkeys. Dystocia resulted in 15 adult female deaths or 10% of the total adult female deaths in the colony. Colony-born squirrel monkeys and Peruvian squirrel monkeys are at greatest risk for dystocia and fetal wastage.

Supported by NIH grant P40 RR01254.

### PS35 Hypoglycemia Induced by Food Withholding in a *Cynomolgus* Monkey

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A 4-year-old, captive-born, female *Macaca fascicularis* showed abnormal behavior (weakness, ataxia, and disorientation) after food was withheld for 24 hours before routine research sampling. Physical examination revealed the animal was hypothermic (temperature = 96.4°F [35.78°C]). Serum chemical analysis revealed a serum glucose concentration of 31 mg/dl and an alanine aminotransferase concentration of 171 U/liter. The monkey subsequently experienced three other incidents of food withholding-induced hypoglycemia and hypothermia that responded to supportive care, which included oral and I.V. glucose replacement. Follow-up diagnostic procedures to determine the underlying cause included complete blood counts, repeat serum chemical analysis, thyroid function profile, preprandial insulin, glucagon, glucose:insulin ratios, liver biopsy, radiography, and ultrasonography. Because of the high serum glucose values and normal insulin values during a 24-h food withholding trial, it was thought that the initial hypoglycemia was due to a lack of regulation of insulin or glucagon prior to the onset of type II diabetes mellitus. The underlying cause of hyperglycemia is often a difficult diagnosis, the major clinical clue being the clinical history of the animal. Because the animal's mother had type II diabetes, the most likely diagnosis is that the daughter is in a prediabetic state, and hypoglycemia was the first sign. Hypoglycemia has been reported in some prediabetic humans, but its cause is not well documented.

Because diabetes is inherited, hypoglycemia could be monitored in the offspring of diabetic monkeys to identify monkeys in a prediabetic state. Such animals might be useful in determining the pathogenesis of hypoglycemia seen in prediabetic humans and its relationship to the development of diabetes mellitus.

#### **PS36 Idiopathic Blistering in Two Macaques (*Macaca mulatta*)**

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Two adult male macaques (*Macaca mulatta*) were found to have multiple blisters and scabbed lesions following prolonged isoflurane anesthesia (>10 hours) for the implantation of a middle ear device. The two animals were second and fourth in a series of seven animals that underwent the surgery. The nonpainful lesions appeared on hands, feet, and trunk and ranged in size from 1 cm in diameter to 10 cm by 7 cm. Lesions were not associated with pressure points. Primary rule-outs for the lesions included burns, drug reaction, embolic shower, and autoimmune disease. All equipment including heating pads and electrocautery were examined and found to be in working order. Histologic examination of punch biopsy specimens revealed a subepidermal blister. The stratum malpighii of the epidermis had areas of coagulative necrosis, spongiosis, and stained eosinophilic. A mild polymorphonuclear cell infiltrate was also seen. Other epidermal layers appeared normal. Microscopic examination of more mature lesions demonstrated necrosis and hemorrhage in the dermis and infiltration of granulation tissue. No histologic evidence of bacterial, viral, or other foreign particles were found. Results of bacteriologic cultures of blister contents were negative. All lesions were nonprogressive and healed with conservative therapy. The signs appear to mimic "coma blisters" in humans, which is a syndrome of bullae and erythematous lesions in people who are in drug-induced unconsciousness. This syndrome is only rarely reported in humans (estimated 5% occurrence in coma victims) and never before in primates or other animals to our knowledge.

#### **PS37 Skin Disease in the Aged Rhesus Macaque**

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Nonhuman primates are frequently used for aging studies. We observed a high incidence of skin disease among a group of geriatric rhesus monkeys (mean age = 25 years; N = 9) used in aging behavioral studies. Gross and histologic changes in skin of the aged rhesus were compared with skin from control adult rhesus monkeys (mean age = 10; N = 4) to characterize age-related skin changes. Complete physical examinations were performed on all animals. Biopsy specimens were taken from four specified skin locations (lateral to bridge of nose, ventral midline, dorsal midline, perineal area) and from additional areas where skin lesions were present. Samples were routinely processed and evaluated by light microscopy. Blood samples were collected and tested for estrogen, thyroid-stimulating hormone, triiodothyronine, thyroxine, and cortisol levels. The axilla was swabbed and samples were obtained from all animals for bacterial and fungal culturing. All nine of the older monkeys had notable dermal lesions while only one of the control animals had abnormal findings. Major gross findings included increased areas of erythematous skin, wrinkling, thinning of hair, callous formation, and proliferative and exudative dermatitis. Histologic skin changes were classified as proliferative, degenerative, or inflammatory. These changes were not associated with hormonal abnormalities or bacterial or fungal pathogens. Histologic changes are compatible with non-specific skin changes observed in elderly humans. This study establishes a

baseline of dermatologic changes of the aging rhesus macaque. These findings indicate that the nonhuman primate is a useful model for understanding degenerative skin changes in humans.

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#### **PS38 Reconstructive Vaginal Surgery in the Female Baboon (*Papio sp.*) with Simian Agent 8**

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Simian Agent 8 (SA8) is a neurotropic, endemic, alphaherpes virus in the *Papio* sp. Clinical lesions associated with SA8 infections usually involve the genitalia of sexually mature baboons. In females, secondary bacterial infections may lead to scarring of the vulva and perineum, with resulting vaginal obstruction. Affected baboons are poor breeders and often develop urinary tract infections due to the retention of urine in the vagina. Reconstructive vaginal surgery was performed on 7 baboons with vaginal obstruction. Four weeks prior to surgery, 50 mg of norgestrel was administered intramuscularly to each animal, to arrest the estrous cycle, reduce swelling, and to allow adequate surgical exposure of the vagina. Ventral and stellate incisions were made around the constricted vagina. The peripheral skin was excised to break down the underlying connective tissue allowing access to the vaginal mucosa. Healthy vaginal tissue was then opposed to the incised skin edges in an interrupted pattern. Once healed, the vaginas remained patent allowing normal mating and urination. Nine months after surgery, 5 females have had babies, one was pregnant, and one was not pregnant. This procedure shows promise, in that baboons unable to reproduce and at risk for urinary tract infections may now become healthy productive breeders.

#### **PS39 B Virus DNA Shedding in the Oropharynx of Apparently Healthy Macaques During Routine Physical Examination**

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Most macaques used in research are latently infected with herpes simiae (B virus). Although B virus causes few or no lesions in these animals, it is capable of causing systemic infection and fatal encephalitis in humans. The potential for acquiring zoonotic infection with B virus is a constant threat to all personnel working with macaques. We have used the polymerase chain reaction (PCR) to determine whether apparently healthy macaques may actively shed B virus in the oropharynx. Twenty macaques that had recently arrived at our animal facility were subjected to a routine physical examination just before being brought out of quarantine. All the macaques were positive for B virus antibodies as determined by enzyme-linked immunosorbent assay. The oropharynx of each animal was rinsed with saline. PCR analysis (in conjunction with Southern blotting and primer extension) of the DNA extracted from the oral rinses revealed that approximately half of the animals were actively shedding B virus at the time of sample collection. These data indicate that a surprisingly large percentage of macaques may actively shed B virus in their oropharynx during routine clinical procedures and probably at other times. The findings also underscore the need for all personnel working with B virus-seropositive macaques to exercise proper biosafety procedures to prevent B virus zoonotic infection.

**PS40 Risk of Venereal B Virus Transmission in Rhesus Monkeys (*Macaca mulatta*) Through Use of Molecular Epidemiology**

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Epidemiologic methods were used in conjunction with polymerase chain reaction (PCR) testing and conventional laboratory diagnostic approaches to quantitatively assess the role of hypothesized venereal routes of B virus (herpesvirus simiae) transmission in a culled population (n=49) of rhesus monkeys (*Macaca mulatta*). Nineteen monkeys were B virus antibody-positive in terminal serum specimens, but no active virus shedding was detected in mucosal (conjunctival, oral cavity, genital epithelium) swab samples collected at the time of death. DNA extract pools of trigeminal and lumbosacral sensory ganglia from these carcasses were tested for the presence of the ICP18.5 (UL28) gene of B virus by PCR, followed by Southern blotting and oligoprobing, using an internal 128-bp sequence specific for the agent. PCR detected B virus DNA in neuronal tissues of 15 monkeys, presumably latently infected, including 6 of 47 (12.8%) trigeminal and 11 of 48 (22.9%) lumbosacral ganglia extracts (two monkeys positive at both sites). Multivariable logistic regression was used to assess risk factors for B virus antibody-positivity, given the history of each cull available in computerized colony records. In this analysis, a monkey history that included breeding use was significantly predictive of B virus infection. On the basis of this result and the sample-wide history of breeding use, the population attributable fraction of B virus infection due to breeding was calculated to be 22.7%, in close agreement with the 22.9% of monkeys PCR-positive for B virus DNA in neuronal tissues subserving the genital region. Sexual contact is an important, but not predominant, mode of B virus transmission between monkeys.

**PS41 Clinical and Epidemiologic Features of Simian Parvovirus Infection in Cynomolgus Monkeys**

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We have recently identified a new simian parvovirus in cynomolgus monkeys. Reported herein are the detailed clinical and epidemiologic findings for an outbreak of simian parvovirus-induced anemia that necessitated euthanasia of 6 animals at the Comparative Medicine Clinical Research Center. The major clinical finding was severe normocytic, normochromic, nonregenerative anemia. Other prominent signs were diarrhea, dehydration, and positive fecal culture for *Campylobacter* spp. All animals were positive for simian parvovirus DNA, and bone marrow lesions typical of parvovirus infection were observed by light and electron microscopy. Affected animals had active infection with type D simian retrovirus on the basis of its isolation from several tissues at time of necropsy. Transfer of animals from single cages into group housing and type D retrovirus-induced immunosuppression appear to have been important factors in the outbreak. Simian parvovirus infection should be considered an important differential diagnosis in monkeys with severe anemia, particularly if a number of animals are affected, and if predisposing factors such as immunosuppression are present.

**PS42 Detection and Cloning of a Novel Papillomavirus (CPV 1) from an Outbreak of Focal Epithelial Hyperplasia-Like Disease in a Chimpanzee (*Pan troglodytes*)**

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An outbreak of focal epithelial hyperplasia (FEH) of the oral mucosa occurred in a chimpanzee (*Pan troglodytes*) colony. Analysis of the DNA from lesions from 10 chimpanzees by Southern blot hybridization showed an episomal papillomavirus DNA with a molecular size of about 8,000 base pairs. Enzymatic restriction analysis of the viral DNA indicated that the same viral type was present in all lesions examined. The whole viral DNA was cloned in two fragments into the *EcoRI* sites of the pGem plasmid vector. Further characterization of this novel papillomavirus, designated chimpanzee papillomavirus by DNA sequence analysis of part of E1 and L1 Open Reading Frame of the viral DNA, showed extensive sequence homology with the newly described pygmy chimpanzee papillomavirus 1 as well as the human papillomavirus 13. The latter virus has been uniquely associated with FEH disease in humans and pygmy chimpanzees. These findings indicate that related papillomaviruses can elicit similar lesions in different primate host species. The chimpanzee may prove to be a good animal model to study the efficacy of antiviral drugs to control papillomavirus-related oromucosal diseases.

**PS43 A Primate Bronchoscopy Model of Silicosis and Coal Workers' Pneumoconiosis**

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A primate bronchoscopy model of coal workers' pneumoconiosis and silicosis is currently being tested to investigate the relationships between dust characteristics, alveolar lining materials, and the development of pulmonary fibrosis. Bronchoscopy was used to instill characterized mineral dusts into a focal area of *Macaca nemestrina* lung. Bronchoalveolar lavage (BAL) was used to recover alveolar lining materials from dust-exposed and nonexposed lungs of the same animals during the development of pulmonary lesions. We found that multiple doses of fibrogenic or nonfibrogenic dust could be bronchoscopically placed into the caudal right lung lobe and that the dust and resulting lesions remained localized to the area of instillation. Generic quartz dust produced marked focal fibrosis and necrosis that resembled progressive massive fibrosis in humans. Generic anthracite coal dust produced marked interstitial macrophage accumulation with slight fibrogenesis that resembled "dust overload syndrome" associated with nonfibrogenic nuisance dusts. Cells obtained by BAL were morphologically representative of the major inflammatory cells within the lesions. This model provides abundant cells and alveolar materials for in vitro examination of pulmonary macrophages, proteins, eicosinoids, free radical products, surfactant, and lysosomal enzymes. It also permits more experimental control over dust variables, lesion development, and individual animal variables than previous inhalation models.

## POSTER SESSIONS

### P01 Substrain Differences in Body Weight and Survival of F344 Rats Housed in Inhalation Chambers

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F344 rats, available from several sources, are widely used in chemical toxicity and carcinogenicity studies. The U.S. National Toxicology Program has recently reported increased body weight and decreased survival over time in the F344/N rat. Two substrains of F344 rats have been used in chronic inhalation studies at our facility. Control data from two large studies conducted at about the same time were compared for differences in maximum mean body weight and survival. F344/N rats from an in-house colony started in 1983 with litters from the NIH Genetic Resource were compared with F-344/CrlBR rats. Biochemical and immunologic genetic monitoring showed both substrains to be identical and consistent with the allelic profile established for the Fischer strain. All animals were housed in H2000 inhalation chambers and fed the same commercial certified rodent diet. The maximum mean body weight attained by F344/N rats during the study was 484 g for males and 322 g for females. Male F-344/CrlBR rats attained a maximum mean body weight of 434 g, and females reached 276 g. The median survival time for F344/N rats was 696 days of age for males and 753 days for females. The median survival time for F-344/CrlBR rats was 747 days for males, and more than 50% of females survived at 781 days when the study was concluded. Thus, it appears that the F-344/CrlBR substrain of the F344 rat has a lower body weight and a longer survival under the same conditions, compared with the F344/N substrain. These differences in substrains should be recognized when designing and reporting studies.

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### P02 Ultraviolet Induced Melanogenesis in Skh:HR II Pigmented Hairless Mice

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The Skh: HR II pigmented hairless euthymic mouse (*Mus musculus*) has been studied as an animal model for dermal melanogenesis as a response to UV radiation. The Skh:HR II mouse develops a full hair coat after birth of brown, agouti, grey, or black, which is shed by three weeks of age. The black juvenile coated animals retain an X-linked dominant trait coding for increased dermal melanogenesis in response to UV radiation. To further define the model, our laboratory used selective breeding techniques in combination with UV irradiation and Warthin-Starry melanin staining and quantitative image analysis for L-dopa melanocyte activity to select for animals that exhibit black juvenile coat and melanin-positive melanocytes. The process of melanogenesis includes melanocytic tyrosinase activation, melanin synthesis and transport, and processing of melanosomes by subjecting the mouse to gradually increasing doses of UV radiation from an artificial source. The tissue techniques described quantitate the amount of melanin present in whole skin sections as a percentage of the total skin (stained) area, clearly demonstrating the increased presence of melanin as a result of exposure to UV radiation. The results for melanin disposition were then utilized to select for "black juvenile coat" breeders dominant for increased dermal melanogenesis. This model may be used for studies involving acute and chronic UV irradiation, photoaging, melanogenesis, and phototumorigenesis.

### P03 The Effects of Ciliary Neurotrophic Factor and Brain-Derived Neurotrophic Factor Co-Administration in Wobbler Mouse Motor Neuron Disease

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Ciliary neurotrophic factor (CNTF) or brain-derived neurotrophic factor (BDNF) slows the disease progress of wobbler mouse motor neuron disease (MND). To investigate the effects of CNTF and BDNF co-administration in this MND, we injected recombinant human CNTF and BDNF (or control vehicle) subcutaneously 6 days/week, alternately 1mg CNTF/kg, 3 times per week, and 5mg BDNF/kg, 3 times per week, for 4 weeks in 14 wobbler mice after the diagnosis was clinically made at 3 to 4 weeks of age. The semiquantitative and quantitative assessments of neuromuscular function were performed weekly. After the treatment, the number of cervical ventral root (C5/C6) myelinated fibers was analyzed. The spinal cord was examined by use of electron microscopy. Paw abnormality and walking patterns did not deteriorate in treated mice as they did in control mice. The average grip strength did not decline in CNTF/BDNF-treated wobbler mice, and in fact 4 of 7 treated mice increased their grip strength from baseline. In CNTF/BDNF mice, open field locomotion, which tests spontaneous motor activity, significantly increased, and time for running 2.5 feet remained unchanged in contrast to that in control mice. Motor axons undergoing acute axonal degeneration (myelin ovoids) in treated mice were fewer in the C5 and C6 ventral roots ( $P, 0.005$ ) than the control. The number of myelinated nerve fibers was 23% greater in treated mice ( $P, 0.005$ ). However, motor neurons of the spinal cord in the treated mice still developed pathognomonic vacuolar degeneration. In conclusion, CNTF/BDNF co-administration significantly retarded the motor dysfunctions in wobbler mice, far more than CNTF or BDNF given individually, indicating that CNTF/BDNF co-administration has additive or synergistic effects on wobbler mouse MND. Although motor axons were significantly preserved with CNTF/BDNF treatment, the mechanisms of the additive effects with CNTF/BDNF co-administration need to be further studied. The investigation with this murine MND not only expands our understanding of MND in general but also suggests important therapeutic implications in human disease.

