Platform and Poster Presentations - AALAS/ICLAS Joint Meeting
Anaheim, California

PLATFORM SESSION

PS01  Use of a Sentinel Program for Infectious Disease Surveillance in Nonhuman Primates

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Using sentinel animals is a well established method of monitoring laboratory animal colonies for the presence of pathogens. NIH has recognized the benefits of disease-defined populations of nonhuman primates by establishing seven breeding colonies of Specific Pathogen Free (SPF) rhesus macaques. Captive breeding populations of macaques may have endemic virus infections such as Type D retrovirus. In order to begin defining the viral status of the research breeding colony of rhesus and longtailed macaques at the California Regional Primate Research Center, a program of viral screening has been initiated. To reduce costs and yet optimize information, a sentinel surveillance program using breeding males was started. Each male may mate with as many as 144 females on an annual basis. The initial screen of 71/934 rhesus and 13/369 longtailed macaques disclosed rhesus, 6 positive by Type D Western blot, 54 negative, and 17 indeterminate with a GP 24 band. Of the longtailed macaques, 13 were negative by Western blot. Annual testing will be initiated to monitor the Type D viral status of the indoor colonies. Western blot-positive animals will be evaluated by virus isolation and repeat serologic testing. This preliminary sentinel screening program will provide information about the retroviral status of our breeding colony.

Supported by NIH grant RR00169

PS02  Epidemiology of Diarrhea-related Morbidity and Mortality in the 1988 Birth Cohort of Rhesus Macaques (Macaca mulatta) at the California Primate Research Center

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Diarrheal disease is a serious clinical and economic problem at the California Regional Primate Research Center and at other facilities housing large numbers of nonhuman primates. We sought to learn the epidemiologic patterns of diarrheal disease in a single large birth cohort (1988) of rhesus macaques in the first 2 years of life and extend previous studies of the relationship between management and diarrhea. We compared the morbidity and mortality of diarrheal disease among groups of rhesus macaques (n=403) maintained from birth in three different management schemes [indoor (n=125), outdoor (n=246), and nursery reared (n=32)]. All groups were managed differently with regard to nursing and weaning practices. In the cohort, 97 (24%) rhesus macaques developed diarrhea, with indoor animals having the highest incidence (38%) compared with outdoor (17%) and nursery-reared (25%) animals. Of the diarrhea cases, 22 (23%) developed chronic diarrhea; indoor animals (35%) had the highest incidence compared with outdoor (10%) and nursery-reared (13%) animals. With regard to mortality, 36 (9%) animals in the cohort died due to diarrhea-related disease. The highest mortality was among the indoor animals (14%) compared with the outdoor (7%) and nursery-reared (3%) animals. The results of this study suggest that the current management practices in the indoor group are a significant risk factor for developing diarrhea-related disease and death.

Supported in part by NIH grant RR00169

PS03  Serologic Assessment of Cytomegalovirus Infection in a Well-defined Breeding Population of Rhesus Monkeys (Macaca mulatta)

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Little is known regarding the epidemiology of cytomegalovirus (CMV) in macaques despite its important role as an opportunistic infection in cases of simian retrovirus-induced immunosuppression and its potential as a model for congenital CMV infections in humans. This study was undertaken to improve basic epidemiologic understanding of CMV in group-housed rhesus monkeys. A three-step Western blot assay was used to assess humoral IgG and IgM antibodies to the agent, employing a preformed avidin-biotinylated horseradish peroxidase complex as the detection system. Isotopic-specific rabbit anti-rhesus immunoglobulins were used as secondary antibodies, followed by biotinylated goat anti-rabbit antibody conjugate. A well-characterized rhesus CMV isolate (ATCC strain 68-1) propagated in MRC-5 cells was electrophoresed and used as test antigen. Frozen serum samples from a closely-monitored breeding cohort of rhesus monkeys collected during a recent prospective study of herpes B virus transmission were evaluated. All (n=123) monkeys aged 12 months and older in the enclosure were seropositive by IgG Western blot. Biweekly testing of all (n=28) available infants born into the population documented persistent anti-CMV IgG/IgM antibodies or seroconversion to the agent in all but one individual during the first year of life. Parallel testing and evaluation of selected cord blood specimens provided for investigation of maternal antibodies versus those acquired from postnatal and possible congenital infections.

Supported in part by NIH grant RR00169

continued
PS04  Fatal Herpesvirus Infection in Debrassa's Monkeys
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A sudden outbreak of herpetic disease occurred in a colony of Debrassa's monkeys (Ceropithecus neglectus) maintained at the Woodland Park Zoo, Seattle, Washington. Clinical signs were noted in seven of eight monkeys and included vesicles and ulcerations of the lips, tongue, and palate. Three affected monkeys died. Histologic examination of the lesions disclosed intranuclear inclusion bodies and electron microscopy revealed nucleocapsids with a herpesvirus-like morphology. Virus isolation from swabs and biopsy specimens of the oral lesions were neutralized with antisera to B virus at low dilutions (1:5), and were not neutralized with antisera to HSV-1, HSV-2, or SA-8. Serum samples were obtained from three monkeys during the acute and convalescent stages of infection: convalescent sera from all three showed neutralization at low dilutions against SA-8 and the original lesion isolate, one had a twofold rise in titer to B virus, and two had a twofold rise in titer to HSV. ELISA, Western blot, PCR, and in situ hybridization experiments are being developed to determine whether the causative agent is B virus, HSV, SA-8, or a previously unrecognized alphaherpesvirus. This episode illustrates the importance of being aware of herpetic outbreaks in different species of primates and the value of following recommended precautions to prevent the spread of diseases between nonhuman primates and humans.

Supported by Grant RR07019-10, RR01203, and RR050062

PS05  Spontaneous Seizures in Baboons (Papio cynocephalus): A Potential Primate Model of Epilepsy
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A group of baboons (Papio cynocephalus) in a large colony at the Southwest Foundation for Biomedical Research have typical grand mal seizures without any obvious provocation. Most of the affected animals also seem to have frequent episodes suggestive of partial complex seizures. Several animals have been monitored with stereotactically implanted deep brain electrodes with prolonged simultaneous video and EEG recordings to correlate the electrophysiologic substrate with the observed clinical seizure activity. The analysis of this data suggests that the origins of these spontaneously occurring seizure events lie in the mesial temporal lobe structures. Neuropathologic evaluation with serial section studies of the brains of these animals, including two that died in status epilepticus, showed no obvious pathology with usual light microscopic studies including Nissl and Golgi stains. GABA receptor binding has been studied in these animals in an attempt to determine the possible involvement of receptor systems in their seizures. The results of preliminary studies indicate lower levels of \(^{[3}H\) GABA receptor binding sites in the cerebral cortex of epileptic baboons relative to control animals. In contrast, GABA receptor binding was not significantly different in the cerebellum and caudate of control and epileptic baboons. We are studying the ligand binding to other sites of the GABA receptor complex and the N-methyl-D-aspartate (NMDA) receptor sites using \(^{[3}H\)MK-801. Analysis of the breeding colony data base has yielded preliminary evidence that the phenomenon may be genetically determined. We believe these animals will provide a valuable new primate model of spontaneously occurring epilepsy that should lead to advances in our ability to understand the pathophysiology of epilepsy and to develop better therapies for the disease in humans.

PS06  Environmental Enrichment Strategies for Baboons
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Successful environmental enrichment strategies vary greatly depending on factors such as age, social rank and housing. A colony of 88 baboons ranging in age from 2 to 15 years was offered a variety of environmental enrichment devices in both gang cage and individual cage settings. Enrichment devices consisted of food items (novel foods or novel presentation of food items), tire swings suspended from chains, chains suspended from the cage ceiling, bowling balls, a Primahedron, forage boards, puzzle feeders and Kong® toys. Without exception, all animals preferred enrichment devices which contained food. High ranking adults in gang cages obtained the most food, but in groups of juveniles, speed and dexterity rather than rank influenced the amount of food procured. Juveniles spent a large part of their time swinging from the chains and running through or jumping on the Primahedron. Rank did not affect the amount of time spent playing with “toys” in the juvenile gang cages. Adult males in gang cages rarely sat on or manipulated tire swings. The majority of adults in gang cages engaged in resting and grooming behaviors in the absence of food. All singly housed adults readily used the forage board. In contrast, puzzle feeders and Kong® toys containing frozen juice were used by only some animals. In summary, food items were preferred over other forms of environmental enrichment in both types of housing. Toys and moveable swings were used predominantly by juveniles in gang cages. Forage boards were used by all singly housed animals. These observed activities are consistent with natural foraging, resting, and grooming behaviors of free-ranging adult baboons, and the “play” behavior of juveniles.

PS07  The Effects of Food Treat Provisioning and Human Interaction on the Behavioral Well-being of Rhesus Monkeys (Macaca mulatta)
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The final regulations from the 1985 amendments to the Animal Welfare Act suggest human interaction with familiar personnel
as an example for providing environmental enrichment to nonhuman primates in laboratory settings. We studied the effects of providing a nutritionally balanced food treat to eight Rhesus monkeys (*Macaca mulatta*) combined with 2 minutes of human interaction per animal three times each week to determine if the incidence of abnormal behaviors would be reduced. The baseline (PRE), experimental (EXP), and postexperimental (POST) phases of the study were each 10 weeks long. Each animal was videotaped once a week for 35 minutes for a total of 5 hours per experimental phase. During the EXP phase, the monkeys were provided with 10 nutritionally balanced food treats, three times a week with the enrichment technician maintaining a submissive posture, lip-smacking, and speaking softly during the interaction period (videotaping occurred on the alternate days). A bar code scoring system was used to record the frequency and duration of the animals’ behaviors. The results of the study indicated a significant decrease in duration of overall abnormal behaviors during the EXP phase ($P < 0.03$). Results also indicated a significant increase ($P = 0.002$) in self-directed behaviors (abnormal and normal grooming) in the POST phase suggesting a “rebound” effect when the food treats and human interaction were removed. When analyzed as independent categories, duration of cage-directed, repetitive locomotion, and stereotypic behaviors significantly decreased during the POST phase suggesting a possible carryover of beneficial effect of food treats and human interaction after they were removed.