### P04 Cloning, Characterization, and Possible Disruption of the Murine Medium-Chain Acyl-CoA Dehydrogenase Gene in Embryonic Stem Cells

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Fatty acid oxidation occurs in the mitochondria to provide energy especially during fasting.  $\beta$ -oxidation of fatty acids consists of a repeating circuit of four sequential steps. Medium-chain acyl-CoA dehydrogenase (MCAD) is involved in catalyzing the initial dehydrogenation of fatty acid acyl-CoA molecules 4 to 16 carbons long. Medium-chain acyl-CoA dehydrogenase deficiency is considered a common inborn error of metabolism. MCAD-deficient patients develop clinical episodes, especially during fasting, resulting in hypoglycemia, disrupted ketogenesis, dicarboxylic aciduria, and hyperammonemia with significant mortality rates during infancy. We are pursuing the development of an animal model of MCAD deficiency via embryonic stem cell technology. The entire mouse MCAD cDNA has been cloned via library screening and reverse transcription-polymerase chain reaction. This cDNA has been used as a probe to clone mouse MCAD genomic fragments via library screening. A MCAD targeting vector designed to disrupt the MCAD locus via insertional mutagenesis has

been electroporated into embryonic stem cells. An embryonic stem cell colony with the putative targeted event has been identified via Southern blotting, using an internal neomycin probe. Further analysis with external probes will confirm the targeted event.

#### **P05 Studies on *Apodemus agrarius* Pallas (Striped Field Mice) as a New Laboratory Animal Model to Study Hantaan Virus**

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This study was conducted to develop wild *Apodemus agrarius* Pallas (Striped Field Mice [SFM]), which are natural hosts of Hantaan virus, as a new laboratory animal model through domestication and pathogenic microorganism control. Therefore, SFM captured at six different local areas in Korea were studied to identify naturally occurring infections of pathogenic microorganisms and parasites. Samples were tested by using ELISA, continued particle agglutination (PA), haemagglutination inhibition (HAI), an automatic microbiologic identification system, and microscopic examination. The results of ELISA for *Mycoplasma pulmonis*, Mouse cytomegalovirus, Lymphocytic choriomeningitis virus, Mousepox virus, Mouse adenovirus, Mouse encephalomyelitis virus, Reovirus 3, and *Encephalotozoon cuniculi* were 3.7 to 25.9% positive. Hantaan virus and Japanese encephalomyelitis test results were 18.5% positive when PA and HAI were used. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Haemolyticus*, and *Mycoplasma* spp., were isolated from trachea, lung, small intestine, and cecum. Tapeworms were found in the liver, small intestine, and cecum, and mites were found on hair samples. SFM were then successfully bred in the laboratory. Subsequent studies were conducted on laboratory-bred SFM to test their susceptibility to Hantaan virus inoculated via the intrapulmonary route. Tissues of lung, kidney, liver, and spleen were examined for Hantaan virus antigen by indirect immunofluorescence antibody test. Prior to inoculation of virus, SFM were negative for antibody to Hantaan virus but 2 weeks after inoculation, they had antibody titers of 1:1024 in the lungs from the first to third week; however the kidney, liver, and spleen did not contain virus antigen. In conclusion, wild SFM possessed several pathogenic microorganisms. Laboratory-reared SFM inoculated with Hantaan virus demonstrated formation of specific antigen in the lung.

#### **P06 Experimental *Clostridium difficile* Infection and Disease in Hamsters Fed a High Fat/Cholesterol Diet**

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Following the observation that hamsters fed a high fat/cholesterol diet had high morbidity associated with *Clostridium difficile* infection, an experimental study was conducted to further characterize the disease. Specific aims were to examine the reproducibility of the disease, describe histologic lesions and relate them to culture and toxin results, and to examine the cecal flora in vitro. Two groups of hamsters were fed either a standard rodent diet or the high fat/cholesterol diet. Both groups were orally challenged with a toxigenic *C. difficile*. Hamsters fed the high fat diet suffered 80% morbidity, whereas the control hamsters had 11% morbidity. The most common lesions of clinically ill hamsters were necrohemorrhagic cecitis and cecal mucosal hyperplasia. Hepatic lipidosis was consistent in all hamsters fed the high fat diet. Toxigenic *C. difficile* was recovered from the ceca in most of the clinical cases as were toxins A and B. Cecal flora studies did not reveal any differences in the microflora between the two groups

of hamsters. In vitro colonization resistance studies indicated that *C. difficile* growth was inhibited by both groups, suggesting that ceca from the hamsters fed the high fat diet were not more susceptible to *C. difficile* colonization. Further studies with this model may aid in identifying the mechanisms by which *C. difficile* colonizes and induces disease in hamsters.

#### **P07 Rats as a Model to Study Vascular Pathophysiologic Responses to *Pasteurella haemolytica* Antigens**

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Pneumonic pasteurellosis is often associated with stressful practices, such as transport, and is a major clinical problem in several species. Vaccines have had a limited impact on the incidence of clinical disease and may be a physiologic stress predisposing to clinical disease. Since vaccination often occurs during stressful periods, this study was conducted to determine whether exposure to *Pasteurella* antigens may cause pathophysiologic disturbances in the pulmonary circulation of rats. The results in rats were compared with those in sheep and calves, since pasteurellosis is a major clinical problem in ruminants. Animals were given i.m. injections of a vaccine-derived strain (biotype A) of *P. haemolytica* (10<sup>5</sup> CFU). Three days later, pulmonary vessels were removed for scanning electron microscopic examination of the endothelial surface. Furthermore, ex vivo biophysical responses to carbachol, an endothelial-dependent vasodilator, were determined. Exposure of animals to *P. haemolytica* caused endothelial morphologic disturbances characterized by leukocyte adherence to the vessel wall, fibrin deposition, and lamellipodia type foot processes on the endothelium. Morphologic disturbances were greatest in calves, although all three species had substantial alterations. In all three species, endothelial-dependent cholinergic responses were substantially decreased. The degree of endothelial morphologic disturbance positively correlates with the impairment in cholinergic responses. These experiments indicate that avirulent vaccine-derived antigens cause morphologic and functional disturbances in the vascular endothelium and that rats are a suitable model to study the response in ruminants. Such disturbances may contribute to the pathogenesis of clinical disease, particularly during periods of stress.

#### **P08 Experimental Intravaginal *Tritrichomonas foetus* Infection in the Estrogenized Mouse.**

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Studies were undertaken to establish intravaginal *Tritrichomonas foetus* infections in BALB/c mice as a model to elucidate parasite and host factors that affect vaginal protozoan infections. *Tritrichomonas foetus* trophozoites were inoculated intravaginally into mice. Vaginal washes were collected weekly for culture of *T. foetus* and estrous cycle staging. Isolate ATCC 30003 demonstrated that infections could only be established in mice in which estrus was induced and maintained. Estrus induction by weekly injection of estradiol cypionate resulted in purulent vaginal discharge and perivulvar abscesses, while silastic implants containing 15 mg estradiol did not cause animal health problems and were effective in induction and maintenance of estrus. Studies with estrogenized BALB/c *nu/nu* mice indicated that establishment of isolate 30003 infection was difficult in *nu/nu* mice and that the course of infection was not lengthened. Estrogenized mice inoculated with recently isolated *T. foetus* strains MU17905 and MUY-32 resulted in prolonged trichomonad infections. Passage of isolate 30003 in mice for 140 days did not alter the course of infection of this isolate. Results suggest establishment and maintenance of

intravaginal *T. foetus* infections in mice is greatly influenced by inherent parasite factors, although host immune responses may contribute to the elimination of vaginal trichomonad infections.

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### **P09 The Generation of a Transgenic Rat Model That Uses a Secreted Reporter Gene Product for the Study of $\beta$ -Amyloid Protein Processing**

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Transgenic mice, and more recently, transgenic rats, have been generated as models for human disease. The utility of transgenic rats in the study of central nervous system disease has been recognized as a natural progression of transgenic technology, since rats are the primary model system for behavioral and pharmacologic studies in this area. Currently, attention has been focused on Alzheimer's disease (AD), which affects over 4 million people in the United States alone. Recent studies have demonstrated the ability of cells to process amyloid precursor protein ( $\beta$ -APP), a major component of the senile plaques found in AD patients, in various ways. Aberrant cleavage of  $\beta$ -APP may lead to the deposition of amyloid. To study the proteolytic activities involved in the metabolism of  $\beta$ -APP, a reporter gene system was developed and used to produce transgenic rats. This novel reporter system mimics the processing of  $\beta$ -APP, and has been validated *in vitro*. This system allows the expression and cleavage of  $\beta$ -APP to be assessed qualitatively and quantitatively through serum analysis. These rat transgenics may provide useful models to study the effects of chronic overexpression of potentially amyloidogenic protein fragments and the development of Alzheimer's-like lesions. The generation of these transgenic rats and the reporter gene system will be discussed.

### **P10 Comparison of Differential Analysis vs. Photometric Assay for Quantitative Measurement of Polymorphonuclear Leukocyte Migration In Vivo**

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Historically, differential cell counts have been the preferred method of quantitating polymorphonuclear leukocyte migration into the peritoneal cavity. However, this method is subject to variation in interpretation and is time-consuming when there are large numbers of samples to process. A method for utilizing an enzymatic photometric assay to quantitate peritoneal lavage fluid polymorphonuclear leukocytes from mice has been developed. The myeloperoxidase specific for polymorphonuclear leukocytes was used as a marker enzyme to determine peritoneal lavage fluid polymorphonuclear leukocyte content. Mice were injected intraperitoneally with 2.0 ml of thioglycollate to induce an acute inflammatory response. Peritoneal lavage fluid was collected at 0, 3, and 5 hours after thioglycollate administration by means of an intraperitoneal lavage (10 ml Hanks balanced salt solution, 0.03% EDTA). Total blood cell counts were performed and then whole cell suspensions of peritoneal lavage fluid were solubilized with hexadecyltrimethylammoniumbromide. Myeloperoxidase content was assayed by using 3, 3', 5, 5'-tetramethylbenzidine in N,N-dimethylformamide as a substrate in the presence of 0.3 mM hydrogen peroxide in the 96-well microplate format. Since the peroxidase content varies between cell preparations, the enzyme released by a known number of cells was determined for each experiment. A cytospin was made of one lavage fluid sample from each timepoint, stained with a Wright-Giemsa stain, and a differential cell count was performed. This was then used to

calibrate the assay to correct for variations in the peroxidase content of different peritoneal lavage fluid preparations at different time points by generating a standard curve that correlates optical density readings to known polymorphonuclear leukocyte concentrations. Optical density readings from the assay correlated with the number of polymorphonuclear leukocytes in a linear fashion from  $1 \times 10^3$  to  $5 \times 10^4$  cells/well with intra-assay coefficients of variation of <10%. Polymorphonuclear leukocyte cell counts determined by myeloperoxidase assay showed a direct correlation between polymorphonuclear leukocyte cell counts obtained by white blood cell differentials of the same peritoneal lavage fluid (correlation coefficient = 0.970). These results suggest that quantitation by myeloperoxidase assay is a suitable substitute for quantitation by differential analysis. The elimination of the need for differentials and the ability to process large numbers of samples in a short period of time make this an ideal assay for research applications.

### **P11 Thioglycollate-Induced Peritonitis in the Mouse as a Model of Acute Inflammation**

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Inflammation plays a key role in many common disease conditions in humans (such as inflammatory bowel disease and reperfusion injury). In order to evaluate the potential of new anti-inflammatory compounds under development, it is imperative that reproducible animal models be used. Thioglycollate is commonly used in a number of animal species for harvesting leukocytes. This model may also be used in the evaluation of new anti-inflammatory compounds. Model development involved studies to determine the most effective volume of irritant (thioglycollate) and characterization of the inflammatory response after thioglycollate administration. Comparison studies were performed by using 1 or 2 ml of thioglycollate administered intraperitoneally. The results of these initial studies suggested that 2 ml of thioglycollate produced a consistent and significant increase in leukocytes. Time course studies were then conducted by injecting 2 ml of thioglycollate and harvesting cells at 2, 3, 3.5, 4, 5, and 24 hours. Leukocytes were harvested by euthanizing the animals (CO<sub>2</sub> asphyxiation) and flushing the peritoneal cavities with 10 ml of Hanks balanced salt solution (+0.3% EDTA). Cells were then counted by using a cell counter or hemocytometer. To normalize the time-related response to thioglycollate, the mean number of cells determined from a natural group (animals not treated with thioglycollate;  $0.4$  to  $0.6 \times 10^6$ /ml) was subtracted from each individual animal treated with thioglycollate. The results of the timing study demonstrated that the cell count was significantly increased at 3 hours ( $0.6 \times 10^6$ /ml) and continued to increase through 5 hours after thioglycollate administration ( $2.5 \times 10^6$ /ml). The cell count did not change significantly at 24 hours (compared with the 5-hour time point). Histologic evaluation of the peritoneal fluid obtained from unstimulated (natural) mice revealed 10 to 15% of the total leukocytes were neutrophils, and most of the remaining cells were macrophages (~43%) and lymphocytes (~35%). Peritoneal fluid from thioglycollate-stimulated mice demonstrated a marked increase in neutrophils ranging from 60 to 70% at 3 and 5 hours. Remaining cells were mostly macrophages (~20%). This thioglycollate-induced model of peritonitis provides a rapid and reliable screening method for *in vivo* examination of potential anti-inflammatory compounds.

## **P12 Fertilization Rate and In Vitro Preimplantation Embryo Development in C.B-17 *scid/scid* Mice**

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The *scid* mutation was first discovered in a colony of C.B-17 mice in 1980. Very little has been reported in the literature about the reproductive biological properties of C.B-17 *scid/scid* mice (*scid*). In this study, we looked at the fertilization rate, the in vitro embryo development to blastocyst, the effect of timing of hormone injections for superovulation, and the approximate time of ovulation following injection of human chorionic gonadotropin (HCG) in the *scid* mouse. B6SJLFl and *scid* female mice were superovulated with intraperitoneal injections of 5 IU pregnant mare serum gonadotropin, followed in 48 hours with 5 IU HCG. Hormone injections were given at 2 p.m. or 5 p.m. Embryos were collected from vaginal-plugged mice at 10 a.m. the following morning and placed into CZB medium. The overall fertilization rate and development to blastocyst were 69.87 and 56.92%, respectively, for Fl mice, and 67.44 and 34.94%, respectively, for *scid* mice. In a second experiment to determine time of ovulation, Fl and *scid* female mice were superovulated by using the 5 p.m. injection schedule as before. A total of 45 mice of each strain were used. Three replicates were performed. Oviducts were removed from 3 mice of each strain every 2 hours from 8 to 16 hours after HCG injection. The number of oocytes per mouse was counted, and the mean number of oocytes collected for each strain for each time period was determined. The mean number of oocytes ovulated for Fl mice peaked between 10 and 12 hours. In *scid* mice, the number of oocytes ovulated peaked between 14 and 16 hours. These studies show that the time of hormone injection may be important to maximize the fertilization rate and preimplantation development in *scid* mice and the time of ovulation may be delayed in *scid* mice following HCG injection.