**PS09** Research Complications Associated with the Detection and Isolation of an “Orphan” Parvovirus

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Since the mid-1980s it has been apparent that many commercial and private rodent colonies are infected by atypical parvoviruses. Attempts to characterize and eradicate these “orphan” parvoviruses (OP) have been hindered by the inability to isolate the virus and by the absence of known effects on animal health or research results. Recently, serious problems developed in ongoing immunologic experiments at our institution. Investigation of the problem led to the conclusion that in vitro cultivated T-cell clones were affected by viral contamination. Although no clinical disease was detected, sentinel health monitoring results revealed evidence of OP infection in the barrier colony based on the presence of partial serum cross-reactivity to MVM. A coordinated follow-up plan was instituted to characterize the putative virus, to determine the prevalence of infection in the facility, and to identify strategies for protecting research projects at risk. Through these efforts we determined that the agent is a lymphotropic parvovirus related to, but distinct from, previously characterized MVM strains. In addition to interfering with in vitro lymphocyte manipulations, the virus has potential to be immunosuppressive and oncolytic. This parvovirus has now been isolated in vitro and should be added to the list of agents known to adversely affect biomedical research. Supported in part by USPHS grants CA-44372 and AI-29531 and Cancer Center Support Grant CA-14599.

**PS08** Sensory Approach to Enrichment Design Increases Use of Cage Space and Activity in Research Monkeys

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Singly housed cynomolgus (*Macaca fascicularis*) monkeys used in research can become bored. Developing a method of enrichment that consistently stimulates naturalistic behaviors requires an approach that effectively stimulates all, or most of an animal’s perceptual sensory modes. We used the OVA concept as defined by Fritz & Fritz [1979, 1986]; OVA is an acronym for olfactory, visual, auditory, and tactile stimuli. A clear, open-topped plexiglass box was designed using the OVA concept. The box is 12 × 12 × 2 inches; twenty 1/2-inch diameter holes were drilled in the solid bottom piece. The box was affixed to the cage top. Food items, colorful toy objects, and paper and cedar chips, were placed in the box. The intention is that a monkey will manipulate the small food items out through the holes and play with the toy objects, thus using scent, sight, and tactile responses. Use of natural locomotor activity is stimulated because the animal uses the vertical cage space. In a test to determine use of cage space, animals with the box device (n = 5), when compared to a non-box control group (n = 5), used the vertical cage space significantly more often (F = .01) than did the control group, which spent much of the time (u = 71%) sitting passively on the cage bottom. We conclude that using the box and the OVA approach effectively stimulated sensory modes, increasing use of vertical cage space, and therefore enriched the animals by allowing more time to be spent in naturalistic activities.

**PS10** Experimental Infection with a Putative Mouse Parvovirus Antigenically Distinct from Minute Virus of Mice

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Serologic evidence has revealed infection of rodents with one or more parvoviruses that bear hemagglutinin(s) distinct from those of known rodent parvoviruses. The newly recognized agent(s) has been called orphan parvovirus(es) (OPV) or parvovirus(es), other. A virus isolated by Fitch and coworkers at the University of Chicago and fitting the criteria for OPV (designated F-OPV) was tested in mice. Neonatal and weanling Senhar mice developed asymptomatic infection after intraperitoneal and/or oral inoculation. F-OPV was also infectious for DBA/2 and C3H/He mice. Virus was recovered from the spleens, kidneys, and lungs of suckling mice during the first week of infection and from the spleen of a BALB/c scid weanling mouse on day 21. In situ hybridization revealed that virus also infected lymph nodes and pancreas. Serum samples from recovered mice reacted with minute virus of mice (MVM) and F-OPV using an immunofluores-
cence assay, but reacted only with F-OPV by hemagglutination inhibition (HAI). Anti-OPV sera obtained from an unrelated colony reacted by HAI with F-OPV, but not with MVM. Virus was transmitted by neonatally-infected mice for up to 6 weeks and was also transmitted by soiled bedding from cages housing infected mice. These results should encourage further investigations of rodent OPV(s).

Supported by NIH grant RR00939 and a grant from the Charles River Foundation

PS11 Molecular Characterization of Newly Recognized Rodent Parvoviruses
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Two newly isolated viruses have been identified and propagated in mammalian cell cultures. One was isolated as a contaminant from mouse splenocyte cultures and the other from infected hamsters after neonatal tooth loss. Our objective was to identify these viruses and characterize them at the molecular level. Examination of viral antigens by immunofluorescence revealed that both isolates reacted with antibody directed against the nonstructural protein (NS1) of MVM, which is well conserved among the rodent parvoviruses, but failed to react with antibody directed against MVM-specific capsid protein. Serum antibodies produced against both viruses were negative in hemagglutination inhibition assays for the known rodent parvoviruses (MVM, H1, and RV). Gene sequences from both viruses were amplified by polymerase chain reaction using primers for the ATP binding region of NS1; however, primers for specific capsid genes in MVM and H1 failed to initiate amplification. Analysis of the viral DNA by Southern blots using a radiolabeled MVM genomic probe demonstrated that genomes of both viruses are single stranded DNA of approximately 5 kilobases, consistent with the known parvoviruses. These data suggest both viruses are parvoviruses distinct from MVM, H1, and RV. Further characterization of these viruses was performed by DNA sequence analysis of the ATP binding region of the NS1 gene, the small splice region located distal to the nonstructural genes, and the allotropic host-range determinant in the capsid gene. Homology between these regions and comparable regions of MVM and H1 was determined. The data generated by this study will aid in future molecular characterizations of these viruses and will lay groundwork for pathogenesis and epidemiologic studies to establish the importance of these viruses in biomedical research.

Supported by the Public Health Service Grant RR07004 and RR06215 and the COR Grant from the College of Veterinary Medicine, University of Missouri

PS12 Asynchronous Expression of Genetic Resistance to Mousepox in Major Target Organs
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Mousepox is a major threat to mouse colonies because most inbred mouse strains are susceptible to lethal infection. C57BL/6 mice are resistant to lethal mousepox because of mechanisms that are expressed in peripheral tissues, skin and regional lymph nodes, and in the spleen, a major target organ. The mechanisms that mediate genetic resistance are unknown but appear to be expressed by radioresistant cells or factors early in infection. It is also not known whether genetic resistance is expressed in the liver, the principal target of lethal infection. The temporal expression of genetic resistance in the liver and spleen was examined by comparing ectromelia virus replication in C57BL/6 and susceptible DBA/2 mice, six mice per strain, 1, 2, and 3 days after intravenous infection with the Moscow strain. Using slot blot hybridization, infectious center assay, and virus titration, ectromelia virus replication was found to be suppressed within 24 hours of infection in the spleens of C57BL/6 mice relative to DBA/2 mice but not until 72 hours after infection in the liver. Because α/binterferon is required for C57BL/6 mice to express resistance, the induction of α/binterferon in the spleens of C57BL/6 and DBA/2 mice was examined in five mice per strain, 1, 2, and 3 days after intravenous infection. DBA/2 mice produced more interferon and produced it earlier than did C57BL/6 mice. These results indicate that genetic resistance to mousepox is expressed in the liver later than in the spleen and that the quantitative induction of interferon is probably not the basis of genetic resistance.

Supported by NIH grant R24-RR02053

PS13 Experimental Sendai Virus Infection in Aged Mice
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Infant mice are more susceptible than young adults to severe Sendai virus (SV) pneumonia, but the effects of SV on aged mice is unclear. We reasoned that deterioration of host defenses with aging could alter the expression of SV infection in aged mice. To test for altered expression, infection was compared in 2-month-old (young) and 2-year-old (aged) BALB/c mice inoculated intranasally with SV. Young and aged mice were equally susceptible to infection. Mice of both ages were inoculated with a dose of SV that reproducibly induced mild bronchopneumonia in young mice and were examined at 6, 10, and 20 days after inoculation. The lungs of young mice had infectious viral antigen on day 6, whereas the lungs of aged mice had virus and viral antigen on days 6 and 10. Viral titers on day 6 were higher in aged mice. Virus and viral antigen were not detected in either group at day 20. Mild pneumonia developed in both groups by day 6. By day 10, pneumonia was more severe in aged mice. On day 20, repair was advanced in young mice whereas lesions persisted in aged mice. The results illustrate age-associated susceptibility to SV infection pneumonia and suggest SV in aged mice as a model for viral pneumonia of elderly humans.