## **P13 Endogenous Opioid Systems and Human Colon Carcinomas Grown in Nude Mice,**

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Cancer of the colon is a common disease and is the third leading cause of cancer mortality worldwide. Factors involved in controlling colon cancer growth are unknown. Endogenous opioids, particularly opioid growth factor (OGF; i.e., [Met<sup>5</sup>]-enkephalin) and its receptor (i.e., zeta opioid receptor) are known to inhibit tumor incidence and growth, as well as to significantly extend the survival time, of mice injected with murine neuroblastoma. This study seeks to establish a model of tumorigenicity and examine for the presence and distribution of OGF and the zeta receptor in human colon cancer xenografts in nude mice. Male athymic 5-week-old *nu/nu* mice were given subcutaneous injections over the right shoulder with  $5 \times 10^5$ ,  $1 \times 10^6$ , or  $5 \times 10^6$  log-phase HT-29 human colon cancer cells growing in vitro. Mice were monitored daily for the appearance of tumors. Tumors were measured every two days by using vernier calipers, and tumor volumes were calculated from the two largest perpendicular dimensions. Tumors appeared initially within 6 days of xenografting. By the 10th day following tumor inoculation, 100% of the mice each given an injection of  $5 \times 10^6$  cells had measurable tumors, with a mean volume of 98.5 mm<sup>3</sup>; only one of the mice given an injection of  $1 \times 10^6$  cells had a measurable tumor at this time. By day 12, all of the mice given  $1 \times 10^6$  cells had measurable tumors, with a mean tumor volume of 27.9 mm<sup>3</sup> recorded; on this day, mean tumor volume for the  $5 \times 10^6$  dosage group was 213.3 mm<sup>3</sup>. Three weeks following tumor inoculation, mean tumor sizes were 1931.3 mm<sup>3</sup>, 713.0 mm<sup>3</sup>, and 548.6 mm<sup>3</sup> for mice inoculated with  $5 \times 10^6$ ,  $1 \times 10^6$

and  $5 \times 10^5$  tumor cells, respectively; only 5 of 7 mice in the lowest dose group had tumors. In order to determine the presence and location of the opioid peptide related to growth (i.e., OGF) and its receptor, immunocytochemical studies were performed with polyclonal antibodies to OGF and the zeta opioid receptor. Immunoreactivity for OGF and the zeta receptor was detected in the cytoplasm but not the nucleus of adenocarcinoma cells. Specimens stained with preabsorbed antibodies or secondary antibody alone were negative, indicating the specific nature of each antibody. These data demonstrate that a dose-dependent tumor burden for human colon cancer could be established in nude mice. Furthermore, an opioid peptide and receptor known to inhibit many animal cancers was detected in a human colon cancer cell line. Thus, this nude mouse paradigm will serve as a useful model for further studies on the modulation of human cancer growth by the endogenous opioid system.

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## **P14 Histologic Localization of Surgically Transplanted Syngeneic Seminiferous Tubule Segments into Testis Using Fast Blue as Tracer**

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Spermatogenesis is a complex differentiative process influenced by both the testicular extratubular and intratubular tissue environment. One method of determining the relative importance of intratubular vs. extratubular factors in cases of deficient spermatogenesis has been syngeneic seminiferous tubule transplantation. Generally, in such a scheme, tubule segments from a testis deficient in spermatogenesis are transplanted into an intact recipient testis, and the progression of spermatogenesis in transplanted tubules is examined histologically. However, this experimental approach has been complicated by the tedious histologic serial sectioning required to locate these transplanted tubules, and the need to distinguish them from recipient testis solely by structural differences. A method is described for the surgical transplantation of seminiferous tubule segments into rat testes, which uses prelabeling donor tubules in vitro with the fluorescent tracer fast blue to facilitate their localization. The technique was evaluated by transplanting cut segments of fast-blue labeled seminiferous tubules from 15-day-old testis into normal adult testis (recipient), then localizing and examining histologically the progression of spermatogenesis in the transplanted tubules for up to 28 days. Transplanted tubules were easily identified in sections of recipient testis by fluorescence microscopy, exhibiting intense fast-blue staining with low background up to 28 days after transplantation. Histologic examination revealed transplanted tubules displayed limited germ cell differentiation in recipient testis for this strain of rat. At 10 days after transplantation, transplanted tubules showed characteristics qualitatively similar in appearance to tubules from 25-day-old rat testis, with increased tubular diameters and abundant germ cells at the pachytene spermatocyte stage. At 28 days after transplantation, germ cell loss was evident in most of the transplanted tubules, with some showing evidence of germ cell progression to various spermatid stages. These results suggest that, at least in the short-term for this strain of rat, this method should make it easier to separate intratubular from extratubular defects in cases of deficient spermatogenesis or the lack of recovery following testicular damage.

## P15 Adhesion Formation in Rats with Surgically Induced Endometriosis

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Endometriosis, a disease of human and nonhuman primates causing pain and infertility, is characterized by extrauterine endometrial glands and stroma, and associated with peritoneal adhesive disease. The cause of endometrial-associated adhesion formation is unknown. Endometriosis has been studied in the rat by suturing autologous uterine tissue to the mesenteric arterial cascades of the small intestine. This study compared adhesion formation in the rat endometriosis model with adhesions resulting from placement of mesenteric sutures only, uterine resection only, and exploratory laparotomy only. Three weeks after surgery, a second-look laparotomy was performed and adhesions scored (0 = no adhesions to 3 = severe adhesions). Adhesion scores were significantly higher in rats with endometriosis (mean  $\pm$  SEM =  $2.2 \pm 0.2$ ) than in rats with mesenteric sutures ( $1.1 \pm 0.6$ ) or uterine resection ( $0.6 \pm 0.1$ ) only; exploratory laparotomy did not result in adhesions. Adhesion formation in the endometriosis model does not result solely from the presence of suture material, nor is it the result of uterine resection alone. This model should permit continued study of the role of endometrial tissue in the formation of adhesions and the pathophysiologic mechanisms of endometriosis.

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## P16 Lesions Caused by *Tritrichomonas foetus* in Estrogenized BALB/c Mice

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*Tritrichomonas foetus* isolate MU Y22 establishes chronic genital infections for greater than 26 weeks in estrogenized BALB/c mice, whereas *T. foetus* isolate ATCC-30003 is unable to maintain infections beyond 7 weeks. This study was undertaken to determine whether uterine histologic lesions induced by these isolates differed. Persistent estrus was induced in 80 BALB/c mice by subcutaneous 17- $\beta$  estradiol implants, and 30 mice each were intravaginally inoculated with either ATCC-30003 or MU Y22; uninfected mice served as estrogenized controls. Weekly vaginal washes were obtained for culture in modified Diamond's medium to monitor trichomonad infection of mice. Five infected mice from each group were euthanized and necropsied at 2, 4, 6, and 10 weeks after inoculation; an additional group of MU Y22-infected mice was necropsied at 26 weeks. Histologic lesions were not seen in infected mice at 2 weeks after infection. At 4 and 6 weeks, endometritis was seen in 40 and 60% respectively, of ATCC-30003- and 100% of MU Y22-infected animals. At 10 and 26 weeks, 53% of MU Y22-infected animals had endometritis. At 10 weeks, no ATCC-30003-inoculated animals remained infected. Our results indicated the severity of uterine lesions was isolate-dependent and suggested a direct correlation between lesion severity and ability to maintain chronic infection.

## P17 Achieving Liver Ascorbate Depletion without Inducing Scurvy in the ODS Rat

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Researchers at this institution wished to investigate the use of ODS (Osteogenic Disorder Shionagi) rats to study the effect of ascorbate depletion on hepatic porphyria caused by halogenated aromatic hydrocarbons. The ODS rat is a Wistar substrain that possesses an autosomal recessive gene defect for l-gulonolactone oxidase, and therefore cannot synthesize ascorbic acid. Previous studies on hepatic porphyria were carried out in nonascorbate-depleted rat and mouse models. This report describes the study used to determine the concentration of ascorbate in the diet that would lead to a decrease in hepatic ascorbate levels sufficient to facilitate the research, while avoiding the complicating effects of clinical scurvy. Other groups have reported studies in ODS rats given diets containing 0, 50, 150, and 350 ppm ascorbate for three weeks. Rats on the 150 ppm diet showed some weight loss after 3 weeks, whereas rats fed diets with  $\leq$  50 ppm ascorbate lost weight and developed clinical signs of scurvy, characterized by bleeding into the joints, aphagia, and unthrifty appearance as compared with rats fed 350 ppm ascorbate diets that had normal growth and development. In our study, 8-week-old female ODS rats were fed diets containing 15, 200, or 800 ppm ascorbic acid. The two low-ascorbate groups contained 9 rats each; the high-ascorbate group contained 10 rats. Rats were weighed twice weekly, and examined daily for signs of scurvy. The rats were kept on the diets for 3 weeks, with porphyrogenic treatments administered after the first week. As we expected, the rats fed 15 ppm ascorbate had 80 to 90% decreases in liver ascorbic acid levels and developed clinical scurvy. The rats fed 200 ppm ascorbate diets showed 40 to 60% decreases in liver ascorbate, but did not lose weight or develop scurvy. The decrease in hepatic ascorbate in the 200 ppm diet group was sufficient to create the desired experimental model. We concluded that the 200 ppm ascorbate diet avoids experimental variables introduced by poor animal health from scorbutic conditions, and is most suitable for conducting these types of studies lasting three weeks or more.

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## P18 The Ferret as a Model of Human $\beta$ -Carotene Absorption and Metabolism

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The ferret has been used as a model for human  $\beta$ -carotene ( $\beta$ -C) absorption and metabolism since 1989, when tissue absorption similar to that in humans was demonstrated in feeding studies.  $\beta$ -C metabolism in the ferret is characterized by absorption of the intact molecule in the lymph and by quantifiable amounts of central and eccentric cleavage products in the intestinal mucosa. In this study, we have evaluated the influence of various amounts of  $\alpha$ -tocopherol (0, 0.2 mM, 5.0 mM, and 10.0 mM) on  $\beta$ -C (5 mM) absorption and metabolism, using an intestinal perfusion model. The effluents of mesenteric lymph duct cannulation and portal vein cannulation were evaluated every 30 minutes during a 3-hour perfusion of a 60-cm long piece of intestine in vivo. Three ferrets were given perfusions at each  $\alpha$ -tocopherol dosage level. Lymphatic absorption of  $\beta$ -C was enhanced by using  $\alpha$ -tocopherol in the perfusate. Similarly, lymphatic absorption of  $\alpha$ -tocopherol occurred in a dose-dependent manner. Neither  $\beta$ -C nor  $\alpha$ -tocopherol was transported in portal blood. This model will permit further in-

vestigation of the relationship between absorption and metabolism of  $\beta$ -carotene and  $\alpha$ -tocopherol.

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### **P19 *Macaca mulatta*: An Animal Model Simulating Orbital Hemorrhage**

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This project was designed to continue the effort of creating a valid animal model to define the effects of orbital hemorrhage on visual function. A tentative time frame for increased intraocular pressure of 180 minutes had been established to induce an optic neuropathy. This study delineates this time period and the deleterious effects it has on the visual system. Base line color fundus photographs, monochromatic photographs, fluorescein angiograms, visual evoked potentials, and intraocular pressure measurements were obtained before surgery and at 2, 4, or 6 weeks after surgery on both eyes of each of 12 *Macaca mulatta* monkeys. An arterial embolectomy catheter was placed retrobulbarly in one orbit and sutured in place. Saline was added to inflate the catheter balloon. This raised the intraocular pressure to greater than 50 mm Hg, suppressed the visual evoked potential, and proptosed the globe. The balloon remained inflated for either 180 or 240 minutes. This study demonstrated optic neuropathy secondary to an acute increase in intraorbital volume at 180 and 240 minutes in 6 of 12 subjects as documented by postoperative photographs, histologic examination findings, and visual evoked potentials. In 2 of the 12 primates, complete nerve fiber atrophy with central retinal artery occlusion was observed. Nerve fiber atrophy extending from the temporal disc to beyond the macula was noted in 4 of 12 primates. These results demonstrate that an increased intraocular pressure for 180 or 240 minutes caused by a retrobulbar hemorrhage may cause optic nerve dysfunction and visual pathway impairment, and that the nonhuman primate serves as an appropriate animal model for such purposes.

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### **P20 Bradykinin Aerosol-Induced Bronchoconstriction in Conscious Squirrel Monkeys**

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When administered by inhalation to asthmatic subjects, bradykinin induces bronchoconstriction. In the present study, we investigated airway responses to inhaled bradykinin in 10 healthy adult male conscious squirrel monkeys (*Saimiri sciureus*) to determine the utility of this agonist in this primate model. The monkeys were acclimatized to the laboratory environment and trained to sit in a chair inside an airtight dual-chamber plethysmograph apparatus. Pulmonary function changes in specific airway resistance (sRaw) were measured as the index of bronchoconstriction, using a noninvasive respiratory analyser. Bradykinin aerosol (1 to 5 mm particle size) was generated by an ultrasonic nebulizer and delivered directly into the face mask to each monkey for a duration of 5 minutes at a flow rate of 2 liters/min during normal tidal breathing. Bradykinin dose-response curves were determined at weekly intervals. Our results showed that bronchoconstriction to inhaled bradykinin was not dose-related. Bradykinin, at doses of 0.1, 1.0, and 10.0 mg/ml increased sRaw values,  $2.3 \pm 0.2$  (71.8%),  $2.6 \pm 0.5$  (86.7%), and  $2.2 \pm 0.14$  (68.7%) cm H<sub>2</sub>O.sec, respectively, above baseline. Moreover, the airways showed a substantially reduced response in some

monkeys after a second or third weekly challenge (72.0, 38.5, and 36.4%, respectively). Similar to what has been reported in other studies, the reactivity to bradykinin varied markedly from animal to animal (e.g., 8.5 vs 94.3% at the same dose). Preliminary results with the neutral endopeptidase inhibitor, thiorphan (1 mg/kg i.v.) combined with the angiotension converting enzyme inhibitor, enalapril (1 mg/kg i.v.) failed to normalize the response to this agonist. The present results indicate that inhaled bradykinin induces variable and inconsistent increases in airway responses (sRaw), in this primate model. The reasons for this are unknown, but are currently under investigation.

### **P21 Cataract Development in the Rabbit Gamma Radiation Cataract Model**

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A series of pilot studies was conducted to investigate cataract development in the rabbit gamma-radiation model. We measured cataract incidence and severity in New Zealand White rabbits at dose levels ranging from 1500 to 3500 rads. An irradiator with a <sup>137</sup>Cs source fitted with a variable slit collimator/attenuator was used. Each rabbit was anesthetized with ketamine and xylazine and placed in a holder so that one eye protruded from an aperture in the holder. The holder containing the rabbit was placed in the irradiator so that the body was protected by a minimum of 13 cm of lead shielding while one eye was exposed to <sup>137</sup>Cs gamma radiation emerging from a 1-cm wide slit in the collimator/attenuator. The dose rate at the eye's location was measured, and the rabbit's time in the irradiator was adjusted to yield the appropriate dose rate. Periodic slit-lamp evaluations were performed on the irradiated eyes at 3 to 20 weeks after irradiation. Posterior subcapsular and/or cortical cataracts of various severity developed within 8 weeks at all dose levels, and a dose-response relationship between radiation dose and cataract incidence and severity was observed. Later-stage mature, nuclear cataracts developed in some cases. This model provides a convenient means of assessing the efficacy of compounds against cataracts.

### **P22 A Subacute Porcine Model of Myocardial Hibernation**

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In patients with coronary artery disease and chronic left ventricular (LV) dysfunction, revascularization procedures have been demonstrated to improve LV function greatly in some cases, suggesting myocardial hibernation in the chronic dysfunctional LV due to persistent hypoperfusion. The purpose of this study was to develop a subacute porcine model of myocardial hibernation and to determine the critical level of acute coronary flow reduction for development of myocardial hibernation without necrosis. In 8 piglets (weight 25 to 35 kg), left descending or circumflex coronary artery was dissected to insert a flowmeter probe and regional coronary flow was reduced to 30 to 65% by graded coronary stenosis, using hydraulic occluder. Regional ventricular wall thickening, myocardial oxygen consumption, and coronary arterial and venous lactate and pH were measured at 15, 30, 60, 90, 120 minutes and 24 hours after the created stenosis. Animals were kept for 4 to 24 hours with fixed coronary stenosis. Low-dose dobutamine (2.5 to 10 mg/kg/min) was given intravenously to recruit contractile reserve at the end of each experiment and pigs were euthanized. The LV was examined by 1.0% triphenyl tetrazolium chloride to identify necrosis. Regional coronary flow was reduced from  $0.87 \pm 0.14$  to  $0.50 \pm 0.24$  ml/min/kg wet tissue ( $P < 0.01$ ) and myocardial wall thickening was reduced from  $29 \pm 4$  to  $14 \pm 6\%$  ( $P < 0.01$ ). Coronary flow and regional wall thickening remained unchanged throughout the experiment, myocar-

dial metabolism changed significantly. Myocardial lactate production increased initially with arteriocoronary venous (AV) lactate difference of  $-1.33 \pm 0.21$  mmole/liter at 15 minutes after coronary stenosis and returned to near the baseline level (AV lactate difference =  $0.07 \pm 0.31$  mmole/liter at 60 to 90 minutes thereafter,  $P < 0.01$ ). There was an associated initial decrease in myocardial pH ( $7.19 \pm 0.08$  at 15 minutes) and then gradual recovery ( $7.29 \pm 0.06$  at 60 to 90 minutes). Regional myocardial oxygen consumption also decreased further ( $2.8 \pm 0.8$  ml/min/100g at 60 to 90 minutes) after the initial stage of stenosis ( $3.1 \pm 0.9$  ml/min/100g at 15 minutes). All these observations indicate down-regulation of the ischemic myocardium to match the hypoperfusion. Intravenous infusion of dobutamine at 2.5 to 10 mg/kg/min resulted in improvement in end-systolic wall thickening from  $14 \pm 6$  to  $21 \pm 15\%$ ,  $P < 0.01$ , suggesting recruitable contractile reserve by inotropic stimulation. Two animals with coronary flow reduction more than 65% of baseline had patchy necrosis. It is feasible to produce a subacute porcine model of myocardial hibernation up to 24 hours under persistent myocardial hypoperfusion. Development of myocardial hibernation without necrosis appears to critically depend on the level of acute coronary flow reduction in this model.