Supported by PHS grant RR00393
PS14  Infectivity, Disease Patterns, and Serologic Profiles of Reovirus 1, 2, and 3 in Infant and Weanling Mice
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Reovirus infections are often detected serologically in rodent colonies without overt disease. The relative prevalence and significance of the three major reovirus serotypes that may occur naturally are unknown. The comparative susceptibility of 2-day-old and 3-week-old Sencar mice was examined following oronasal inoculation with reoviruses 1, 2, or 3. Blood and tissue samples were collected on days 3, 5, 7, 10, and 21 after inoculation for virus isolation, histology, and serology. Disease patterns in infant mice were distinctly different. Reovirus 1 induced mild focal necrosis of the liver and heart; reovirus 2 induced mild enteritis; whereas reovirus 3 was most virulent, inducing encephalitis and myocarditis. Weanling mice seroconverted, but did not develop lesions. Uniform transmission of virus to cagemates or mothers of infants did not occur, indicating low contagiousness of all three virus serotypes. The relative oronasal median infectious dose of the three virus serotypes was established in infant and weanling mice. Infant mice were susceptible to low doses of all three serotypes, whereas weanling mice became infected only with the highest dose. Sera from experimentally infected mice were tested in virus serotype-specific enzyme immunoassays. Cross-reactivity of antibody among the three virus serotypes was found, but antibody titers were always highest with homologous antigen. These studies confirm that infant laboratory mice are susceptible to infection with all three serotypes of virus; weanling mice are resistant to infection; the viruses induce different patterns of disease in infant mice; and infecting virus serotype can be distinguished serologically.
Supported by PHS grant RR00393

PS15  Genes Encoding Rotavirus Outer and Inner Capsid Proteins Do Not Determine Host Range Restriction and Virulence in Mice
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Rotaviruses are one of the leading causes of viral gastroenteritis in humans and animals worldwide as well as an endemic problem in laboratory mouse colonies. They are also an excellent model system to study the molecular basis for pathogenesis of a mucosal pathogen. To study the genetic determinants of rotaviral virulence and host range restriction (HRR) in the mouse model, a library of in vivo reassortant viruses was produced by coinfecting infant mice with the simian rotavirus RRV and the murine virus EDIM-Ward. Reassortant viruses were isolated by plaque-purifying the progeny virus obtained from mouse pup intestines on MA104 cells. These plaque-purified reassortants were evaluated for diarrhea dose 50 (DD50) and for their ability to spread and cause diarrhea in unoinoculated littermates. The parental RRV strain had a DD50 of 103 plaque-forming units per milliliter while the EDIM-Ward parental strain had a DD50 of less than 1 tissue culture infectious dose per milliliter. RRV never spreads from inoculated to unoinoculated littermates to cause disease. Twenty-two reassortants were tested. Of great interest was reassortant D1 which derived genes 4, 6, and 7 (encoding the outer and inner capsid proteins) from RRV. This virus had a DD50 similar or identical to EDIM-Ward and spread efficiently from inoculated mouse pups to unoinoculated pups. Previously, characteristics associated with rotavirus virulence and HRR had been linked to the 4th rotavirus gene and its product, VP4. From our data, we conclude that the major structural proteins, VP4, VP6, and VP7 are not primarily responsible for host range restriction or virulence in this mouse model.
Supported by NIH grant AI21362

PS16  Experimental Infection of Murine Cytomegalovirus in Severe Combined Immunodeficient Mice
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The susceptibility of T and B lymphocyte-deficient C.B-17 severe combined immunodeficient (SCID) mice to experimental murine cytomegalovirus (MCMV) infection was studied. Twenty-two, 9-week-old female SCID mice received 1 of 11 different doses of MCMV by intraperitoneal injection ranging from 117 to 120,000 plaque forming units. Time of death was recorded and tissue samples were processed for histology and for staining with the modified Steiner silver stain (MSSS). All mice died between 12 and 23 days postinoculation. Time until death was dose-dependent with the lowest dose group of animals surviving the longest. Splenic necrosis, hepatitis, glomerulonephritis, and pneumonia were noted. Adrenal glands exhibited mild multifocal suppurative adrenitis after high-dose injection, but were severely hemorrhagic and necrotic after low-dose injection. Infected cells were readily identified in salivary gland tissue after staining with MSSS. The results indicate that SCID mice are extremely susceptible to MCMV infection and that MSSS can be used to identify the virus in some tissues.
Supported by NIH grant 1-P30-AI28662

PS17  Murine Cytomegalovirus Tissue Distribution and Associated Histopathology in Severe Combined Immunodeficient and BALB/c Mice
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Cytomegalovirus infections are often more severe in immunocompromised hosts. Murine cytomegalovirus (MCMV) tissue distribution and related histopathology were studied in continued ▶
tissues of C.B-17 severe combined immunodeficient (SCID) and BALB/c mice. Twenty 9- to 11-week-old female SCID and BALB/c mice were infected by intranasal inoculation of 1000 plaque forming units of MCMV. Animals were sacrificed at 7-day intervals and selected tissues prepared for plaque assay, immunoperoxidase histochemistry, and histology. Plaque assay showed that viral titers in all tissues of SCID mice increased until these mice were moribund. Viral titers were undetectable or decreased after an initial increase in BALB/c mice and these mice remained clinically normal. Viral antigen was detected by immunoperoxidase histochemistry only in spleen cells of BALB/c mice; histologic lesions were not present in any tissues of these animals. Viral antigen was present in all organs examined in SCID mice, and tissue lesions included severe, hemorrhagic, necrotizing adenitis and multifocal hepatitis. MCMV infection was severe in SCID mice but was clinically inapparent in BALB/c mice.

Supported by NIH grant 1-P30-AI28662

PS18 Characterization of Acute Rat Virus Infection by In Situ Hybridization

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Acute infection of infant rats with rat virus (RV), a common parvovirus of rats, can be lethal or lead to persistent infection despite the development of anti-RV immunity. The early course of RV infection was examined in suckling rats to identify portals of entry, characterize the pathogenesis of infection, and search for sites of viral persistence. In situ hybridization with strand-specific probes, to distinguish virion from replicating RV DNA, was used in conjunction with virus titration. Results strongly suggested that virus entered through the lung and that early viremia led rapidly to pantoecic infection. Although cells derived from all three germ layers were infected with RV, cells of entodermal and mesodermal origin were the predominant targets. Infection of vascular endothelium was widespread and was associated with hemorrhage and infarction. Convalescence from acute infection was accompanied by mononuclear cell infiltrates at sites containing virion DNA. Four weeks after inoculation, viral DNA was still detected in endothelium, fibroblasts, and myofibers. Further examination of these cell types as sites of persistent infection is warranted.

Supported by NIH grant RR04047

PS19 In Vivo and In Vitro T Cell Immune Dysfunction During Infection with Murine Hepatitis Strain A59

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Mouse hepatitis virus (MHV) is known as a common natural pathogen in most animal colonies worldwide. Previous reports have documented that MHV can markedly modulate the course of responses to other agents indicating that inadvertent infection with MHV can potentially alter observations in a variety of biomedical research. We have used intranasal inoculation of MHV-A59 in BALB/c mice to study the effect of the viral infection on the immune system and observed that the animals underwent an acute phase of infection followed by a long-lasting immune dysfunction. In the first week, mice underwent rapid weight loss and their fur was ruffled. During that time, although the thymus became involuted (primarily a loss of CD4+ CD8+ cells), the proportions of T cell subsets and B cells in the peripheral lymphoid tissues remained normal. When spleen cells from infected mice were cultured with mitogen, proliferation to Con A (but not LPS) was reduced. Culturing cells with anti-CD3 mAb also caused significantly decreased responses. The results of cell mixing experiments with enriched T cell and accessory cell preparations indicated that the diminished proliferation originated in the T cell population. At later timepoints (days 35 and 100) when the animals no longer had acute clinical signs, a decrease in anti-CD3 mAb-mediated proliferation continued to exist. Notably, mice at this stage also rejected skin grafts 2 to 5 days slower than did normal mice. Additional in vivo responses are currently being examined by using T-cell-dependent and T independent antigens. Also, experiments are in progress to evaluate immune competency of animals infected for more than 100 days and to address the mechanisms of immune dysfunction at the different timepoints postinfection.

Supported by NIH grant RR04326

PS20 Posterior Paralysis as an Alternative to Death as an End Point in SCID Mice Bearing Disseminated Burkitt's Lymphoma

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Clinical signs offer an acceptable alternative to death as an end point for evaluating therapeutic regimens in animal models of human neoplasia. After intravenous injection of human Burkitt's lymphoma (Daudi) into SCID mice, neoplastic cells predictably proliferate in the lungs, kidneys, ovaries, spleen, and bone marrow. In the vertebrae, neoplastic cells replace marrow, and destroy the vertebrae by stimulating marked osteolysis of the vertebral bone. The cells then invade the spinal canal compressing the cord leading to posterior paresis and paralysis. In a titration experiment, the mean survival time of mice injected intravenously with 10^4 to 10^7 Daudi cells was dose-dependent. All animals developed paralysis before death. Plots of mean survival time and mean paralysis time against the log of tumor cells inoculated are linear and parallel. In this animal model, paralysis accurately predicted death, leading us to conclude that it can be used to evaluate therapy for xenografted tumors.

Supported by NIH grant RR00890
PS21  Serial Backcross Analysis of Rmp-2, a Gene on Mouse Chromosome 2 that Mediates Resistance to Lethal Ectromelia Virus Infection

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Most inbred strains of mice are susceptible to lethal mousepox infection. Multiple dominant genes impart resistance to C57BL/6 mice including some under gonadal control that are differentially expressed in females and males. Rmp-2 is a gonad-dependent resistance gene, linked to the Hc locus on chromosome 2. As a means of isolating Rmp-2 from other resistance genes and to develop a bilineal congenic resistant strain, C57BL/6 mice were serially backcrossed to susceptible DBA/2 mice using survival from ectromelia virus infection as the method of selection and Hc as a marker locus. Only male backcross mice were used to avoid passive immunity. Survival rates were greater than 70% in generations 1, 2, and 3, declined to 30% in generation 4, and remained near that level in generations 5 and 6. The number of surviving mice that carried Hc (C57BL/6) in each generation exceeded the number expected if Hc was a random passenger gene, indicating that Rmp-2 was being selected. Thirty-nine percent of survivors sired by mice that carried Hc did not carry Hc either because of crossovers between Rmp-2 and Hc or because an additional resistance gene, epistatic to Rmp-2, was also being selected. These alternatives were tested by typing surviving progeny of Hc-positive mice for alleles at the β2m locus, 26 centimorgans distal to Hc and near the predicted location for Rmp-2 if the latter was the only resistance gene being selected. Fewer survivors carried the C57BL/6 allele for β2m than for Hc, evidence that a second resistance gene was carried by most or all Hc-negative survivors. Survival rates of mice sired by Hc-negative survivors were significantly lower than those of mice sired by Hc-positive survivors, further evidence that two resistance genes were being selected. Susceptibility was tested in female and male mice in generation 7. Females that inherited resistance alleles from Hc-negative mice, unlike male mice, were fully protected. These results suggest that Rmp-2-mediated protection of male mice requires an epistatic resistance gene, presumably named Rmp-A, which can fully protect female mice in the absence of Rmp-2.

Supported by NIH grant R24-RR02053

PS22  Behavioral and Physiologic Effects of Wound Infection in Rats

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There is a common notion that rats are resistant to postoperative wound infection because many recover with little apparent pain and distress from surgery performed under nonsterile conditions. As a result, nonaseptic surgical techniques are often used in rat surgery. Our aim was to document that nonsterile surgery may cause wound infection and to determine whether this infection, which may be inapparent by casual observation, creates measurable changes in rat physiology and behavior. Rats subjected to craniotomies or laparotomies and inoculated with 10⁷ Staphylococcus aureus or Pseudomonas aeruginosa or sterile saline were tested for open field activity, freezing behavior, home cage behavior score, and wheel running activity. Physiologic indices determined included: lactate dehydrogenase (LDH), blood glucose, plasma fibrinogen, complete blood counts, wound histology scores, body temperature, and body weight. Although postoperative observation disclosed no clinical signs, rats inoculated with bacteria were significantly less active in the open field and exhibited shorter duration of freezing behavior. Plasma fibrinogen, serum glucose, total white blood cell counts, and wound histology scores were significantly altered in the bacteria-inoculated rats. These results underscore the need for sterile techniques in rat surgery to avoid experimental confounds.

PS23  Stimulation of Mucosal Immunity to Pasteurella multocida Heat-labile Toxin in Rabbits

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Elaboration of heat-labile toxin (PMT) is an important virulence factor in some isolates of Pasteurella multocida from rabbits. We hypothesized that local respiratory tract immunity plays a role in host defense of rabbits against PMT. To test this hypothesis, five groups of five male rabbits were immunized intranasally (IN) at days 0, 7, 14, and 21 with 5 μg heat-inactivated PMT (IPMT), 5 μg IPMT with 30 μg cholera toxin (CT), 25 μg CT, or phosphate-buffered saline (PBS). Serum was collected before initial immunization (day 0) and weekly thereafter. Nasal lavage samples were collected at days 0, 7, 10, 14, 17, 21, 24, and 28 and tracheobronchial lavage was performed terminally on day 28. Samples were assayed for specific anti-PMT IgG and IgA by ELISA. Groups of similar immunized rabbits were then challenged intranasally with 28 μg of native PMT after developing anti-PMT immunity (day 16), necropsied 7 days later along with 5 immunized nonchallenged rabbits, and histologic lesion severity judged on a numerical scale. Immunization with IPMT stimulated marked anti-PMT responses in nasal secretions (IgA), serum (IgG), and in tracheobronchial lavage samples (IgA). Coadministration of IPMT with cholera toxin enhanced anti-PMT titers in all samples. Challenge animals immunized with CT or PBS experienced anorexia and adiposis and had severe pneumonia, pleuritis, hepatic necrosis, and testicular atrophy. Rabbits immunized with IPMT or IPMT + CT remained clinically normal and had less severe lesions, particularly when CT was coadministered with IPMT. The results show that protective immunity develops after immunization with IPMT and that this protection is enhanced by coadministration with CT. Supported by the American Rabbit Breeders Association
PS24 Clinical Assessment of an Adjuvant Comparison Study in Rabbits for Polyclonal Antibody Production

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Four adjuvants were evaluated to elicit an acceptable polyclonal antibody titer and to compare the clinical assessment of each adjuvant in the rabbits. Using a 27-mer peptide conjugated to limulus hemocyanin, four adjuvants were compared: Freund's (complete, followed by incomplete), AdjuPrime (Pierce), MPL/TDM/CWS (Ribi), and TiterMax (CytRxCorp). The rabbits received 1 ml of the antigen/adjuvant given intradermally along the back at multiple sites, between 0.06 ml and 0.12 ml per site. Three rabbits per adjuvant group were injected with 200 µg of peptide followed by a booster injection using 100 µg of peptide 21 days later. Serum was collected for immunologic assays at days 30, 42, 50, and 72. Dermal inflammation responses using this peptide adjuvant combination in descending priority were: TiterMax, Freund's, Ribi, and AdjuPrime. Rabbits that received the antigen in phosphate buffered saline elicited no inflammatory response. The immunologic assays identified Freund's adjuvant producing an antibody titer tenfold higher than all other adjuvants. Although the quality of the IgG produced by all adjuvants was similar, the yields of IgG produced by the other adjuvants were significantly lower. These results confirm the merit of comparative adjuvant evaluation per antigen in rabbits.

PS25 The Use of Freund's Complete Adjuvant in Polyclonal Antibody Production

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The tissue destruction that results from using Freund's Complete Adjuvant (FCA) in antibody production has led to recommendations limiting the injectable volumes and routes of injection. The object of this study was to evaluate the antibody response and tissue damage resulting from the injection of a set dose of FCA-mouse IgG by a variety of routes and volumes per site. Nine groups of rabbits were injected with 100 µg of mouse IgG emulsified in FCA (0.5 ml total volume). Four groups received intradermal injections with volumes per site varying from 0.025 ml to 0.25 ml. Four groups of rabbits were injected subcutaneously with volumes varying from 0.05 ml to 0.5 ml per site and the final group was injected with 0.5 ml in a single intramuscular site. Antibody titers were determined weekly using an enzyme-linked immunosorbent assay (ELISA). Intradermal lesion sizes were measured weekly using calipers while subcutaneous and intramuscular lesions were measured with ultrasound and, in a few cases, magnetic resonance imaging. The rabbits were euthanized at 15 weeks and the lesions evaluated histopathologically. The antibody responses of the intradermally injected rabbits were significantly higher than those injected subcutaneously or intramuscularly. Ulcerations occurred at all intradermal injection sites but healed uneventfully. Granulomatous lesions remote to the initial injection sites were present in all rabbits injected subcutaneously or intramuscularly. Overall, the intradermal route of FCA administration produced the highest antibody response with the least tissue destruction. Supported by NIH grant RR06222

PS26 Comparison of a New Immunoadjuvant Used for Stimulating Antibody Production in Rabbits

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An alternative immunoadjuvant to Freund's complete adjuvant (FCA) is now commercially available which uses a water-in-oil emulsion and a new block copolymer. The new test adjuvant (TA) is reported to stimulate high, sustained antibody (Ab) titers without the severe inflammatory reactions sometimes associated with FCA. We compared FCA and TA used as adjuvants in conjunction with mouse thymocytes to immunize rabbits and evaluated the resulting purified antisera in terms of immunoglobulin yields, as well as in vitro and biological activity of the resulting T-cell antisera. Groups of three New Zealand White rabbits were immunized subcutaneously on days 0 and 14 using mouse thymocytes and either FCA or TA and were then plasmapheresed on days 21, 28, 35, and 42. The resulting plasma was pooled per group and purified by anion exchange column chromatography. Both groups of rabbits tolerated their respective adjuvant well, but surprisingly, there was slightly more purulence noted in the TA group. Immunoglobulin yields were greater in the FCA group, resulting in 1.69 gm/l purified IgG compared with 0.71 gm/l for the TA group. The cytotoxicity assay, an in vitro measurement of complement dependent Ab activity, demonstrated slightly higher Ab strength for the FCA antisera. Using a quantitative method (the Effective Dose 50% assay) to determine biologic potency, a marked difference between antisera was evident, with the FCA Ab being nearly twice as potent as the TA Ab. Our study raises questions as to whether TA is a worthy alternative to FCA in producing anti-T-cell Ab in rabbits.