### **P23 Colonization of *Pasteurella multocida* With Retroperitoneal Abscess Formation in a New Zealand White Rabbit (*Oryctolagus cuniculus*).**

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A case of retroperitoneal abscess secondary to chronic *Pasteurella multocida* infection was diagnosed in a 24-month-old doe. According to the breeding records, after failing to conceive in three consecutive matings, this rabbit was removed from the breeding stock to become a research subject. At culling, clinical examination revealed a slight induration and distention of the uterus. Further health surveillance overlooked this initial finding and 4 months later, when it was examined, the animal appeared obese to the extent of advanced pregnancy. The doe was alert and moderately febrile without relevant signs of disease. Palpation revealed a large firmly adhered abdominal mass. Radiographic evaluation revealed an intrapelvic mass occupying one third of the abdominal cavity. Differential diagnosis included abscess or neoplasm. The rabbit was euthanized and necropsy revealed adherent to the bladder, uterus, and the pelvic floor, a large mass approximately 9 cm long. Careful excision of this mass disclosed a clear attachment to the caudal vena cava, and the mass weighed 190 g. Incision of the fibrous wall resulted in an abundant discharge of a thick, creamy exudate from which a pure culture of *P. multocida* was isolated. Histologic examination revealed fibrous connective tissue and a few mononuclear inflammatory cells. This case describes a rarely reported intra-abdominal abscess, resulting from chronic infection of *P. multocida* in a healthy doe.

### **P24 Exophthalmia in the Rabbit after Chronic External Jugular Catheter Placement**

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Chronic catheterization of the external jugular veins in rabbits allows for repeated venous sampling and administration of agents. Following external jugular catheter placement, we have noticed a unilateral exophthalmos on the ipsilateral side, which appears to subside naturally within 24 hours after surgery. Although acute glaucoma was a primary differential diagnosis, there were no other classic signs suggestive of the condition (i.e., episcleral engorgement, mydriasis, blepharospasm, and pain), and the rab-

bits appeared to maintain normal activity. Fundic examinations have revealed no gross abnormalities. Intraocular pressures (IOP) were monitored with a Schiotz's tonometer after induction of anesthesia (30 mg ketamine/kg and 6 mg xylazine/kg) and 7 to 8 hours after surgery. The IOP was 13 mm Hg before surgery, and 16 mm Hg after surgery (ipsilateral globe). The IOP of the contralateral globe did not change after surgery. On four occasions, external jugular catheters were replaced in rabbits, resulting in the occlusion of the contralateral vessel. A resulting bilateral exophthalmos appeared with significantly elevated IOP (25 to 29 mm Hg) and no obvious fundic changes. The globe size of these rabbits decreased transiently following the initial use of mannitol at a rate of 1.0 g/kg, I.V., SID for two days. Pilocarpine (2.0%) given topically TID resulted in the globe returning to normal size in 2 weeks. The exact cause of the exophthalmia is unclear, but venous drainage of the rabbit's head appears to be primarily via the external jugular veins and may account for this phenomenon. Collateral circulation with the internal jugular veins and possibly other vessels appears to compensate within 24 hours after occlusion of a single external jugular vein.

### **P25 A Granulocytic Sarcoma in a Specific Pathogen-Free New Zealand White Rabbit**

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A 1.5-year-old specific pathogen-free rabbit was observed to have a firm tissue mass extending from the tail head cranially over the sacrum and dorsolaterally to the left rear leg. The rabbit had been housed in an AAALAC-approved facility for 10 months and was on a standard polyclonal antibody regimen. The rabbit was maintained on a commercial high fiber diet and water ad libitum. Initially, the mass measured 4.0 x 2.0 cm. It was firmly attached to the underlying tissues, and signs of pain were not induced by palpation. Within two months the mass had doubled in size and caused right lateral deviation of the tail. The rabbit was euthanized and necropsied. A firm white mass measuring 7.5 x 7.0 x 3.5 cm infiltrated the musculature ventral and lateral to the tail and encircled the tail head. Cut surfaces were white to yellow with foci of hemorrhage. Histologic examination revealed neoplastic granulocytes that invaded and replaced the regional skeletal muscle and invaded the dermis of the overlying skin. The tumor was comprised of myeloid cells with large eosinophilic cytoplasmic granules and was classified as an eosinophil granulocytic sarcoma. Verification is anticipated with special stains and electron microscopy. To our knowledge, this is the first report of a granulocytic sarcoma in lagomorphs.

### **P26 Encephalitozoon Eradication in a Commercial New Zealand White Rabbit Breeding Colony**

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*Encephalitozoon cuniculi* (ECUN) is a protozoan parasite that has a predilection for the kidney, and later the brain, during an infection in rabbits. An aggressive breeding program was developed to eradicate this organism from a commercial, production/maturation, New Zealand White (NZW) colony with a mean daily population of 8500 rabbits of all ages up to three years. Initial incidence rates (43%) were determined by serologic sampling of groups of 100 breeding females. To collect samples, rabbits were properly restrained, and 3 to 5 ml of blood was withdrawn from the medial ear artery by use of a 20-gauge needle. Samples were spun in a centrifuge, and 1.0 ml of serum was poured into a conical vial. Each vial was labeled to coincide with each breeder ear tag number. The samples were packed on

dry ice and shipped to the laboratory for either enzyme-linked immunosorbent assay or indirect fluorescent-antibody assay to screen for antibody to ECUN. Upon receipt of the results, all breeders that proved to be positive for ECUN were removed from the breeding colony, as well as any litters. All adjacent does were placed on "hold" (all production was stopped) and retested to ensure that the breeders were still seronegative. Once these results were received and all breeders that were on "hold" determined seronegative, production could begin again. Once a seropositive doe (or buck) was removed from the breeding colony, the cage and feeder were disinfected with a quaternary ammonia and hot water. The cage was heated to 180°F and remained empty for 5 days before the next breeder was put into that cage. After several trials, and considering the facility and caging design, it was determined that groups of 20 NZW breeders in each of the 8 family genetic lines would be bled. The groups were bled across the eight family lines, leaving a three-cage barrier between each group. When a positive for ECUN was reported, the rabbit was removed from the colony. All remaining breeders in that group were retested and production stopped until those results were received. When all 20 does in any group were consistently ECUN-negative, the technicians proceeded to bleed the next sequence of 20 NZW breeder does. The sequential testing-and-removal technique for eradicating ECUN from an NZW breeding colony proved to be successful.

#### **P27 Alopecia and Endocrine Abnormalities Associated with Testicular Interstitial Cell Tumor in a Ferret**

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A three-year-old albino male ferret used in a long-term gastric microflora study developed progressive thinning of the hair coat affecting the lumbar region and tail. Ten months following the onset of alopecia, the left scrotal sac became slightly fluctuant and enlarged. Both testes were of normal size but were somewhat firm and irregular on palpation. Bilateral orchiectomy was performed. Histologic examination revealed both testes contained neoplastic tissue of interstitial (Leydig) cell type, which had displaced most of the normal tissue. Eight weeks after castration, the hair coat had returned to normal. Pre- and postsurgical (10 weeks) serum concentrations of cortisol, estradiol, progesterone, 17  $\alpha$ -hydroxy-progesterone, dihydroepiandrosterone, androstenedione, and testosterone were determined and compared with concentrations in 3 sexually intact normal male ferrets. Presurgical levels of progesterone, 17  $\alpha$ -hydroxy-progesterone, and dihydroepiandrosterone, were at least 4, 5, and 12 times the level in control individuals. Concentrations of hormones assayed, with the exception of cortisol and estradiol, decreased 90- to 800-fold after castration. These results suggest that interstitial cell tumors in ferrets may produce steroid hormones that cause alopecia.

#### **P28 Characterization of Spontaneously Generated Vulvar Carcinomas in 129/J Mice**

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Clinical examination of female 129/J mice from an in-house production colony revealed protrusions and tumor-like masses of the vulva in 47 of 106 (44%) animals. These neoplastic-like lesions were initially recognized in 5- to 9-month-old mice, but were subsequently found in females as young as 6 weeks. Cultural, serologic, histologic, and electron microscopic examinations were performed in an effort to characterize and determine the cause of these lesions. Cultural and serologic results were negative for the

known murine bacterial mycoplasmal, parasitic, and viral pathogens. Microscopic examination revealed invasive carcinomas of the ventral portion of the vulva, characterized by multiple coalescing nests of epithelial cells extending downward from the surface. These proliferative lesions were very site-specific and characteristically located caudal to the vaginal opening between the orifices of the urethra and clitoral gland duct. Invasive cells exhibited predominantly a well-differentiated squamous morphology forming solid nests, with scattered foci of proliferative epithelium with cystic or mucoid differentiation. Electron microscopy did not reveal any distinguishing diagnostic features nor evidence of viruses. Endometrial hyperplasia was invariably present in all tumor-bearing mice; interstitial cell hyperplasia of the ovary was seen less frequently. No other important findings were revealed by examination of other major organs. Assays of feed, water, bedding, and environmental air were negative for the presence of microbial pathogens and for detectable amounts of toxic chemicals including pesticide residues, formaldehyde, and ammonia. Sporadic cases of vulvar squamous cell carcinoma have been reported in cattle, sheep, non-human primates, and humans, and have been associated with exposure to sunlight or papillomaviruses. Vulvar carcinomas are rarely seen in mice and have not been reported in the 129/J strain. The cause of these tumors remains unknown; however, an estrogenic effect is being investigated.

#### **P29 A Novel Presentation of *Clostridium piliforme* (Tyzzer's disease) in Nude Mice**

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Two deaths due to *Clostridium piliforme* infection (Tyzzer's disease) were documented in a *nude* mouse colony. Because previous reports suggest that *nude* mice are resistant to clinical Tyzzer's disease, the pathogenesis of this outbreak was investigated. Sixty *nu/nu* females, 10 *nu/nu* males, and 10 *nu/+* females were obtained from this colony. Over a three-month period, 30 homozygous mice died at sporadic intervals. Heterozygotes and weanlings were not affected. Clinical signs were infrequently found, and when evident, death ensued within four hours. Gross and histologic examination of affected animals confirmed *C. piliforme* infection of the lower portion of the intestinal tract and the liver. *Clostridium piliforme* propagated in tissue culture from these mice demonstrated marked cytotoxicity. This outbreak was unique in that mice homozygous for the *nude* mutation displayed an increased susceptibility to overt disease, disease expression was limited to adult animals, and the progression from clinical signs of infection to death was peracute. The immunodeficiency of homozygous *nude* mice and/or the host specificity and cytotoxin production of this *C. piliforme* isolate may account for these findings.

#### **P30 Histochemical, Immunohistochemical, and Histomorphologic Characterization of Uterine Smooth Muscle Cell Neoplasms (Leiomyomas/Liomyosarcomas) in B6C3F1 Mice**

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Twenty-four female B6C3F1 mice diagnosed as having uterine mesenchymal neoplasms were identified in the National Toxicology Program's 2-year bioassay database. Archival paraffin blocks of uterine tissue were obtained, and tissue sections were prepared and stained by using hematoxylin and eosin, Masson's trichrome, and desmin stains. A qualitative comparison of the proliferative activity of cells in benign (leiomyoma) versus malignant (leiomyosarcoma) uterine smooth muscle cell neoplasms

was done by using an immunohistochemical technique for detecting proliferating cell nuclear antigen. The localization of transforming growth factor- $\alpha_{34-43}$  in the neoplasms was also assessed immunohistochemically. Six of the 24 uterine mesenchymal neoplasms were found to be of smooth muscle cell origin on the basis of red staining of tumor cells with Masson's trichrome stain and positive immunoreactivity for desmin intermediate filament. Histologic examination revealed the leiomyomas (4/6) had organized and noninvasive growth patterns. The tumor cells were spindle-shaped, had eosinophilic cytoplasm, and elongated or round to oval vesicular nuclei. Conversely, the leiomyosarcomas (2/6) appeared to have disorganized cellular growth patterns. The tumor cells were large and spindle-shaped with eosinophilic to basophilic cytoplasm, and centrally located elongated or round to oval nuclei that were either hyperchromatic or vesicular. The nuclei of the malignant tumor cells contained single or multiple prominent nucleoli or mitotic figures. There was minimal expression of proliferating cell nuclear antigen observed in the nuclei of benign (leiomyoma) versus malignant (leiomyosarcoma) tumor cells. An increase in transforming growth factor- $\alpha_{34-43}$  immunoreactivity was seen in the leiomyosarcomas and not in the leiomyomas. Mouse uterine smooth muscle cell neoplasms have typical histomorphologic features of leiomyomas and leiomyosarcomas described in other organs, including the uterus of other species. It appears that uterine smooth muscle cell neoplasms in the mouse may serve as a model for studying the interactions of polypeptide growth factors and uterine smooth muscle cell growth. Also, mouse uterine smooth muscle cell neoplasms may be useful in helping to understand the role of genetic factors and/or the environment in mediating the growth and development of fibroids (leiomyomas) that develop in women.

### **P31 Cranioventral Abdominal Mass in a Macaque**

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A colony-born, single-housed, sexually intact female, Herpes B-positive *Macaca mulatta* was examined during routine quarterly tuberculin testing under ketamine sedation and found to have a large, firm medial cranioventral abdominal mass and 12% body weight loss. Other physical variables were normal. Radiography did not identify the organ associated with the mass. Differential diagnosis at this time included neoplasia, granuloma, cyst or abscess of hepatic, splenic, or renal origin, endometriosis, and gastroenteric intra- or extra-luminal mass. Results of CBC, chemical profile, and urinalysis were within normal limits, except concentrations of alkaline phosphatase and gamma glutamyl transferase were high. Historical tuberculin test results were negative. Ultrasonic examination suggested that the mass was adjacent to the spleen, not involving the liver, kidneys, or reproductive tract. Exploratory surgery revealed a large, 12 x 6-cm trichobezoar, which occupied 90 to 95% of the gastric lumen and was successfully removed by gastrotomy. Trichobezoars are considered to be infrequent in nonhuman primates. Primates may exhibit stereotypic behaviors that favor formation of trichobezoars, such as social grooming or excessive self-grooming associated with inappropriate adaptation to captivity, or boredom. A thorough physical examination whenever anesthesia is used and an effective environmental enrichment program for captive primates should help with early identification of gastric bezoars and lessen their frequency.

### **P32 Retroperitoneal Hemorrhage in Two African Green Monkeys**

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A 12-year-old male grivet (*Cercopithecus ethiops ethiops*) was reported to be weak and not eating. The clinical signs were vague and nonspecific. The monkey was anemic, and after a short clinical course, the animal died. Retroperitoneal hemorrhage was the major gross observation at necropsy. The underlying problem was polycystic kidney disease with hemorrhage into a cyst of the right kidney and then into the retroperitoneum. Six months later, a 15-year-old vervet (*Cercopithecus ethiops pygerythreus*) had a similar clinical course, and the gross diagnosis was retroperitoneal hemorrhage. The underlying disease was muscular dysplasia of the renal arteries. The source of the hemorrhage was rupture of a segmental branch of the right renal artery. Retroperitoneal hemorrhage is recognized as a rare life-threatening complication of polycystic kidney disease among people. Renal artery dysplasia is another known risk factor for retroperitoneal hemorrhage in people. The right kidney is more often involved than the left.