PS27 Practical Considerations for Rodent Health Quality Assurance in a Large, Diverse Academic Vivarium

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The most common problem with rodent health surveillance is not interpreting diagnostic results, but the design and implementation of the overall program. The realities of an academic environment often include uncontrollable variables such as communication difficulties, unpredictable traffic of research personnel and animals, and the vagaries of academic
politics. Because of these variables, standard health surveillance sampling which may be appropriate for large breeding colonies or toxicologic exposure studies of defined duration are of little use. To minimize logistic and communication problems, and maximize the usefulness of diagnostic results from an often limited number of animals, we have implemented a health surveillance system based on the continuous regeneration of sentinel animals. Beginning with a breeding pair of inbred rodents originating from the barrier of origin for study animals, sentinels breed and raise one litter. At weaning, one male and one female from the litter become the new sentinel breeding pair to replace the original sentinels. The original breeding pair provide samples for virus and mycoplasma serology, microbiology, parasitology, and limited histopathology. The remainder of the litter provides samples for a more extensive histopathologic survey of target tissues as well as microbiologic and parasitologic examinations. Thus, small diagnostic accessions every 65 to 70 days allow us to accumulate much temporal information about a specific rodent colony. Together with high husbandry standards and a vendor approval process, this health surveillance system has resulted in a significant decline in both morbidity of experimental rodents and the impact of infectious disease on research results.

PS28 Effect of Buprenorphine on Lameness and Immune Response in Rats

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A model of chronic glomerular nephritis was induced in rats by footpad inoculation of kidney tubular brush border emulsified in Freund’s complete adjuvant (FCA). Inflammation of the tarsus and lameness, beginning 14 days post inoculation (PI), is a frequent complication. We sought to determine if oral administration of the opioid, buprenorphine, would alleviate lameness without diminishing immune response to the antigen. Ten days PI, 16 male Lewis rats, 8 weeks old, were given 1 ml of cherry-flavored gelatin twice daily. From PI days 15 to 27, eight randomly chosen rats had buprenorphine (0.4 mg/kg) added to their jello. An observer, blinded as to treatment, measured food and water consumption, weight gain, thickness of the tarsus from PI days 10 to 27, and ranked the degree of lameness (0-3). Delayed type hypersensitization to mycobacteria was assessed by comparing swelling 24 hours after inoculation of 25 μl of tuberculin into the right ear pinna and PBS into the left on PI day 26. Delayed type hypersensitivity was greater in treated rats as was swelling of tarsal joints (P < 0.05) and lameness (P < 0.05) but there was no quantitative difference in IgG deposits in kidneys. The results indicated that buprenorphine enhanced inflammatory response and was not a suitable treatment for alleviating complications in adjuvant-induced glomerulonephritis in rats.

Supported by NIH grant P40 RR1203

PS29 Comparison of Tribromoethanol, Ketamine/ Acetylpromazine, Xylazine/Telazol, Pentobarbital, and Methoxyflurane Anesthesia in HSD:ICR Mice

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Variation in the duration of surgical anesthesia in mice prompted an evaluation of various commonly used anesthetic regimens. Using bio telemetry technology, we evaluated the effects on temperature and activity of six different anesthetic regimens [2.5% tribromoethanol (Avertin), 0.16 ml/kg; ketamine/ acetylpromazine, 44 mg/kg; 0.75 mg/kg; high-dose (45 mg/kg; 7.5 mg/kg—a 6:1 ratio) xylazine/Telazol®; low-dose (7.5 mg/kg; 45 mg/kg—a 1:6 ratio) xylazine/Telazol®; pentobarbital, 60 mg/kg; and 4% methoxyflurane]. Six groups of four male HSD:ICR mice underwent one of the anesthetic regimens or an equivalent volume of saline, intramuscularly. Induction time and duration of anesthesia (loss of response to interdigital toe pinch) were evaluated. Methoxyflurane (0.66 min ± 0.29 SD) and both low-dose (0.66 min ± 0.29 SD) and high-dose (1.23 min ± 0.63 SD) xylazine/Telazol® combinations produced the shortest and most repeatable induction times. Ketamine/acetylpromazine- and pentobarbital-treated groups never completely lost interdigital toe pitch. Superior duration of anesthesia occurred in the xylazine/Telazol®-treated (97 min ± 30.6 SD, low; 36 min ± 19 SD, high) groups. A direct correlation exists between length of anesthesia and magnitude and duration of body temperature reduction. The results suggest low-dose xylazine/Telazol® is a good choice for prolonged anesthetic procedures in mice. Duration of anesthesia can be used to predict degree of hypothermia.

PS30 Correction of Lymphopenia by Injection of Diabetes-resistant Splenocytes Prevents Diabetes and Improves Breeding Efficiency in Diabetes-Prone BB/Wor Rats

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The BB/Wor rat is an animal model of Type 1 diabetes mellitus. Diabetes-prone (DP) rats are lymphopenic and >80% develop diabetes before 120 days of age. Diabetes-resistant (DR) rats are not lymphopenic and do not develop diabetes if maintained under VAF conditions. Since pregnant and lactating DP rats frequently develop diabetes, breeding colonies are difficult to maintain. Previous studies have shown that reconstitution of DP rats with DR splenocytes corrects lymphopenia and prevents diabetes. In this study, we wished to determine if injections of DR splenocytes would prevent diabetes and improve breeding efficiency. One spleen equivalent of fresh DR splenocytes was injected i.p. into
21 to 40-day-old DP rats. Spleen cell recipients (SCRs) were mated at 62 to 65 days and tested for diabetes until 150 days of age. The incidence of diabetes and litter size was compared with unmanipulated DP rats (controls). Diabetes among SCRs was 16% (97/600) versus 86% (817/949) in controls (P < 0.001). SCR females produced 7,669 pups, average litter size at weaning 7.7 vs 4,295 pups, average litter size at weaning 4.3 for controls (P < 0.001). The incidence of diabetes among progeny of SCRs and control matings was comparable. We conclude that reconstitution of BB/Wor DP rats with DR splenocytes prevents diabetes and improves breeding efficiency.

**PS31 Use of Vancomycin for Treatment of Clostridium difficile Enteritis in Syrian Hamsters**

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As part of an 18-month oncogenic study, more than 600 Syrian hamsters received daily gavage doses of an experimental miticide. Mortality associated with severe enteritis was noted beginning at 4 months of age and ranged from two to six deaths per month until about 10 months of age, when 38 deaths occurred in 1 month. Anorexic and postmortem findings were consistent with those reported for antibiotic-induced enterocolitis in hamsters. Culture of cecal contents revealed the presence of toxigenic Clostridium difficile in 93% of the samples analyzed. Since C. difficile in hamsters is reported to be susceptible to vancomycin, daily treatment at 20 mg/kg was initiated in the tenth month. Deaths associated with enteritis were eliminated within 3 weeks and treatment was continued for 3 months. Because withdrawal of vancomycin from hamsters with C. difficile is reported to result in high mortality, a trial withdrawal for a subset of 64 animals was initiated. C. difficile-associated enteritis recurred within 2 weeks and caused seven deaths. The animals were returned to daily vancomycin treatment for the remainder of the study with minimal clinical effects. In a separate blood level study, no detectable levels of vancomycin were present in the plasma of hamsters after oral dosing. These findings suggest that long-term vancomycin treatment does not systemically expose hamsters, but can protect against fatal C. difficile enteritis without major side-effects.

**PS32 Hereditary Renal Carcinoma in Long-Evans Rats: A Unique Familial Cancer Model to Study Genetic/Environmental Interactions**

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We have characterized a familial cancer syndrome in rats in which renal cell tumors develop due to the inheritance of a mutation in a tumor susceptibility gene. Rats that inherit one mutant copy of this gene develop bilateral multifocal renal cell carcinoma (RCC) at a young age. When homozygous, the mutation is lethal during embryogenesis, indicating that at least one normal copy of this gene is required for normal growth and development. In addition to RCC, splenic vascular and uterine sarcomas develop as second primary neoplasms later in life. The primary renal cell tumors and cell lines derived from them share molecular and cytogenetic alterations with human RCC, thus providing a useful animal model for this important urologic malignancy in humans. We have shown that this animal model is a useful tool to study the role of tumor susceptibility genes in chemical carcinogenesis. Because the genetic mutation is not yet identified, and because it is lethal in the homozygous state, this animal model presents a significant challenge for colony management. Breeding stock must be identified by the early detection of renal neoplasia as a phenotypic marker of the mutation. Detection of these tumors requires laparoscopy, imaging techniques, or unilateral nephrectomy. Degenerative nephropathy shortens breeding life and is exacerbated in this strain by unilateral nephrectomy. Carcinogenesis bioassays with this mutant rat model require the breeding of wild-type animals, a difficult task without genetic markers. Rodent small colony management becomes of paramount importance in the establishment and use of specialized genetic models for carcinogenicity studies.

**PS33 Age-related Immune Response and Lesions in C3H/HeJ Mice Inoculated with Borrelia burgdorferi**

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The host immune response to B. burgdorferi, the causative organism of Lyme disease, is poorly understood. We characterized and correlated the immune response and lesions in mice infected with B. burgdorferi (Strain 297 human isolate) at different ages. Three groups (18 to 26 mice/group) of C3H/HeJ mice, aged 3 days (group 1), 21 days (group 2), and 54 days (group 3), were inoculated i.p. with 3×10⁶ B. burgdorferi and euthanized at 2, 4, or 5 weeks postinjection. B. burgdorferi organisms were isolated from the spleen, urinary bladder, heart, joint and back, and ear skin of infected mice. The isolation rates were 70% (76/108), 82% (128/156), and 80% (111/138) for groups 1, 2, and 3. The organism was recovered most frequently from ear skin and least frequently from spleens. Redness and swelling of bicipital joints were observed in 89% (16/18), 100% (26/26), and 13% (3/23) of groups 1, 2, and 3. Histopathologically, groups 1 and 2 had severe supplicative periartritides and tendonitis and mild synovitis. The lesions in group 3 were much less severe and suppurrative. The antibody response measured by ELISA and Western blot analysis was age dependent, with the lowest response in group 1 at 2 weeks postinjection and the highest response in group 3 at 6 weeks postinjection. Control mice were negative for B. burgdorferi organism, lesion, and antibody. These results show that lesion development is correlated inversely with high antibody response. Supported by NIH grant RR00890
PS34  A Rabbit Model of Quantitative Bleeding Time Measurement: Elevation with Antithrombotic Therapies  
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In order to better predict the bleeding consequences of in vivo administration of antithrombotic agents, we have developed a bleeding time model in anesthetized rabbits that closely simulates clinical bleeding time measurement (BTM). Anesthetized rabbits were subjected to a series of cuticle and ear incision BTMs. BTMs were accomplished by either cutting a hind foot toe nail 5 mm from the cuticle base or inserting a #11 scalpel blade (1 cm wide) through the middle portion of the ear transecting the smaller blood vessels between the central ear artery and marginal ear vein. Blood from the incisions was gently absorbed onto BT blotting paper (surgiclut) by capillary action every 30 seconds. Cessation of bleeding was determined in two ways: complete cessation representative of complete hemostatic, fibrin-rich clot formation and when more than a 90% reduction in blood flow had occurred representative of initial hemostatic platelet-rich plug formation. Treatment was administered via the contralateral marginal ear vein and blood samples were obtained via femoral vein catheter. Blood samples were processed for measurement of hematologic and coagulation parameters. Ex vivo platelet aggregation with platelet-rich plasma was unaffected during the experimental time course. BTMs were accomplished at 0, 15, 60, and 90 minutes after steady-state administration of heparin at 100 U/kg bolus + 2 U/kg/minute constant infusion, a dose which completely inhibits venous thrombus formation in rabbits, or with an equivalent volume of phosphate-buffered saline. Control infusion of phosphate-buffered saline (n=6) showed a cuticle BTM of 2.7 ± 0.3 minutes by blood flow and 3.6 ± 0.5 minutes by complete cessation, and an ear BTM of 2.3 ± 0.4 minutes by blood flow and 2.7 ± 0.5 minutes by complete cessation. Infusion of heparin (n=6) showed a cuticle BTM of 11.8 ± 3.3 minutes by blood flow and 18.9 ± 3.5 minutes by complete cessation, and an ear BTM of 6.3 ± 1.4 minutes by blood flow and 8.9 ± 1.9 minutes by complete cessation. This model has been shown to be both qualitative and quantitative in its ability to measure elevation of bleeding time after administration of antithrombotic agents.

PS35  Development and Implementation of a Comprehensive Biosafety Program  
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To ensure compliance with federal, state, and local regulations, and institutional health and safety policies, a comprehensive biosafety program was developed and implemented at Stanford University. The program was initiated by a review of all research and teaching activities involving the use of biohazardous agents and recombinant DNA experiments by the Institutional Biosafety Committee. We defined biohazardous agents as any infectious agent that is either: (A) a Class 2, 3, 4, or 5 bacterial, fungal, parasitic, viral, rickettsial or chlamydial agent; or (B) other infectious agent, toxin-producing agent, either isolated naturally or constructed by recombinant DNA, that has the potential to cause disease in healthy individuals, animals, or plants. Elements of the biosafety program included: 1) training; 2) mechanisms to report workplace hazards; 3) systems for laboratory personnel to conduct regular, periodic inspections of workplaces to identify and evaluate workplace hazards and unsafe work practices; 4) means of correcting discovered hazards and/or protecting individuals from the hazards; and 5) record keeping systems to document compliance with statutes, regulations, and standards. Our program, headed by the biosafety officer with strong institutional support for environmental health and safety, has met regulatory requirements and provides a safe work environment for employees and students. The program serves as an example for creating comprehensive biosafety programs at other institutions.

PS36  Development of an Effective Safety Program in a Laboratory Animal Environment  
CC Hayden, HJ Klein, G Semler  
Merck Research Laboratories  
A program to improve the safety performance of our large laboratory animal resources department was undertaken in response to a trend toward an increasing number of accidents during several years. An analysis of the problem led to development of a comprehensive program encompassing all aspects of laboratory animal science, from general safety awareness to handling a wide variety of species including nonhuman primates, biosafety, radiation safety, and chemical safety. The program was designed to involve all levels of animal care, technical, veterinary, and management staff, and was headed by a two-person team with direct responsibility, accountability, and authority for its function. Short-term goals of enhancing safety awareness were met through formation of an active safety committee, animal facility inspections, weekly training, competitions, employee recognition, and incentives. The long-term goal of reducing accident rates was accomplished through employee participation in the development of operating procedures and involvement in animal facility and equipment designs. As a result, the overall per capita accident rate decreased by 50%, and lost time accidents decreased 100%, even while the total manpower roster increased by 14%. Increased employee involvement and the team approach taken by veterinary, management, and animal care staffs will result in definitive improvements in safety performance.

PS37  Operating Theater Pollution: A Case Study  
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Acute or chronic exposure to waste gases of inhalant anesthetics may contribute to dysfunction of a variety of organ systems.  
continued →
Waste gases were monitored in two surgery rooms during isoflurane and nitrous oxide anesthesia in guinea pigs. Anesthesia was induced and maintained by administering 1 to 4% isoflurane in 2 liters per minute total flow of a 1:1 mixture of oxygen and nitrous oxide using a face mask and coaxial circuit. Room air was collected for 38 minutes by monitors worn by both surgeon and anesthesiologist; an additional collection of room air was made from a remote site. Isoflurane in air was adsorbed on charcoal tubes during the sampling period and then analyzed by NIOSH Method 1003. Real time levels of nitrous oxide were measured by infrared spectrophotometry at the surgeon’s breathing zone, nitrous oxide source and distribution system, and various room locations. Isoflurane exceeded the maximum recommended exposure (MRE) level of 2 parts per million (ppm) for the surgeon in one of the rooms. Nitrous oxide levels at or above the MRE of 25 ppm were detected at the face mask, surgeon’s breathing zone, and exhaust hose connections in both rooms. Strategies for pollution control (e.g., changes in room ventilation, replacement of faulty components) are discussed. The findings underscore the importance of quality control measures for surgical facilities where inhalants are in use.

Supported in part by grants RR01046 and RR07036

PS38 An Allergy Testing Program at Hazleton Laboratories

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Allergies to laboratory animals are a well recognized problem for people working in laboratory animal facilities. Measuring and monitoring workers' sensitivity are important from both a human safety and legal disability perspective. Since 1988, Hazleton Laboratories has conducted yearly skin test screens on all staff who come in contact with laboratory animals. Through the 1990 testing period a total of 242 individuals were allergy tested for an accumulated number through the 3-year period of 408 tests. Results indicated that 35 (14.5%) of those tested were allergic to one or more animal species. Most of these were also allergic to at least one of the common allergens such as ragweed, house dust, trees, grasses, and molds which were tested simultaneously.

The most commonly detected response by skin test was to rats followed by cats, dogs, rabbits, mice, guinea pigs, and cattle. Retesting of 138 staff in 1991 indicated that 21 (15.23%) additional animal allergies developed during the past year. Testing conducted in 1991 for the first time on 56 new staff indicated that 14 (25%) were allergic to one or more animals. These data have been used to quickly develop a program to prescreen selected applicants before employment. An additional result is the benefit to human health in effectively identifying and providing medical information for employees with developing or increasing sensitivities.

Supported by Hazleton Laboratories, Madison, WI

PS39 Experimental Engineering Laboratory to Evaluate Environmental Characteristics of Animal Rooms

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During the design phase of a new pharmaceutical research facility, an experimental engineering laboratory was constructed to provide full-scale rodent, rabbit, dog, and primate animal room designs. The objective was to optimize the distribution of air within the animal rooms. Different engineering design features included: air exchange rates per hour; the number, location, and type of supply and exhaust diffusers; and the effect of cage arrangement on room air distribution. Each room had either cages or mock-up cages and heating coils were used to simulate total animal heat output. The rooms were carefully calibrated for air balancing and each room could be adjusted to provide either 15, 20, or 30 air changes per hour. Multiple exhaust ports could be opened or closed so as to provide a variety of configurations per room. Likewise, the supply air could be adjusted to provide air along the length of the room or perpendicular to it. Detachable flat or convex supply diffusers were also evaluated. During the test periods, measurements were recorded in approximately 70 cubic grids per room: temperature, velocity, and direction of air. Fog tests were also accomplished, documenting air clearance time. This Experimental Engineering Laboratory provided valuable final design criteria for the new facility.

PS40 Validation of Newly Constructed Animal Facilities

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The physical plant and supporting equipment in newly constructed or renovated animal facilities need to be fully tested to assure animal health, quality of data, and compliance with animal welfare and good laboratory practice regulations. In 1990 and 1991, Hazleton Laboratories in Madison, Wisconsin added a 134,000 sq ft addition. Approximately 50,000 sq ft were animal rooms. During construction, a plan was made and subsequently implemented to fully test the facility and support systems before studies were begun. Validation included lighting, plumbing, and electrical systems, emergency electrical generators, automatic water systems, heating/ventilating/air conditioning systems, cage sanitation equipment, safety hoods, pest control, computerized data acquisition systems, and necropsy facilities. Calibrated instruments were used when quantitative data were required for validation. In addition, 4-week studies were done of rats, mice, and dogs to assess effects on study animals. Complete facility inspections were done by the Institutional Animal Care and Use Committee, and notification was sent to the United States Department of Agriculture and American Association for Accreditation of Laboratory Animal Care. A comprehensive
report was prepared and made available to clients. The planning and execution of the validation effort and supporting documentation assured that quality care and compliance were an integral part of completing the construction process.