### **P33 Evaluation of Visual Acuity Following Cataract Surgery and an Intraocular Lens Implant in an Aged Rhesus Monkey**

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The match-to-sample performance test is an experiment used to evaluate short-term memory in aged monkeys. This test involves visual recognition of an object followed by a delay and then distinguishing the same object from a group of similar objects. Although this test is designed for memory evaluation, visual acuity can also be evaluated. The decline of performance on the match-to-sample test of a 33-year-old female Rhesus monkey prompted a complete physical examination. The ophthalmic evaluation revealed bilateral complete mature cataracts, normal intraocular pressures, normal pupillary light reflexes, and mild peripheral corneal and limbal infiltrations (arcus). No other physical abnormalities were found. Electroretinography revealed normal electroretinogram potentials in scotopic light conditions. Following axial eye length and corneal curvature determinations, a 43-diopter lens implant was selected for replacement. Cataract surgery was performed by phacoemulsification and the lens was implanted. Three weeks following the surgery, performance on the match-to-sample test improved from 30 to 40% correct responses as seen before the surgery to 65 to 70% correct responses. This study documents the diagnostic and therapeutic methodology involved to restore vision in a valuable aged monkey, using an intraocular lens implant.

### **P34 Atraumatic Preputial Abscesses in Young Squirrel Monkeys (*Saimiri* sp.)**

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Atraumatic abscesses of the prepuce were found to be unexpectedly common among young male *Saimiri* in a domestic breeding colony. Colony records were surveyed for the period from 1985 to 1993 for information regarding cases of nontraumatic preputial abscess by year, age of onset, and subsequent reproductive performance of affected animals. In 19/21 cases (90%), abscesses were found in animals less than 3.5 years old. A

mean of 2.3 cases were found per year. Clinical signs included preputial pain, erythema, and swelling. Five of the 10 nonruptured abscesses (50%) had a tuft of hair floating free in purulent fluid in the abscess pocket. Treatment consisted of surgical removal of hair from the pocket and/or establishing drainage. Recovery in all cases was uncomplicated. Review of breeding records suggests that post-abscess breeding performance was not affected. The mechanism for hair migration into the subcutaneous tissue of the prepuce is under investigation. Examination of young male *Saimiri* should include inspection of the penis and prepuce. Supported by NIH grant P40 RR01254.

### **P35 Transient Blindness in a Rhesus Monkey: Use of Computed Tomography for Diagnosis**

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A five-year-old female rhesus monkey (*Macaca mulatta*) developed clinical illness after twenty months of uneventful cranial instrumentation. Signs of illness included lethargy, anorexia, and dysmetria. Hematologic evaluation, serum chemical evaluation, and thoracic and abdominal radiography revealed no abnormal findings. Supportive care and antibiotic injections were given. Two weeks later, the animal appeared to be blind. Differential diagnoses included cerebrovascular infarction, meningoencephalitis, cerebral edema, and hydrocephalus. Her condition remained unchanged and clinical laboratory values remained within normal ranges. Results of an ophthalmic examination of the monkey under anesthesia and a cytologic evaluation of cerebrospinal fluid were normal. Computed tomography (CT scan) of the skull revealed dilated ventricles. The history along with these findings indicated outflow obstruction leading to hydrocephalus. Fifty-one days from the first reported signs, the monkey regained sight. A CT scan performed six months from the original CT scan revealed normal ventricle size. This unusual clinical neurology case with emphasis on the use of computerized tomography to aid diagnosis will be presented.

### **P36 A Technique for Measurement of Corneal Topography in Conscious Laboratory Rabbits**

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Traditionally, rabbits have been very popular as animal models in a variety of ophthalmic investigations. Data collection is often performed with instruments that have been designed for specific applications in human clinical medicine. In many cases, the specific procedure or technique must be adapted to accommodate obvious discrepancies in both the anatomic features and temperament of rabbits, as compared with humans. In studies involving the cornea and/or anterior chamber, it is important to be able to document alterations in corneal topography. Devices for mapping the surface of the cornea are available but require the subject to stare at a specific point while the measurement is taking place. In most animal subjects, this is not feasible and generates results that are not accurate or repeatable. A system was developed for the measurement of corneal topography in conscious laboratory rabbits, using a corneal modeling system. The study involved 8 adult male Dutch-belted rabbits in which the curvature of the cornea had been altered. Postmanipulation measurements involved the routine mapping of each eye for a minimum of 6 months. The system uses a cylindrical photokeratoscope that generates a 20-ring image covering approximately the central 6-mm diameter of the cornea. The image of the reflected rings is acquired from real-time video onto an integrated computer system. Topographic data and calculations are generated by scan-

ning the resulting corneal video images. In order to successfully utilize this technology in rabbit subjects, a special positioning stand was designed for both the instrument and the rabbit. The target image was centered on the animal's pupil to ensure that the same area was mapped during each examination. This technique was easily performed in awake animals with minimal restraint and produced very consistent results.

### **P37 Techniques of Water Cystometry in Miniature Swine**

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Our studies involve a new surgical technique for augmenting the capacity of the contracted or diseased urinary bladder. After augmentation, urinary bladder capacity and stability were followed for five months. This information is determined by the procedure called cystometry. In order to simulate the procedure as performed in humans, we have developed a cystometry protocol in unsexed female minipigs. Blind digital technique is used to place a Foley catheter into the bladder. Once the catheter is in place, urine will flow from the catheter at which time the balloon can be inflated to hold it in place. With the bladder empty, the transducer and the infusion lines are attached to the dual-lumen Foley catheter. Warmed sterile saline is delivered by a nonpulsatile pump, which fills the bladder while intravesicle pressure is relayed through the transducer and printed on the cystometer. Infusion is stopped when the pig begins to void. The technician assists the pig in voiding around the balloon catheter, by sliding the catheter in away from the urethra as it voids. After voiding, the Foley is disconnected from the transducer, and any residual saline is aspirated by syringe from the Foley. The cystometrogram is then analyzed for pressure, volume, and occurrence of abnormal pressure spike. Urine obtained before the procedure is evaluated by urinalysis and culture. A total of 48 normal and 93 augmented cystometrograms were obtained from 15 minipigs. In the normal bladder, pressure remains relatively low and increases slightly before the urge to void. When the pig voids, a pressure spike occurs on the tracing caused by a bladder contraction. The bladder pressure drops back to baseline when the bladder is empty. In the abnormal bladder, instability and/or hypersensitivity causes resting pressure to be high and rise sharply before the urge to void. Maximum capacity and the capacity at which there is a desire to void are reduced. This technique brings us one step closer to an animal model of ambulatory cystometry.

### **P38 Postoperative Use of Adjustable Cervical Collars in Rabbits**

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There have been numerous techniques described to prevent suture removal and/or self-trauma in animals following surgery, including chemical restraint agents such as tranquilizers or noxious tasting agents (e.g., bitter apple, tabasco sauce) and mechanical restraint devices such as body casts, limb bandaging, plastic buckets, and Elizabethan collars. In recent years, our laboratory has been tasked with providing major survival surgical support services to investigators who are using a large number of rabbits in their research. We needed the means to prevent suture removal and/or self-mutilation in the postoperative period and not inhibit the animal's recovery. We found that the aforementioned techniques either failed to prevent self-mutilation or caused other adverse effects such as not eating or drinking, or the ability to move around. Like other animals, rabbits have a strong propensity for self-mutilation of surgical incision sites. Unfortunately, many

rabbits not only remove skin sutures but often continue to mutilate themselves to underlying bone and muscle tissues. We present an easily prepared, adjustable cervical collar that we have developed and used successfully following surgical procedures in >1500 rabbits. We make the "scratch guard" from 4 x 4 gauze sponges rolled into a 5-cm diameter tube approximately 36 cm long and connected end to end to form a circular ring. The ring is then reinforced and made water resistant by sequential wrapping with surgical adhesive tape and self-adhering commercial product. When used, the collar is slid over the animal's head and behind the ears to secure into place. The collar can be adjusted to each individual animal by pinching the collar to form a yoke and securing this cinch with tape. Of the 1500 surgical cases, approximately 2.5% have resulted in mutilating episodes primarily due to the rabbit's ability to remove the scratch guard. Further mutilation can be deterred by modifying the collar to larger diameters and/or tighter yokes. Our scratch guard enables the rabbit to eat, drink and move normally while preventing suture removal and/or self mutilation.

### **P39 Modification for the Improvement of Canine Gastric Cannula Model**

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While the canine gastric cannula model is well established for research use, there are several complications that preclude it from being an ideal model. Postoperative complications, clinically seen as swelling, discharge, and infections, often lead to rejection of the cannula, which causes the model to be nonfunctional. We developed a series of modifications to decrease the overall rejection rate. A new cannula design and new suturing pattern was tried. The diameter of the flange on the cannula, for placement against the stomach mucosa, was increased, and a plastic collar was placed over the exteriorized end of the cannula and secured with a set screw. Six equally spaced sutures of PDS II were used to connect the stomach to the abdominal wall at a distance of 1.5 cm from the edge of the cannula flange. Another modification was postoperative feeding of a prescription diet, alone or mixed with dry chow, fed two times a day at six-hour intervals for a period of two weeks. It was hoped these modifications would better secure the cannula and promote healing. Comparison of this model to our previous model showed a decrease in postoperative complications. There was a decrease in swelling and discharge at the cannula exit site, and an increase in model longevity from three to ten months because of less cannula rejection. Additional benefits were a decrease in the total number of dogs used per experiment and a greater degree of comfort and well-being for the animal. These new modifications resulted in a chronic gastric cannula model with less postoperative complications and subsequent cannula rejection.

### **P40 Optimal Jugular Catheter Length and Diameter for Blood Sampling in Rats**

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Silicone rubber catheters of various lengths and diameters were aseptically implanted into the right jugular vein and subcutaneously tunneled so that they exited between the scapulae in male, Sprague-Dawley origin rats. The catheters were flushed daily with 10 units heparinized saline/ml and left filled with this solution. Patency for blood sampling was recorded daily for each rat. Histologic examination of the jugular vein, cranial vena cava, and heart was performed. Patency for blood sampling was lost within a few days after surgery when the tip of the catheter was located either in the cranial vena cava or in the right ventricle. Microscopic examination revealed cranial vena cava catheters were blocked by tip-associated thrombi

that formed one-way valves that allowed for infusion but prevented blood withdrawal. The lumen of cardiac catheters was blocked by clotted blood. Patency for blood sampling was continued only when the tip of the catheter was located at the junction of the cranial vena cava and the right atrium. Microscopic examination revealed thrombotic sheaths around catheters of all lengths. However, when the tip was located at this junction the sheath did not cover the tip. Also, the lumens of the catheters placed at this junction did not become blocked with clotted blood as did catheters with tips in the right ventricle. The diameter of the catheter did not affect patency for blood sampling as long as the tip was located at the junction of the cranial vena cava and the right atrium. However, larger diameter catheters were associated with more surgical and postsurgical complications.

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### **P41 Surgical Techniques Used in the Generation of Transgenic Rabbits**

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Transgenic rabbits originating from the English (British) half-lop (EHL) were produced in order to study the effects of overexpression of particular apolipoproteins on the pathogenesis of atherosclerosis. The EHL rabbit, derived from the Watanabe heritable hyperlipidemic (WHHL) breed, was used because of the reported lower variance among siblings of the level of hyper-cholesterolemia when compared with WHHL siblings. The surgical techniques we developed were designed to collect single cell embryos from the anesthetized rabbit allowing recovery after surgery. Great care was taken to minimize trauma and adhesions produced by the surgery, and postoperative analgesia was administered (0.02 mg buprenorphine/kg, SC, BID for 2 days). Since the EHL is not yet available commercially, we have justified the use of the dose in multiple survival surgeries. Techniques used in the collection of the embryos involved superovulation, cannulation of the ampulla of the proximal oviduct with "flared" polyethylene tubing, cannulating the distal oviduct with a 22-gauge indwelling catheter, and retrograde flushing of the embryos into Petri dishes for collection. After transgene insertion, the embryos are reimplanted into New Zealand White recipient females bred to a vasectomized male. The reimplantation is done with the rabbit under general anesthesia, using cannulation of the proximal oviduct with a typical pulled glass transfer pipette. Here we describe and depict the methods used for the production of vasectomized males, superovulation of recipient females, embryo collection and reimplantation, and postsurgical care. The intimate relationship of the microinjection suite and the operating theater were developed to facilitate these procedures.

### **P42 A Technical Feasibility Study for Chronic Intracerebroventricular Drug Delivery in the Cynomolgus Monkey**

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Centrally active compounds that do not cross the blood-brain barrier require direct delivery to the central nervous system. Chronic intracerebroventricular (ICV) drug delivery was evaluated in cynomolgus monkeys. Each animal was maintained under isoflurane anesthesia with its head held in a stereotaxic device. A 22-gauge, stainless steel cannula was implanted in the right lateral cerebral ventricle. The anatomic coordinates for cannula placement were determined and fluid flow in and out of the ventricle was used as a guide for ventricle location. After cannulation,

a 2-ml, saline-filled osmotic minipump with a delivery rate of 2.5  $\mu$ l/h was placed subcutaneously on the dorsal back region and attached to the cannula via polyethylene tubing. During surgery, the animals were monitored under veterinary supervision. The surgical procedure lasted approximately 2 hours and the postsurgical recovery was uneventful. Minipumps were replaced at approximately 2-week intervals for up to 5 months. Minipump replacement surgery was performed with the monkey under sedation and lasted approximately 30 minutes. Routine veterinary supervision, including antibiotic therapy to minimize potential infection, was provided during the study. At the time of minipump replacement, the delivery apparatus was inspected for disconnection or cracked tubing and replaced as necessary. This method can be used successfully in cynomolgus monkeys for chronic intracerebroventricular delivery of potential centrally acting therapeutic agents that do not cross the blood-brain barrier.

### **P43 A Surgical Tilting Device to Achieve Trendelenburg and Reverse Trendelenburg Positions without Table Rotations**

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Endoscopic-assisted laparotomy and thoracotomy are becoming standard human and veterinary surgical methods. Laboratory animal veterinarians are consistently requested to assist in surgical training laboratories involving animals for physicians. Endoscopic surgery often requires tilting of the animal so that the head is higher than the feet, resulting in forward positioning of the abdominal organs while the animal is on the surgery table (Trendelenburg position). For cranial abdominal surgeries, the reverse Trendelenburg position may be required. To reduce the number of animals required, multiple procedures may be performed prior to euthanasia. Standard, commercially available, veterinary surgery tables require the table to be rotated to achieve both positions during a single surgery. If the animal is monitored by an EKG, intubated and connected to a gas anesthesia machine, grounded for electrocautery and provided with suction, rotation of the table is impractical. We developed a modification to a commercially available surgery table that allows for both the Trendelenburg position and the reverse Trendelenburg without rotating the table. The modification is a mirror image of the single tilting device manufactured from surgical grade stainless steel. The device creates new ranges of motion for standard surgery tables by creating a double-hinge scissor action. For tables lacking hydrolytic lift, this modification is capable of operating as an inexpensive height adjustment. This tilting device is relatively simple and can be manufactured by most metal fabricators at a modest cost. Institutions performing endoscopic training procedures may consider modification of their existing surgery tables.

### **P44 Laparoscopic Dexterity Training Program: An Alternative to Animal Use for Laparoscopic Surgical Training**

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Laparoscopic surgery is popular with general surgeons because it is less traumatic to the patient and results in decreased recovery time. Training for surgeons usually involves a two-day laboratory session, which is expensive and requires the use of a porcine or canine animal model. The Laparoscopic Dexterity Training Program is an inexpensive, nonanimal laboratory session for surgeons and third-year medical students. During the two-hour course, the participants develop basic skills required in a variety of laparoscopic procedures. First, each student performs specific tasks inside a clear-topped laparoscopic training box, fully utilizing the capabilities of the laparoscopic instruments to maneuver objects. Next, stu-

dents work together completing tasks on the laparoscopic task board, contained in a blind box. The task board offers an unfamiliar, inexpensive environment in which the students gain dexterity with the laparoscopic instruments while viewing their motions two dimensionally on a monitor. The tasks require the same motions that are used for actual laparoscopic procedures. Each person participates as camera operator, assistant, and surgeon. Only one instructor is needed for the program. The Laparoscopic Dexterity Training Program decreases the use of live animals while developing the laparoscopic skills and knowledge of surgeons and third-year medical students.