Supported by Hazleton Laboratories, Madison, WI

PS41 How to Determine and Justify Animal Facility Staffing Levels

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The 90s are proving to be times of economic concern for administrators at academic institutions. With research budgets being critically evaluated and reduced by many funding sources, it is mandatory that animal facilities be cost effective. The highest level of animal care must be provided at the best value. Salaries are one of the highest controllable cost items at animal facilities. To control that expenditure, administrators need to staff animal facilities at the level appropriate for the workload. To determine that level, a normative study was done using the Delphi engineering technique, verified with time checks, at both a centralized and decentralized animal facility. For each animal species, a standard time for animal care was developed by determining the time necessary to complete a well-defined list of weekday and weekend care tasks. Consideration was given to species- and choredependent delay and fatigue time, as well as the skill and effort of the animal technician. Indirect times for janitorial chores, continuing education, meetings, and investigator interactions were also included as part of the staffing formula. The study resulted in a series of time standards which were used to determine the appropriate staffing of animal technicians. Since the time standards were established based on animal species, per diem rates could be more accurately adjusted to cover salary expenses. The time standards established in this study could be used at any facility as a baseline to gauge the appropriateness of the current staff level, while the methodologies could be used to modify these standards for specific institutional needs.

PS42 The Development of a Comprehensive Training Program for Laboratory Animal Resource Personnel

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New technology in the field of laboratory animal science created a need to establish a comprehensive training program for all laboratory animal care personnel. The need for this program was reinforced with the Laboratory Animal Resources Department's transition to specific pathogen free rodent facilities, revisions to the Animal Welfare Act, and the department's anticipated occupation of a new animal facility in 1995. Historically, most training for new laboratory animal personnel was provided by the "buddy system," which involved the use of existing personnel with minimal instructions from supervisors. An evaluation of this type of training identified several areas of concern: accountability, inconsistencies in standard operating procedures, and a decrease in productivity and quality of care and services. In 1991, a joint committee of supervisors and animal caretakers worked as a team to develop a training program. The committee used a process called "Training for New Technology" (TNT), which evaluated the present skill levels and competence of our personnel in existing technology and future technologic skill requirements. The scope of TNT involved identifying the skills necessary to perform the job, assessing the current skill levels, identifying gaps in current knowledge and skills, identifying future technical skills, and finally, designing an implementation strategy to ensure skill development. The project required 1 year to complete and was followed by an evaluation to determine the program's effectiveness. The conclusive results are that the new departmental training program provides a thorough and effective method for training in basic animal care.

PS43 A Comprehensive Training Program for Animal Technicians in the Pharmaceutical Environment

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A quality training program has been operational at Sterling Winthrop Pharmaceuticals Research Division (SWPR) at Alnwick, England for more than 8 years. During that time the program has undergone constant development to ensure that customers' needs, both human and animal, were met. The program was designed to ensure that all personnel caring for, or using animals in nonclinical laboratory studies did so according to rigorous inhouse standards, fully compliant with the Good Laboratory Practice Act and with full regard for animal welfare. Training is conducted by a limited number of experienced designated trainers who are responsible for organizing training seminars and standardizing training topics. Training is supported by standard operating procedures and comprehensive training manuals. All experimental procedures are reviewed regularly and follow-up training is ongoing. A standardized system of training documentation has been adopted, enabling instant examination of competence and training status. The system is flexible enough to cover all aspects of animal care and use. Further individual training records are easy to maintain. This system is currently planned for adoption on an international basis to ensure harmonization of practices worldwide.

PS44 Magnetic Resonance Imaging: Basic Introduction, Safety Precautions, and Representative Research Images for Laboratory Animal Technicians

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Magnetic resonance imaging (MRI), being a noninvasive means of obtaining images of superior clarity for diagnosis and research, is an important asset to the medical field. Understanding the basic continued ▶
principles of image acquisition and the variety of image parameters available can be a valuable tool for research technicians. Definitions of some of the more common terms used when discussing MRI, including TR, TE, T₁, and T₂ will be provided. The magnetic field generated by the MRI machine poses an unusual safety hazard that many technicians may not be familiar with. Objects can be drawn into the magnetic field with great force resulting in injury to people or animals and damage to the magnet. Basic knowledge of the field's strength can prevent potential problems. By being familiar with animal anatomy, laboratory animal technicians can be very helpful in directing the course of scanning, thereby saving time and acquiring better images. With MRI becoming an increasingly valuable instrument to researchers, it is necessary that laboratory animal technicians be informed and aware of the important role that is available to them.

PS45  Respiratory Disease Attributed to Bordetella bronchiseptica in a Colony of Domestic Ferrets (Mustela putorius furo)

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Domestic ferrets are susceptible to respiratory infection with some human viruses, and affected animals frequently have secondary bacterial infections. An outbreak of respiratory disease occurred in 10 to 16-week-old ferrets in a colony of approximately 2000 6-week to 9-month-old animals. Animals affected early in the epidemic responded to penicillin, but some groups developed a more severe persistent syndrome of sneezing, mucopurulent nasal discharge, anorexia, and weight loss. B. bronchiseptica, the only pathogen identified, was cultured from the nasopharynx, trachea, and lung of several animals. Central nervous system signs occurred in some animals without neurologic lesions. Affected ferrets recovered a few days after treatment with chloramphenicol was started. When subsequent groups of young animals entering the housing unit were given two doses of B. bronchiseptica, no disease occurred in these ferrets, although new cases continued to develop in older, previously unaffected animals. Research or pet ferrets are at risk of developing severe respiratory disease when exposed to B. bronchiseptica.

PS46  Chlamydia Trachomatis Strain FeCo Serovar E Isolated from Proliferative Colitis in Ferrets

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Profound diarrhea, associated with hyperplastic intestinal cells containing intraepithelial campylobacter-like organisms (ICLOs) in a variety of mammalian hosts, has been studied for 6 decades, but an etiologic agent has not been isolated and characterized. In our study, typical chlamydia inclusions were visible by light microscopy in ferret proliferative intestinal tissue adjacent to and distinct from the ICLO. An intracellular bacterium isolated from proliferative bowel tissue of two ferrets was shown by a variety of techniques to be Chlamydia trachomatis and is designated C. trachomatis strain FeCo. 16SrRNA sequence determination and comparison indicated that C. trachomatis strain FeCo is 99.4% similar to C. trachomatis L2/434 and 95.2% similar to C. psittaci. McCoy cells infected with C. trachomatis strain FeCo demonstrated brightly fluorescent inclusions when examined with fluorescein-labeled species-specific monoclonal antibodies to C. trachomatis serovar E. The chlamydial inclusions in tissue culture also stained with iodine which is a unique characteristic of C. trachomatis. Restriction enzyme analysis using Hinc II, Pvu II of two isolates of FeCo and C. trachomatis, serovar E (ATCC #VR-348B) differed by one or two bands with each restriction enzyme. This is the first report of a natural infection of C. trachomatis in animals. Its role in proliferative bowel disease of animals, potential as a zoonotic disease, and the use of C. trachomatis infection in ferrets as an animal model to study related diseases in humans requires further study. Supported by NIH grants RR01046 and RR07036; NCI grant CA26731; NIDR grants DE08303 and DE04881.

PS47  The Effect of Anesthetics on Hematology Parameters in Ferrets

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Recent studies of isoflurane-anesthetized ferrets demonstrated significant reductions in hematocrit. This phenomenon has not been previously described during isoflurane use in ferrets or other species. We evaluated the effect of anesthetic agents and regimens on hematocrit and characterized the magnitude and kinetics of this effect. Six ferrets were anesthetized for 45 minutes at two-week intervals with isoflurane alone and isoflurane with atropine as a premedicant and 2% xylcaine viscous as an endotracheal tube lubricant. Blood samples were collected for complete blood count at pretreatment and 15, 30, and 45 minutes after induction of anesthesia, and 45 minutes after discontinuation of anesthetic administration. Complete blood counts were also obtained at various intervals from ferrets anesthetized with ketamine and xylazine. During isoflurane anesthesia, significant reductions in hematocrit and total protein were observed at the 15 minute postinduction timepoint. Hematocrit decreased from 52.0 ± 2.5 to 33.3 ± 4.4%, and total protein decreased from 7.3 ± 0.5 to 5.8 ± 0.4 g/dL. These parameters remained relatively unchanged for the duration of anesthesia and then rebounded after 45-minute anesthetic recovery. Decreases in hematocrit and total protein of lesser magnitude were also observed after ketamine/xylazine anesthesia. In conclusion, isoflurane, and to a lesser extent, ketamine/xylazine anesthesia in ferrets was associated with reductions in hematocrit and total protein which appear to be rapid in onset and reversible. Further studies are ongoing in attempts to elucidate the mechanism of the observed effect. Supported in part by NIH grants RR01046 and RR07036.
PS48 Estimation of Glomerular Filtration Rate and Evaluation of Renal Function in Ferrets (Mustela putorius furo)