### **P45 Aspirin Disposition in Rabbits**

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Limited information exists on the disposition of aspirin in rabbits. Such information is of value not only for treatment, but also for the development of experimental protocols with human application. This study evaluated the pharmacokinetic properties of aspirin at different doses by monitoring the serum levels of the major metabolite, salicylic acid (SA). Four groups of six rabbits received a dose of 2.5 mg/kg, 10mg/kg, 20mg/kg, or 50mg/kg. Aspirin was given once daily via the oral-gastric route and blood samples were obtained after the third dose. Samples were collected before the last dose then 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after dosing. The SA analysis was completed by using a validated assay on an HPLC system. The mean maximum concentration was 7.54 mg/ml, 22.65 mg/ml, 43.2 mg/ml, and 70 mg/ml, respectively, for the 2.5 mg/kg, 10 mg/kg, 20 mg/kg, and 50 mg/kg doses. The total systemic clearance was not altered by increasing doses, but statistically significant differences were noticed in volume of distribution. The elimination half-life also increased proportionally with the dose. Bleeding tendency developed in rabbits given the highest dose. This study suggests that the 20 mg/kg and 50 mg/kg doses produced serum drug levels that simulate results observed in humans after 600 mg and 1.2 g doses, respectively.

### **P46 Topical Tetracaine Anesthesia for Canine Ear Tattooing**

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Although ear tattooing of dogs by means of tattoo pliers is an approved and common method of identification, it is a potentially painful procedure and can result in aggression of dogs. Applying tetracaine in dimethylsulfoxide (DMSO) topically can produce dermal anesthesia. This study tests the hypothesis that topically applied tetracaine/DMSO can alleviate pain associated with canine ear tattooing. Groups of 8 adult, mixed-breed dogs underwent ear tattooing following either no treatment, a 45-minute contact with 1 ml of DMSO applied topically to the ear with a cotton swab, or a 45-minute contact with topically applied 25% (wt/wt) tetracaine base in DMSO. Pain evaluation was made by heart rate elevation and by a subjective numeric behavior scale where 0 = no flinch or vocalization, 1 = flinch and mild vocalization, 2 = flinch and moderate vocalization, and 3 = flinch and vigorous vocalization. Results indicate the application of DMSO and tetracaine produces a significant ( $P \leq 0.05$ ) reduction in pain associated with ear tattooing. In summary, topical application of tetracaine/DMSO appears to reduce pain associated with canine ear tattooing and might reduce the hazard to personnel performing this procedure in aggressive animals.

#### **P47 Evaluation of Ketamine-Xylazine-Acepromazine as a Combination Anesthetic Regimen in Mice**

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A study was conducted to evaluate the effectiveness of a ketamine-xylazine-acepromazine combination anesthetic regimen in mice. The consistency and safety were evaluated for use in minor recovery procedures using large numbers of mice. Thirty-three age-matched B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice underwent one anesthetic episode. The anesthetic dose was accurately calculated on a per gram body weight basis, and consisted of ketamine (30 mg/kg), xylazine (6 mg/kg), and acepromazine (1 mg/kg) combined and administered via intraperitoneal injection. Anesthetic depth and duration were monitored by recording time from anesthetic injection to loss and recovery of both righting and rear pedal withdrawal reflexes. Anesthetic depth was also evaluated by observation of response to minor procedures (orbital sinus blood collection and subcutaneous tumor injection). Anesthetic induction was smooth with mean time to loss of righting reflex 5.0 ± 0.33 minutes, and to loss of pedal withdrawal reflex 12.6 ± 0.74 minutes. The mean anesthetic time from loss to recovery of pedal withdrawal reflex was 8.0 ± 0.97 minutes. The mean recovery time of righting reflex was 43.8 ± 1.34 minutes after injection. Although there were minor individual variations in response, this regimen was considered safe and adequate for minor procedures requiring short-duration anesthesia.

#### **P48 Pharmacokinetics of Gentamicin in the Ferret (*Mustela putorius furo*)**

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Gentamicin is an aminoglycoside antibiotic, which has a narrow therapeutic index, with vestibular ototoxicity and renal tubular toxicity reported in a variety of species. Safe and effective therapy requires accurate pharmacokinetic information. The present study was designed to determine the pharmacokinetic properties of gentamicin in six healthy ferrets of both sexes. A parenteral preparation of gentamicin sulfate (5% aqueous solution) was administered intravenously at the dosage level of 5 mg/kg of body weight. Venous blood samples were taken at 0, 5, 10, 15, and 30 min, and 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after gentamicin administration. Serum gentamicin concentration was measured by a radioimmunoassay technique. The gentamicin concentration was found, with the aid of a nonlinear least squares program, to be best described by a two-compartment model with  $r^2 = 0.991$ . Half-life as determined from the terminal phase was 42.1 ± 4.9 min (mean ± SD). Calculation of the total body clearance rate provided a mean of 3.55 ± 0.51 ml/min/kg of body weight. The volume of distribution calculated from the area under the curve was determined separately for each animal and had a mean value of 0.213 ± 0.025 liters/kg. The data are consistent with those reported for other small mammals. These animals have high body clearance rates, which reflect high glomerular filtration rates and result in short dose intervals.

#### **P49 The Effect of Ketamine Hydrochloride on Creatine Kinase Blood Values in Cynomolgus Monkeys**

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Creatine kinase (CK) values were 4 to 12 times higher than normal in all monkeys (n = 42) that were being screened prior to being assigned to a toxicology study. The events involving this group of animals prior to blood collection were examined in an attempt to determine the cause of the CK elevations. The afternoon prior to blood collection, the animals had been given ketamine hydrochloride to permit ophthalmoscopic examinations. The poster/presentation will describe a series of experiments conducted that demonstrated that ketamine, one of the most common chemical restraints used in nonhuman primate colonies, was responsible for the elevated CK values. Experiments were designed to characterize this effect more fully. The first study was intended to determine whether the amount of ketamine affected the magnitude of the CK elevation and to determine the time course of the increasing values. The study demonstrated that the amount of ketamine was not a factor in determining the magnitude of the CK increase. All animals showed gradually increasing CK values with peak concentrations being reached generally between 4 and 8 hours after dosing. The mean CK value prior to treatment was 136 units/liter. Following ketamine injection, peak values ranged from 2,475 to 12,870 units/liter (mean = 8,563 units/liter). The next study was intended to determine whether the elevations were the result of muscle damage following intramuscular injection. Three groups of 4 animals were given either saline, ketamine, or xylazine by intramuscular injection. Serial blood samples collected over the next 48 hours demonstrated again that CK values in the animals receiving ketamine were markedly increased, with peak values occurring around 8 hours after dosing. Values were not increased in the saline group, but mild sporadic increases were noticed in some of the animals receiving xylazine. Subsequent studies were performed to compare the effects of ketamine by intravenous injection and intramuscular injection and to determine the effects when given on a milligram per kilogram basis. The results of these studies will be presented and discussed.

#### **P50 The Possible Role of Coprophagy in the Maintenance of Intestinal Flora**

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Coprophagy has been proved to play an important role in the nutrition of rabbits, rats, mice, and other animals, whereas the relation between coprophagy and intestinal flora is still obscure. This problem was examined in mice in detail. Male 8-week-old ICR mice (N = 8) were individually housed and were prevented from reingesting their feces by the specially contrived restrainer for 3 weeks. Fresh feces collected from each animal twice during coprophagy-permitted or -prevented period, respectively, were incubated anaerobically and each population of twelve kinds of microorganisms was counted. All animals gained weights during the coprophagy-prevented period and no animals had adverse clinical signs from the prevention treatment. Total bacteria, *Bacteroidaceae*, lactobacilli, and staphylococci in feces were significantly decreased in number, and numbers of eubacteria were significantly decreased after the prevention of coprophagy. Lecithinase-positive clostridia were slightly decreased in number. These findings seem to corroborate that coprophagy plays an important role in the maintenance of intestinal flora in coprophagous animals.

## **P51 A Summary of Normal Urinary Proteins in a Variety of Laboratory Mice**

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A complex of proteins in the urine of *Mus musculus* has previously been described, which migrates faster electrophoretically than serum albumin. These proteins have a low molecular weight, exhibit stable electrophoretic polymorphism, and have been useful as genetic markers in various inbred strains of mice. Traditionally, this has been accomplished by means of slab gels and traditional electrophoresis apparatuses. This process requires individual housing of subjects and the collection of pooled urine specimens over time in order to obtain a sample of sufficient volume for analysis. A study was performed to examine the feasibility of using microbore column chromatography to accomplish the same objective with a much smaller initial sample volume. The use of high-pressure liquid chromatography (HPLC) to investigate rodent urinary proteins offers many advantages over traditional techniques, specifically automation, speed, versatility, sample conservation, sensitivity, and resolution. Voided urine samples were collected by using nonheparinized microhematocrit capillary tubes from manually restrained mice, and analyzed by using reverse-phase HPLC with photodiode array and fluorescence detection. Mean urinary protein concentration and peptide components were compared in a variety of common laboratory mice. The results obtained were consistent with those reported in the literature. Common peptide fractions were identified, and characteristic patterns could be demonstrated in some strains. Sexually intact males had significantly higher concentrations of total urinary protein, which appeared to correlate with individual body weight, in contrast to females in which no such correlation could be demonstrated. Protein excretion was also highly variable in concentration in males and contained a greater amount of total protein and low molecular weight proteins (m.w.  $\leq$  49,000) when compared with that in females. Preliminary results indicate that HPLC has great potential for further investigations in this area.

## **P52 Study of Swine Tattooing for Humane, Permanent, and Rapid Identification**

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Ear notching, ear tagging, and ear clamp tattoos are presently being used for swine identification. These procedures are not totally adequate. Loss of tags, difficulty in reading notches, and rapid fading of clamp tattoos are encountered. The purpose of this work was to evaluate an electromechanical tattoo device, two tattoo sites, and two pigments for humane, permanent identification of laboratory swine. In study 1, 1-day-old Landrace-Yorkshire cross-bred gilts were tattooed on the outside of both ears by using black pigment 242. At 19 months of age, the tattoos were pale but legible. In study 2, Litzky White Diamond Pigs of both sexes were used. Tattoos were done lengthwise on the dorsal skin of the neck at 3 to 5 days of age, using black pigment 242 and black 242 Concentrate B. Pigment 242 Concentrate B resulted in darker tattoos. Forty pigs were tattooed per hour, and tattoos were easily readable at 10 weeks of age and at 230 pounds. The dorsal skin of the neck was more easily readable than the ears. Best legibility was obtained with pigment 242 Concentrate B. Neck tattooing was practical for humane, rapid, permanent, and safe identification of swine.

## **P53 Tail Tattoos for Humane, Safe, and Permanent Identification of Pigmented Mice**

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Identification of albino mice has been accomplished by using tail tattoos with black pigment 242. However, tattoos on pigmented mice were difficult to read. A 2-year study was conducted to evaluate 3 tattoo pigments at two tattoo sites on 320 B6C3F1 mice. Weanling mice were tattooed on the dorsal or ventral skin of the tail by using physiologic saline (controls), black 242, black 242 concentrate B, and blue 270. Mouse weights and tattoo readability scores were recorded monthly. Gross and histologic examination of the tail and proximal lymph nodes was conducted when the study ended. There were no differences in weight or mortality. Black 242 concentrate B and blue 270 resulted in easily legible tattoos. Ventral tail tattoos were easier to read early in the study, but both dorsal and ventral sites were equally legible after 9 months, as skin color became lighter with age. Microscopic examination of tissues revealed pigments deposited largely in the subepithelial dermis within macrophages. Pigments were essentially inert and caused no adverse effects on tissues or macrophages. Tail tattooing by use of black 242 concentrate B or blue 270 was a safe, permanent, and minimally invasive procedure for identification of pigmented mice. These results extend data obtained in a preliminary study.

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## **P54 Vaginal Cytology of the Lesser Bandicoot (*Bandicota savilei*)**

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The Lesser Bandicoot (*Bandicota savilei*) is a wild tropical rat maintained for research purposes at the Armed Forces Research Institute of Medical Sciences, Bangkok. To define the female estrous cycle, vaginal smears were obtained from 5 animals twice daily for 28 days and stained with Giemsa stain. Epithelial cells were categorized as parabasal, superficial epithelial, intermediate epithelial, and keratinized epithelial cells. Leukocytes were also quantified. The external appearance of the vulva and the color of the tip of vaginal swab specimens were recorded. Age of the animals ranged from 9 to 18 weeks. The vaginal cytologic features of the bandicoots included a cyclic change and a pattern similar to the estrous cycle in the laboratory rat. Estrous cycle length was 3 to 4 days. One of the females was time-mated and whelped a litter 26 days after the first day of detected estrus. Grossly, the tip of the swab looked yellow at diestrus, and a white caseous material was seen at estrus. No changes were observed in the external genitalia. We conclude that the stages of the estrous cycle in the Lesser Bandicoot rat can be determined by vaginal cytologic examination and that further study is needed to determine the exact length of each stage.

### **P55 Recombinant Sendai Virus Antigen for Use as a Diagnostic Reagent**

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The most common diagnostic technique for detection of Sendai virus infection in rodents is enzyme-linked immunoassay (ELISA) serologic analysis, using semi-purified preparations of whole virions as antigens. This assay often suffers from lack of specificity. The goal of this project was to develop more specific antigens for diagnostic testing by producing recombinant antigens in baculovirus-infected insect cells. To identify viral proteins immunoreactive in multiple laboratory rodent species, Western blots (immunoblots) of viral polypeptides were probed with immune and nonimmune sera from mice, rats, hamsters, and guinea pigs. The nucleocapsid protein (NP) reacted with immune sera from all species tested. To construct the recombinant, complementary DNA was prepared by reverse transcriptase polymerase chain reaction from Sendai virus RNA by using primers from 5' and 3' termini of the coding region. Amplified DNA was cloned into a baculovirus transfer vector (pBluBacHis) and co-transferred with wild-type baculovirus into insect cells. Baculovirus recombinants containing the NP gene were identified by polymerase chain reaction with NP-specific primers. Evaluation of recombinant proteins expressed in insect cells by Western blot analysis revealed specific reactivity with immune sera. Comparison of ELISAs with whole virions and recombinant NP indicated that ELISAs with recombinant protein were more specific and sensitive.

### **P56 DNA Fingerprinting of Rodents in the NIH Repository**

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Genetic monitoring of inbred, outbred, and transgenic rodents is important in order to determine the genetic authenticity of strains used in research. Currently, the most widely used techniques involve analysis of animal tissues such as blood, intestinal tract, and liver for the presence of biochemical markers. Alterations of the genotype that do not affect the assayed markers are not detected. Immunologic markers to determine tissue antigens may also be used as an alternative to biochemical assays. A more accurate way to determine the genotype of an animal is by reading the physical patterns of DNA treated with various restriction enzymes—a process referred to as DNA fingerprinting. During the past year, we have extracted DNA from tail tips and livers of 130 different inbred and congenic rats and mice. Using nonisotopic as well as isotopic multilocus probes, we have been able to detect genetic differences that were not identified with biochemical assays. DNA fingerprinting has also confirmed the genetic authenticity and individuality of 24 albino mouse strains, 13 black mouse strains, and 34 different albino rat strains, in addition to other closely related strains. Reproducible band patterns are used at the present time as reference profiles.

### **P57 New Diagnostic Testing for Giardia**

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*Giardia*, an intestinal flagellate found in many host species, including humans, can cause severe diarrhea. It is the most frequently reported parasite in the United States in humans, water samples, and some animals. Zoonotic potential is not clearly defined but may be significant. Animals and humans may harbor and excrete the parasite without clinical signs of disease. Until recently, identification methods required the presence of cysts in feces or water samples. The cyclical nature of the cyst shedding rendered these techniques of questionable reliability. From 1990 to 1993, *giardia* was the most common parasite found in dog fecal samples submitted to the Michigan State University Animal Health Diagnostic Laboratory. During July 1993 to December 1993, two enzyme immunoassays designed for the detection of *giardia*-specific antigen in human feces were compared with the sucrose fecal flotation technique on dog fecal samples. Dogs successfully treated for *giardia* infection were negative by all tests two weeks following treatment. Other species testing positive by assay were horse, cow, goat, bison, and chinchilla. *Giardia* can easily be missed when testing a single fecal sample by use of the sucrose fecal flotation technique. Both enzyme immunoassays displayed cross-species sensitivity. Both were significantly more accurate than the sucrose flotation technique.

### **P58 Production Parameters and Clinicopathologic Features of Monoclonal Antibody Production in Murine Ascites**

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Although murine ascites production has been a commonly utilized and useful technique for generating monoclonal antibodies, animal welfare concerns have been raised. The objective of this study was to characterize production parameters and clinicopathologic changes in mice associated with the *in vivo* production of monoclonal antibodies, and to compare these results between cell lines. Three different hybridoma cell lines were grown in groups of 20 mice. Mice were primed with 0.5 ml pristane intraperitoneally 14 days prior to inoculation of  $1 \times 10^6$  hybridoma cells. Fluid was collected from the abdomen of each mouse a maximum of 3 times for collection of ascites. A sham control group of 12 mice was similarly treated. Clinical observations, pre- and post-abdominal fluid tap body weight measurements, ascites volume measurements, and postmortem examinations were performed. Although total ascites volumes were similar ( $m = 111$  ml), different trends were observed between groups in comparison of ascites volume and antibody concentration from the first to the third abdominal fluid tap. Total antibody produced per group ranged from 445.57 to 996.64 mg as determined by enzyme-linked immunosorbent assay. Incidence and severity of clinical abnormalities increased with time. Survival to the third abdominal fluid tap ranged from 75 to 100%. Disseminated intraabdominal seeding with irregular soft tissue masses and/or solid tumor masses were observed on postmortem examination. These results documented significant clinicopathologic changes in mice associated with monoclonal antibody production in ascites and demonstrated differences observed between different hybridoma cell lines.

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## **P59 Hollow Fiber Bioreactors as an Alternative to Murine Ascites for Nonoclonal Antibody Production**

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Hollow fiber bioreactor technology has recently been applied to small-scale ( $\leq 1$  g) hybridoma cell culture. The objective of this study was to compare monoclonal antibody production in hollow fiber bioreactor systems and in murine ascites to determine the feasibility of using bioreactors as alternatives to mice. Three hybridoma cell lines were grown in each of three different bioreactor systems and in 20 mice. Mice were monitored clinically throughout the study, and abdominal fluid from each mouse was tapped a maximum of 3 times for collection and evaluation of ascites. Bioreactors were harvested 3x weekly for 60 days and monitored by cell counts, cell viability, and media glucose consumption. Time and materials logs were maintained. The quantity of antibody produced, as determined by enzyme-linked immunosorbent assays, in 20 mice versus the mean production for 3 bioreactors was as follows: cell line 2B11, 454.50 mg vs. 168.35 mg; cell line 3C9, 445.57 mg vs. 560.15 mg; and cell line RMK, 996.64 mg vs. 1247.87 mg, respectively (bioreactor N = 2 for cell line RMK). Although time and materials costs were greater for the bioreactors, results of production data suggest that hollow fiber bioreactor systems merit further investigation as alternatives to monoclonal antibody production in murine ascites.

Supported by Grants RR01046 and RR07036.

## **P60 Polyclonal Antibody Production in the Rat, Using Subcutaneous Chambers**

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Rats are not often used for antibody production, presumably because it is difficult to collect blood from them, and they have small blood volumes. However, alternative species to the rabbit are occasionally needed. We have adapted the whiffle ball technique previously described for rabbits to the rat for production of antibody. A single hollow plastic ball, 2 cm in diameter, containing holes, implanted subcutaneously in each test rat, created a chamber for fluid accumulation. Implant rats were immunized by injection of the antigen, without adjuvant, into the ball. Control rats, which did not have implants, received antigen/adjuvant mix subcutaneously. Antigen concentration and immunization schedule were the same in both groups. Blood from control rats and chamber fluid from implant rats were assayed for antibody. At the conclusion of immunization, titers from implant rats were higher than titers from control rats: approximately 2-fold greater at 2 weeks; and 4-fold greater at 8 weeks after immunization. In addition, approximately 6-fold greater volume (3 ml) was recovered from implant rats at each sample time. Results indicate that the implant technique is useful for antibody production in rats and has the same ease of sampling and minimal animal stress as previously described for rabbits.

## **P61 Characterization and Quantification of Microenvironmental Contaminants in Isolator Cages with a Variety of Beddings**

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Microenvironmental contaminants were measured within isolator type cages housing DBA/1J mice on 8 contact beddings. Each cage contained 850 cm<sup>3</sup> of bedding and 5 mice randomized by weight. Seven test and 2 control cages without mice were evaluated per bedding. Macroenvironmental conditions were defined and controlled. Macroenvironmental and microenvironmental temperatures, relative humidity, carbon dioxide, and ammonia levels were determined daily during each of 3 seven-day test periods. The gas detector tube system was used to measure hydrogen gas, butanol, acetone, ethanol, carbon monoxide, acetic acid, hydrogen sulfide, sulfur dioxide, and formaldehyde on the final day of each test period. Gas chromatographic analysis was used to detect additional volatile alcohols and ketones. Ammonia concentrations ranged from 0 to 410 ppm, depending on the bedding type and day of measurement. On the basis of the mean ammonia concentrations in the test cages, the beddings were ranked from the highest to lowest ammonia concentrations: aspen shavings, pine shavings, reclaimed wood pulp bedding, virgin pulp loose bedding, hardwood chip bedding, paper bedding, virgin cellulose pelleted bedding, and corn cob bedding. The temperature, relative humidity, and carbon dioxide levels were similar between beddings. No other contaminants were detected except acetic acid ( $m = 0.86$  ppm) and sulfur dioxide ( $m = 0.42$  ppm) in both test and control cages in which the corn cob bedding was placed. In summary, the level of ammonia varied significantly among different contact beddings.

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## **P62 An Appropriate Food Withholding Period Alternative to Overnight Food Withholding for Acute Oral Toxicity Study with Rats**

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Overnight food withholding, recommended for the acute oral toxicity study with rats in guidelines of Organization for Economic Cooperation and Development and U.S. Environmental Protection Agency, seems to disorder homeostasis of animals and consequently to be obstructive in disclosing toxicities of chemicals. The present study was conducted to find an effective shorter food withholding period comparable to overnight food withholding. Fischer 10-week-old rats were randomly allotted to 6 groups (N = 6/sex/group): food not withheld, food withheld 4 hours after lighting, food withheld 4 hours before lighting, food withheld 6 hours (4-hours before lighting and 2 hours after lighting), food withheld 8-hours (4-hours before lighting and 4-hours after lighting), or food withheld overnight. Animals were necropsied just after the food withholding period or 4 hours after lighting, and weights of body, gastric contents, and major organs were measured. The food withholding overnight group showed the most reduced body, liver, and thymus weights, but adrenal glands tended to increase. The 4-h (before lighting) and 6-h food withholding groups had approximately 2-fold gastric contents, compared with the overnight food withholding group, and showed slightly reduced liver and thymus weights. The 8-h food withholding group showed the same weight of gastric contents as the overnight food withholding group, but degrees of reduction in organ weights were the same as in the 4- or 6-h food withholding group. From these results, it is possible that an 8-h food withholding period is less distressful to animals and comparably effective in emptying the gastric lumen, compared with an overnight food withholding period.

### **P63 Free Flight And Breeding Pigeon Room**

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To enable pigeons free flight in a room and permit the investigator and technicians to enter the room daily to check, feed, and water, we constructed a divider wall that gives the pigeons an area measuring 8ft 6in x 5ft x 7ft in which to fly, and provides an area measuring 6ft 6in x 5ft x 8ft 6in for a person to enter the room and observe the birds. The wall frame with a door was made of PVC pipe, plastic diamond mesh, stainless steel hinges, and zip ties. We mounted plastic milk crates on the inside walls with self-drilling hollow wall anchors with hooks. Three T-shaped perches of different height were constructed of 1 1/4in PVC pipes and braced on the walls. Sections of the plastic diamond mesh were cut out to allow J-feeders to be attached close to the floor. Thirty-two-ounce plastic water bottles with sipper tubes were attached to the diamond mesh close to the floor both feeder and bottle are secured with stainless steel spring clips. The use of J-feeders and water bottles keep the food and water from becoming soiled from the flying birds. Hardwood chips were used on the floor as bedding and nesting material and were changed weekly. Plastic milk crates were placed on the floor to provide a haven for nesting birds and newly hatched chicks. Total cleaning of the pigeon area is handled by opening the door and moving the pigeons to the outer area and is done monthly.

### **P64 Effects of Incubation Temperature on Growth of Desert Tortoise (*Gopherus agassizi*) Hatchlings in a Laboratory Animal Research Facility**

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Twenty-eight desert tortoise hatchlings (*Gopherus agassizi*) from three incubation temperature regimens (30, 31, and 328C) were weighed and carapace lengths and widths measured to determine whether incubation temperature affects posthatching growth rates. Hatchlings were weighed to the nearest gram and carapace length and width measured to the nearest millimeter weekly over a period of 22 weeks after hatching. The data suggest that incubation temperature may play a role in weight gain of tortoise hatchlings. Animals incubated at 328C demonstrated the least weight gain (1.7 g/week), while those incubated at 30 and 318C demonstrated the greatest weight gain (2.1 g/week). There was no statistical difference between the three incubation regimens relative to carapace length and width. Animals demonstrated an increase in mean carapace length of 0.9 mm per week, and an increase in the mean width of 0.6 mm. This study is the first report of growth in captive laboratory-incubated, hatched, and raised desert tortoises. With the recent listing of the desert tortoise on the Endangered and Threatened Species List, desert tortoise husbandry and captive management may become crucial to the survival of the species.

### **P65 An Enriched Housing Unit for Grey Squirrels (*Sciurus carolinensis*)**

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Scant information exists regarding the housing of arboreal squirrels in research facilities. Presented with a request to house wild-caught grey squirrels (*Sciurus carolinensis*) indoors for a study investigating energy regulation patterns of this species, we designed a housing unit to securely house the animals and provide them with opportunity to exhibit typical species behavior. Pens measured 3.5 m (l) x 1.5 m (w) x 2.3 m (h) and exited to an anteroom through a locking aluminum storm door. Interior walls of pens were constructed of cement board panels within aluminum frames. The floor was constructed of linoleum. Cords to remote monitoring equipment were contained within armored cable. Existing drains and room water lines were covered and sealed. Squirrels were provided a covered nest box measuring 0.1 m (l) x 0.20 m (w) x 0.22 m (h) and elevated 1.7 m off the floor. Suspended branches and ropes were provided for activity. Animals were allowed ad libitum access to a variety of nuts, seeds, fruits, and vegetables and to water in a drinking bowl. Plastic cat litter trays filled with either wood shavings or a sand and gravel mix were provided as caching substrates. Photoperiod reflected natural day length and was adjusted in 15-minute intervals weekly. Use of these units for 7 months showed that squirrels displayed frequent use of suspended branches and chains and housing boxes. No wear of caging materials due to chewing or scratching was observed. Squirrels remained clinically normal, exhibited normal caching behavior, and underwent normal seasonal changes in reproductive condition over the entire 7-month period of housing. In summary, the enriched caging system described here is useful for long-term maintenance of the arboreal grey squirrel.

### **P66 Maintenance of Axenic Mice in Filter Top Cages**

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Since variations in the composition of the intestinal flora are known to profoundly affect rodent metabolism, we are interested in finding practical methods to maintain a standard flora. Although flexible film isolators are routinely used to maintain gnotobiotic or axenic mice, the development of a simpler alternative method seems desirable. Previous studies showed that axenic mice could be maintained for 4 weeks in a filter top cage. We have therefore evaluated the feasibility of maintaining axenic mice, for longer periods of time, using similar methods. In the present study, a class II-A biohazard hood was used to provide the work space. Sterilization of the hood and its contents immediately prior to and during any manipulations was achieved by spraying a metastabilized chlorus acid/chlorine dioxide sterilant. Personnel wore shoe and head covers, gown or lab coat, face mask, and gloves. Five breeder pairs of 5-week-old, axenic, Balb/c mice were transported from the NCSU Core Axenic Facility in a sterile container. Each pair was placed in a cage that had been autoclaved as a unit containing food and bedding. Water-filled bottles were autoclaved in a separate cage and placed into each cage. Once a week the mice were transferred to new autoclaved cage units, water bottles were added, and fresh feces were obtained for the purpose of microbial monitoring. Methods used to determine microbial status included gram staining and inoculation of feces into thioglycolate. Following a 24-hour incubation period at 37°C, the sample was subcultured onto Columbia blood agar-aerobic, and pre-reduced Columbia blood agar-anaerobic plates. The mice in these five

cages have been maintained axenic for three months, thus far. Three litters of axenic mice have been produced and the other two females are pregnant. Now that we are confident that mice can be maintained axenic for at least 3 months, we are evaluating Altered Schaedler Flora associated gnotobiotic mice to determine whether we can maintain this standard flora by using similar methods.

### **P67 Use of Shredded Rubber-Filled Polyester Mattresses to Prevent Injury to Dairy Cattle Housed in Tie Stalls**

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Dairy cattle housed in tie stalls commonly experience trauma to the legs, udder, and feet from concrete flooring and posts. To reduce injury in 84 dairy cattle housed in tie stalls for 20 hours per day, woven fiber mattresses were constructed with shredded (1 inch diameter) nonsteel-belted tire rubber and a heavy-weight industrial fabric and placed in each tie stall. Approximately 250 pounds of shredded rubber were used per mattress at a cost of \$.05/pound. Three different fabric materials were examined. Type 1 fabric was a 9-oz (per square yard) polypropylene bag with rolled lap seams. The filled bag was attached to the tie stall with 2 x 4-inch treated lumber and eye hooks/bolts. Fabric type 2 was 14.4-oz woven seamless polypropylene, and type 3 was 20-oz woven seamless polyester/nylon. Types-2 and -3 mattresses were constructed by placing 2 x 4 treated lumber around the tie stall, filling the platform area with 250 pounds of shredded rubber, and covering the rubber with fabric. Wood screws were placed through the fabric and a tacking strip to fix the mattress to the tie stall. Approximately 1.5 man-hours were spent per mattress. Type 1 materials cost \$33.50/stall, required less time and lumber to construct, but lasted only 1 to 4 months. Type 2 cost \$34.30/stall, required more time and lumber, but lasted 3 to 6 months. Type 3 cost \$29.50/stall, required the same time and lumber as type 2, and lasted 4 to 9 months (to date). All three mattresses greatly reduced the incidence of leg and udder injuries. Mattress surfaces stayed dry and could be easily cleaned. Although all mattresses required a wood shaving cover to reduce friction and absorb moisture, the total amount of bedding used was greatly reduced. It was concluded that the shredded rubber-filled mattresses improve cow health and comfort in cattle housed in tie stalls and that the heavier-duty, polyester woven fiber materials appear more suitable for heavy use.

### **P68 An Efficient, Economical Multicompartment Tank for Housing *Xenopus laevis***

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Housing *Xenopus laevis* in quantity, especially when they must be divided by investigator, presents problems in housing and husbandry due to their needs for an aquatic environment. The use of individual pens on a solid shelf rack, entails potential personnel safety issues such as slipping or back injuries from dealing with water-laden pens. Housing in individual pens also can increase husbandry costs. A mobile tank was designed to address these concerns. It was constructed of 5/8" plexiglas subdivided into 12 x 18 x 18-in. sections, each capable of holding up to 5 frogs, and was mounted on a frame of 0.25 x 2-in. welded aluminum equal angle fitted with locking swivel casters. Each section has a drainage port consisting of a bottom-mounted 0.5-in. brass ball valve and latex tubing. Draining, cleaning, and refilling can be accomplished without lifting or carrying. Sections are refilled with carbon-filtered, UV-treated tap water. The plexiglas and aluminum construction is inexpensive, durable, and functional. We have experienced no breakdowns or deterioration of 6 tanks during 4 years of continu-

ous operation. The multicompartment tank offers a simple, efficient, inexpensive, and safe system for housing and husbandry.

### **P69 Comparison of Pellet Hardness Measurements from Nonautoclavable and Autoclavable Rodent Diets Processed by Different Vendors**

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Very little is known about the optimal physical characteristics of rodent diets. Feed pellets from different diets as well as pellets from the same diet formulation manufactured by different rodent diet vendors may vary in size, shape, texture, and pellet hardness. Normal pellet hardness measurements of commercially available rodent diets have not been reported; however, a standard procedure for measuring pellet hardness of rodent diets has been published. A test stand with a flat horizontal receptacle base and a DFI-500 gauge load cell with a large round-flat upper test jaw (12.7 mm diameter) were used to collect pellet hardness measurements (kilograms of force). Test diets were purchased from four different rodent diet vendors: A, B, C, and D. Autoclavable diets were autoclaved at 2508F (1218C) at 15 to 18 lb pressure for 20 minutes. Results from 90 pellets (3 replicates of 30) from each mill date for each diet were compared for pellet hardness. Moisture content was also determined for each diet. Mean pellet hardness values for nonautoclavable diets are given in parentheses. Mean pellet hardness values for the pre- and post-autoclaved diets are given in brackets: Vendor A NIH-07 (78.01 6 11.98), NIH-31 [73.49 6 9.86], [94.79 6 12.46]; Vendor B NIH-07(42.82 6 7.67), NIH-31 [41.21 6 7.18], [55.66 6 11.37]; Vendor C NIH-07 (82.91 6 22.85), NIH-31 [84.17 6 19.16], [93.16 6 26.13], # 7012 -LM-485 [41.90 6 6.48], [43.23 6 8.30], # 8626 (43.97 6 5.34); Vendor D NIH-31 [42.09 6 8.07], [47.93 6 10.24], # 5001 (56.99 6 7.91), # 5002 (82.58 6 17.52), # 5015 (48.98 6 6.29), # 5058 (45.22 6 6.06), # 5010 [42.77 6 6.81], [46.69 6 9.11], and # 5021 [38.78 6 6.86], [45.90 6 9.43]. Moisture content was similar for both nonautoclavable and autoclaved diets. It was concluded that rodent diets significantly differ in pellet hardness, pellets from the same diet processed by different rodent diet vendors significantly differ in pellet hardness, steam sterilization of diets increases pellet hardness, and in order to compare pellet hardness measurements from different diets, a standardized procedure must be used and the diet's vendor specified.

### **P70 Effects of Temperature and Cage Wash Chemicals on Sanitization of Soiled Cages**

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An important consideration in reducing or eliminating the cross transmission of infectious or contaminating agents, particularly bacteria, is adequate sanitization of caging systems. We tested several different wash procedures on various caging system types to determine the extent of bioload reduction from each operation. Cage racks, pans, and polycarbonate cages were washed at 1408F (608C) and rinsed with hot (1808F [828C]) or warm (1408F) water only, warm water with chlorine dioxide sanitizer (acid activated), and hot or warm water with organic acid only. To determine pre-wash bioload burdens, samples were taken for bacterial culture from several sites prior to treatment by using a direct-contact agar plate. Culturing was then repeated following each of the wash treatments. It was found that microorganism load was reduced with each method as compared with the pretreatment results. Use of water alone caused significant reduction of

colony numbers, although there was some growth. There was no significant difference between the two water temperature treatments when cage wash chemicals were not used. The addition of acid wash chemical, regardless of wash and rinse water temperatures, further reduced the microbial load, and there was no difference seen between chlorine dioxide and acid only (both negative). These results indicate adequate sanitization of caging was achieved by acid wash alone, regardless of the wash water temperatures and was comparable to the use of chlorine dioxide. These results further indicate that the use of acid wash with a warm water rinse may improve cage washer efficiency, reduce chemical use, and eliminate disposal and safety issues pertaining to the use of halogen compounds, thereby providing for a safer working environment.

#### **P71 Use of Computational Fluid Dynamics to Assess Air Distribution Patterns in Animal Rooms**

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Satisfactory ventilation of animal rooms requires a sufficient quantity of air (measured in air changes per hour) combined with a proper air flow distribution pattern. Provision of sufficient air changes without proper air distribution may result in unacceptable ventilation. Computational Fluid Dynamics (CFD) technique has been validated to model the air flow patterns in animal rooms. CFD enables computer simulation and analysis of airflow distribution patterns while altering the size, number, and location of air supply and exhaust systems. In this study, CFD was used to compare five potential airflow patterns in animal rooms under renovation. Results from the original design demonstrated unsatisfactory air distribution patterns with airflow approaching zero (stagnant air pockets) in several areas at the animal level. The design alterations tested provided several alternatives resulting in more uniform air distribution patterns. CFD provided clear visualization of air distribution patterns, which enabled engineers, architects, veterinarians, administrators, and laypersons to reach a rapid consensus on the most acceptable design. CFD is a valuable tool to optimize the ventilation of animal rooms before or during construction.

#### **P72 Disposal of Radioactive and Nonradioactive Animal Carcasses by Alkaline Hydrolysis**

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The alkaline hydrolysis process involves the solubilization in a specially designed stainless steel reaction vessel of animal carcasses and tissues that contain low levels of radioisotopes and of nonradioactive carcasses for their proper and safe disposal as liquid waste. For this, the carcasses are heated in the hermetically sealed digester to 110°C at slightly elevated pressure (10 to 15 lb/in<sup>2</sup>) and digested with a circulating solution of 1 N aqueous sodium hydroxide, using an amount of NaOH equivalent to the peptide bond content of the tissues. Within 2 hours, most tissue constituents are in solution. Hydrolysis and degradation of proteins, as well as of lipids, nucleic acids, and most carbohydrates continues during the heating process. The pH of the reaction mixture drops continuously because of sodium hydroxide consumption. After 16 to 18 hours, the digest reaches a pH value between 11.5 and 12, which is considered neutral (2, pH, 12.5) by EPA wastewater standards. The sterile, liquid tissue hydrolysate containing the isotopes in soluble form is strained free of debris and discharged from the reaction vessel. The alkaline hydrolysis process liberates the 70 to 80% water content of the animals, producing a liquid waste that more than meets 10CFR20 maximum permissible concentrations for disposal of

low-level radioactive waste into sanitary sewer systems. The only insoluble biologic remains are the inorganic portions of bones and teeth, which amount to approximately 3% of the original carcass weight. Foreign materials such as plastics, ceramics, metal, and cellulose-based materials such as plant fibers, gauze sponges, and paper products with which the carcasses may have been contaminated are now sterile and can be disposed of appropriately. Once the digester is installed, the cost of disposal is less than 0.3% of the cost of disposal at the Barnwell, S.C. low-level radioactive waste site and approximately 20% of the cost of disposal of nonradioactive carcasses as regulated medical waste.

#### **P73 Lasers: Technology and Safety**

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During the 1990's, it is estimated that one million workers will be exposed to the use of lasers. As the technology evolves, these numbers are expected to increase. Surgeons, researchers, and technicians will be at increased risk of incidental injury. Today, the most popular lasers in use are the Argon, Carbon Dioxide (CO<sub>2</sub>), Neodymium: Yttrium Aluminum Garnet (Nd:YAG), frequency doubled YAG, or Potassium titanyl phosphate (KTP), and more recently the Holmium. To minimize risk, it is necessary for anyone using a laser to be familiar with basic laser biophysics and understand how the radiation beam affects tissue. Various wavelengths, measured in nanometers, affect tissue differently. For example, the Argon and Nd:YAG laser beam will pass through clear fluids or structures. This characteristic allows retinal surgeries, and tumor vaporization in urologic procedures. Unfortunately, these lasers could also cause extensive eye damage if protective goggles are not used. In addition to tissue damage, laser beam scatter and laser smoke plume are hazards. The laser operator and personnel within the procedure area must observe safety precautions. Safety standards have been established by the American National Standards Institute. Other regulatory agencies include the Center for Devices and Radiological Health, a division of the Food and Drug Administration, the Occupational Safety and Health Administration, and state and local regulations. Laser safety committees have been established in a number of institutions to review protocols involving laser use. These committees should closely scrutinize laser use to ensure researchers are appropriately credentialed for laser operation, and technical staff is properly trained. Frequency of laser use is also a concern. For example, it could become necessary to review annually the training of infrequent laser users because the technology is changing so rapidly. Laser safety training courses are strongly encouraged for all personnel exposed to lasers, and in some cases, offered by the manufacturers. The future of laser technology could necessitate a continuing education program since present standards will change.

#### **P74 An Incentive Program for Unionized Employees**

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An incentive program was developed to recognize and encourage above-standard performance among unionized laboratory animal care personnel. The union contract establishes a wage rate for employees in each job classification. Salary increases and monetary awards based on meritorious performance of individual employees are not provided for under the union contract. This incentive program provides an alternative mechanism for rewarding such employees. Employees earn points by demonstrating above-standard animal care and teamwork, achieving laboratory animal technician or laboratory animal technologist certification, voluntarily participating on projects or committees, maintaining an accident-free workplace,

implementing safety suggestions, or having an excellent attendance record. The points may be exchanged for preselected awards displayed in a catalog specially designed for the program. A chart showing the number of points earned by each employee is prominently displayed in a central location to further recognize and encourage above-standard performance. Measurable improvements in work performance have been observed since the implementation of the program.

### **P75 The Design and Implementation of a Computerized Veterinary Medical Record System**

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The need to maintain accurate veterinary medical records and other animal tracking information led to the development of a veterinary database utilizing commercially available software. The system is easy to learn and use, and facilitated the design of a customized veterinary medical record format for several species. A personal computer network provides departmental personnel with easy access to the database for data entry or information searches. An Animal Inventory file record is generated for each animal upon receipt and contains detailed descriptive information including species, age, sex, identification number, source, receipt date, housing location, IACUC protocol number, and investigator information. The Animal Health History file is the primary veterinary medical record for the colony, and each file record provides a chronologic history of medical and surgical treatment for an individual animal. Three additional files serve to provide more detailed information about observations related to clinical examinations, surgery, and experimental procedures. Information can be displayed in a variety of different layouts, which is useful for colony health management and project planning, as well as for the generation of animal census data and the provision of animal tracking information on regulated species for compliance with USDA requirements. This use of the software program has facilitated the development of a versatile, highly efficient database for veterinary medical records and animal tracking.

### **P76 A Graphical User Interface-Driven Transgenic Mouse Database**

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The use of transgenic technology to generate animal models of human diseases has become increasingly popular. Rodents are particularly useful as transgenic animal models because of their fecundity and relatively short life cycle. The production of transgenic mouse lines has engendered the need to breed, characterize, and keep track of large numbers of animals. To automate this task, a graphical user interface-driven database was created by using commercially available database management software. This approach substitutes graphical objects for arcane computer code. The result is a user-friendly computerized environment for efficient record keeping. This database was created in response to the requirements of the transgenic production colony to allow users access to all information about every transgenic animal. This includes clinical evaluation, and pathology reports relating to the phenotypic consequences of transgene expression, regulatory data such as animal usage and numbers to comply with IACUC requirements, and other mouse pedigree, genetic, and molecular biological data. Combined with a portable computer in the animal rooms, and electronic animal identification, the database provides a powerful new tool for scientific, veterinary, and regulatory personnel to make more effective use of transgenic mouse models.

### **P77 Developing A Database For Tracking Research Personnel Training and Animal Exposure**

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According to the PHS *Guide for the Care and Use of Laboratory Animals*, "It is an institutional obligation to ensure that professional and technical personnel and students who perform animal anesthesia, surgery, or other experimental manipulations are qualified through training or experience to accomplish these tasks in a humane and scientifically acceptable manner." In a large research institution with many investigators and research personnel, this becomes a monumental task. At our recent AAALAC site visit, it was suggested that we extend our current occupational health program to all research personnel with animal contact. A database was developed to keep track of information pertaining to research personnel training and their substantial animal contact. The input to the database comes from three sources: The Animal Experimentation Protocol Review Form, the One on One Training Log, and formal presentations given by the Veterinary Staff and the Training and Compliance Coordinator. This database is available on a network server with read-only access. Significant information on occupational health such as serum banking, immunizations, and other tests required along with the date given and training information as to species, procedures, dates trained, and trainer can be retrieved from this system. Periodic reports such as reminders of needed training and immunizations can be generated and sent to investigators. The use of this database enables tracking of research personnel to ensure both adequate training and occupational health surveillance.

### **P78 Animal Welfare: A Bibliographic Guide, a Valuable Resource for Animal Alternative Searches**

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Investigators are required by law to consider alternatives to procedures that may cause more than momentary or slight pain or distress to vertebrate animals. We have developed an Animal Welfare Bibliographic Guide at the Yale Medical Library to make this potentially complex search user-friendly. The Guide recommends resources for creating a list of keywords and subject headings to search public indexes and databases. Questions and subject headings also are presented that should be considered when searching for alternatives and search hints are recommended, including access to subject headings through Index Medicus/MEDLINE and AGRICOLA. Information on other resources such as textbooks and reference materials are given and instructions on how to locate books and journal titles through Online Research and Bibliographic Information System (ORBIS), Online Public Access Catalog (OPAC) on-line catalog, and techniques for finding articles in journals. Databases and indexes that may need to be searched to provide a thorough investigation of the literature are identified. Several indexes are available in print in CD-Rom for one to search on their own. Search analysts also are available through a Computer Search Service.

## **P79 An Interpretive Summary of the Report of the AVMA Panel on Euthanasia**

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The Report of the AVMA Panel on Euthanasia was revised in 1993. The changes in content that were made necessitated review and analysis by our Institutional Animal Care and Use Committee (IACUC) to ensure that practices at our institution were in compliance with the recommendations in the report. In addition, the summary tables of agents and methods of euthanasia in the report were not satisfactory for distribution to investigators at our institution since they did not contain drug dosages or supplementary information from the body of the report that had not been included in the report's tables. To address these problems, the entire report was summarized in tabular form. The suitability of each agent or method of euthanasia for each species was listed in the summary table. Pertinent information from the body of the report and drug dosages were incorporated into the summary table as footnotes. It also was deemed necessary to bring 5 issues to the IACUC for further discussion. 1) Should scientific or other justification and IACUC approval be required for all conditionally acceptable methods of euthanasia or only for cervical dislocation and decapitation as recommended in the report? 2) Can unacceptable methods sometimes be permitted if adequate justification can be provided? 3) Should decapitation and cervical dislocation of rodents be classified conditionally acceptable, as recommended by the panel, or acceptable to obviate the need for scientific justification? 4) Should the IACUC require assurance that safety precautions on the use of ether from the *Guide for the Care and Use of Laboratory Animals (Guide)* will be followed? 5) Should the IACUC permit the use of chloral hydrate for euthanasia of rodents or sustain the panel's classification of this agent as unacceptable? After discussion, the IACUC concluded that investigators would be required to provide scientific or other justification for all conditionally acceptable methods of euthanasia and that cervical dislocation and decapitation would specifically require scientific justification. Unacceptable methods of euthanasia would be considered under unusual circumstances if adequate justification could be provided. Investigators would be required to provide assurance that they would follow safety precautions in the *Guide* when using ether for euthanasia. Finally, chloral hydrate for euthanasia of rodents would be classified as unacceptable, but its use would be permitted if adequate justification were provided.

## **P80 Providing Career Exploration for High School Students in a Research Facility**

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At The Ohio State University Laboratory Animal Center, two programs are provided for students of a Columbus area high school. In the first program, six students participate 2 1/2 to 3 hours once a week, for eight con-

secutive weeks in Career Exploration. In addition, two senior students participate in the Senior Service Program in which they contribute 60 hours of work to the research facility over the course of the academic year. By means of discussions, demonstrations, seminars, field trips, audiovisual aids, and hands-on experience, students are able to get personal, in-depth exposure to the careers of research technicians, animal behaviorists, lab technicians, and veterinarians. Not only do the students benefit, but the research community as a whole benefits. First, the work that the Senior Service students contribute gives much needed support in areas such as animal enrichment. Second, both programs help attract students to careers that are research related. Third, there is a motivational aspect for the animal caretakers and research technicians who are able to share their knowledge and experience. Last, in view of the great interest in animal welfare, students are introduced to the system of checks and balances that protect research animals, as well as the benefits derived from using animals in research.

## **P81 The DuPont Merck and Warner Elementary School Partnership Program**

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Because of the increasing amount of negative publicity that the animal rights groups have presented to elementary age students, we decided it was time for us to get into the school system and let the students hear what we do and why. Our overall goal was to get the students interested in science and understand the importance of science and math in their daily life. To do this we selected an elementary school that had administrative support as well as the teachers' support. We met with the teachers numerous times to find out what they needed/wanted from us, as well as to let them know what our goals were. Once the programs were chosen, we solicited volunteers from our research organization to implement the following programs: Coteaching, Mentoring, Let's Visit A Research Lab, Educational Partnership Program for Teachers, and Tours for Teachers. We had 52 volunteers and were able to implement these programs successfully. The feedback from the parents, students, and teachers has been overwhelming. Through classroom presentations we were able to expose the students to a wide variety of sciences and dispel the myths and stereotypes about scientists and their jobs. Through the mentoring program we were able to do one on one interactions and experiments with the students to help them learn the scientific process and the importance of recording data.