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The usefulness of renal function tests used in other species has not been evaluated in ferrets. In the clinical setting, blood urea nitrogen and serum creatinine tests have provided indirect characterization of glomerular filtration rate (GFR), but these values may be influenced by extra-renal factors and only document decreases in GFR when 50 to 75% loss of renal function occurs. Three methods of determining GFR were performed in adult ferrets aged 9 months to 7 years. Endogenous creatinine clearance was determined for 29 ferrets by using serum and urine creatinine values obtained during 24- and 48-hour collection periods in metabolic cages. Radioactive inulin and exogenous creatinine were administered to 12 female ferrets by constant intravenous infusion during isoflurane anesthesia. Serial 20-minute urine collections together with midipoint serum samples provided measures for clearance calculations for these substances. Mean endogenous creatinine clearance was 2.50 (S.E. 0.28) ml/min/kg body weight. There was no significant difference (P > 0.05) between the 24- and 48-hour clearance rates. Mean inulin clearance was 2.66 (S.E. 0.24) and mean exogenous creatinine clearance was 2.80 (S.E. 0.36) ml/min/kg body weight. Analysis of variance using least squares means adjustment did not yield any significant differences (P > 0.05) between mean clearance values for each method. Infused inulin clearance is generally the preferred method for GFR calculation in mammalian species. Inulin is freely filtered by the glomerular capillary membrane, is an inert substance, and does not undergo renal tubular resorption or excretion. The results of this study indicate that endogenous and exogenous creatinine clearance both provide reliable estimates of GFR in ferrets when compared with inulin clearance. The GFR values obtained in our study closely approximate the values obtained using these methods for dogs and cats. Supported in part by grants RR01046 and RR07036

PS49 High Prevalence of Helicobacter-associated Gastritis in Purpose Bred Beagles

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It is known that some dogs' stomachs are colonized by at least two types of spiral-shaped bacteria — Helicobacter felis and Gastrospirillum hominis. Both of these bacteria have characteristic spiral morphology and are strongly urease-positive. Helicobacter felis have been cultured and identified by standard biochemical and molecular methods; G. hominis has not been cultured but preliminary RNA sequence data suggest it is a Helicobacter species. Stomachs from two groups of beagles (n=22, n=10) from different commercial sources were assayed at necropsy for the presence of Helicobacter-associated gastritis. Each stomach was opened along the greater curvature and urease mapping and phase microscopy performed on the cardia, fundus, body, pyloric antrum, and pyloric canal. Selected stomachs had mucosal scrapings blotted in Whatman filter #541 paper for bacterial analysis using Helicobacter-specific DNA probes. All 32 dogs were heavily colonized with gastric spiral organisms based on positive urease results, phase microscopy, and identification of spiral organisms in Warthin-Starry-stained stomach tissue. Helicobacter genus-specific DNA probes confirmed the identity of the organisms. Colonization was more pronounced in the cardia, fundus, and body of the stomach. Histologic lesions associated with the gastric spiral bacteria consisted of lymphoid follicles in selected regions of the stomach, particularly in the acrdiac and antral mucosa. A relatively diffuse lymphocytic infiltrate was also present in the subglandular region of the stomach. The uniform presence of Helicobacter-associated gastritis in dogs indicates that some of the pathophysiological responses of the canine stomach noted in the veterinary and toxicology literature need reassessing. Supported by NIH grants RR01046 and RR07036; NCI grant CA26731, NIDR grants DE08303 and DE04881.

PS50 Colonization of Cats by Potentially Zoonotic Helicobacter-like Organisms: Implications for Animal and Public Health

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The bacterial genus, Helicobacter, includes several species which colonize the gastric mucosa of mammals. Natural and/or experimental gastric pathology has been correlated with colonization in humans and a wide variety of animal species. Historical reports in the literature have suggested that a high percentage of cats are colonized by large, spiral, Helicobacter-like organisms (HLOs). One of these organisms has been isolated on artificial medium (Helicobacter felis) and has been shown to cause gastritis in gnotobiotic dogs. We assessed the prevalence of HLO colonization in random-source cats based on urease assay and histologic examination, and to determine if any associated pathology was present. Results obtained to date suggest that over 65% of cats are colonized and that such colonization is associated with histologically evident chronic gastritis. Although further study is needed to determine what specific role helicobacters play in feline gastrointestinal disease, these results indicate that Helicobacter colonization should be considered when interpreting histologic findings in feline gastric tissues. The high prevalence of infection is also important because cats have recently been implicated as a potential reservoir for human infection by Helicobacter-like organisms.
PS51 Left Ventricular Hypertrophy in a Closed Colony of Cats

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Left ventricular hypertrophy is a common clinical condition in both cats and humans. It can be a primary idiopathic condition or secondary to a variety of metabolic, infiltrative, or morphologic causes. The primary form of the disease, hypertrophic cardiomyopathy, represents approximately 35% of reported cardiac disease in cats, and is a common cause of sudden death in humans. Recently, a defect in the beta-myosin heavy-chain gene has been recognized as a cause of hypertrophic cardiomyopathy in one human family. During routine clinical examination, heart murmurs were detected in 14 of 21 Persian ancestry cats which were either hetero- or homozygous carriers of Chediak-Higashi Syndrome, an autosomal recessive disease characterized by a lysosomal fusion defect that has not been previously associated with heart disease. Examination of colony health records revealed many unexplained anesthetic deaths. A foundation breeding male had experienced thrombosis of the iliac aorta associated with left ventricular hypertrophy. Echocardiographic evaluation of the remaining colony members indicated that 13 animals had mild-to-moderate left ventricular hypertrophy; animals as young as 3 months of age had evidence of disease. Serum biochemistries, complete blood counts, and serum thyroid hormone levels were within normal limits in 12 adults which were assayed. Indirect doppler blood pressure measurements were normal to slightly elevated relative to published normal values. This colony of cats represents a population of animals with apparently inherited left ventricular hypertrophy and may serve as a model of the disease in humans.

PS52 Assisted Reproduction in Feline Animal Models of Human Disease


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Domestic cats are used as a model for human genetic diseases, but propagation often is compromised by impaired reproductive performance and physical limitations. Production of cats expressing recessively inherited defects usually is achieved by heterozygotic matings. In this study, artificial breeding methods including electro-ejaculation, artificial insemination (AI) and semen cryopreservation were used to determine the fertility of feline models. Semen was evaluated in nine types of models: mucopolysaccharidosis, types I (MPS-I) and VI (MPS-VI); α-mannosidosis; Gm1 gangliosidosis; Ehlers- Danlos syndrome; porphyria; spasticity syndrome; Chediak-Higashi syndrome; and Niemann Pick-Type C. A total of 104 ejaculates was collected from 19 cats affected by genetic disease (n=59 ejaculates) and 17 cats that were unaffected-carriers (n=45 ejaculates). After assessing sperm motility, concentration, and morphology, semen was washed in Ham's F10 medium and resuspended in either Ham's F10 or egg-yolk/lactose/glycerol diluent for pellet freezing. Forty females were treated with hormones and inseminated in utero with fresh (n=34) or frozen-thawed (n=6) sperm. All males produced spermic ejaculates with high rates of mean percent sperm motility (range, 32 to 81%). A wide range in mean sperm concentration (20 to 650x10⁶/ml) and normal sperm/ejaculate (30 to 66%) was detected among cat groups. A lower proportion (P < 0.05) of normal sperm was detected in affected MPS-I (49%) and MPS-VI (30%) males compared with unaffected-carriers (MPS-I, 60%; MPS-VI, 52%). AI procedures in the MPS models (n=32) resulted in 13 pregnancies (40.6%). Pregnancy rate was lower (P < 0.05), however, using affected MPS sperm (6 of 20, 30%) compared with carrier MPS sperm (7 of 12, 58.3%). Both AI attempts using sperm from affected males with either porphyria or spasticity syndrome resulted in pregnancies. Frozen-thawed sperm from six MPS males revealed a 79% recovery of sperm motility (prefreeze, 78%; postthaw, 62%). Pregnancies were achieved in two of six females (33.3%) inseminated with frozen-thawed MPS sperm. The results of this study indicated that all nine feline models produced sperrmic ejaculates; pellet freezing resulted in a high recovery of postthaw motile sperm; and intrauterine AI using fresh or frozen-thawed sperm resulted in pregnancies, proving the efficacy of artificial breeding in feline models with genetic diseases.(NIH grants RR00045, DK25759, RR02512; Lucille P. Markey Charitable Trust)
P01 Development of a Competitive Inhibition ELISA for Identification of Bacillus piliformis Isolates in Laboratory Animals

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We have developed a group of monoclonal antibodies that specifically react with isolate-specific flagellar epitopes of Bacillus piliformis. In the study, we devised a solid phase ELISA competitive inhibition assay that is based on the capacity of antibodies in serum from infected animals to specifically block the binding of monoclonal antibody to flagellar epitopes. Serum from experimentally infected laboratory animals inhibited monoclonal antibody binding (30 to 95% inhibition) to the flagellar epitopes of the homologous B. piliformis isolate confirming the assay's specificity; serum from noninfected animals or animals infected with heterologous isolates of B. piliformis did not specifically inhibit monoclonal antibody binding. The results of further studies with ammonium sulfate-precipitated serum globulins and purified serum IgG fractions indicated that inhibition of monoclonal antibody binding to flagellar epitopes was due to specific antibody and not to nonspecific serum factors. Thus, this competitive inhibition assay provides an ELISA to identify the serologic isolates involved in B. piliformis infections of laboratory animals.

This work was supported by a COR grant from the College of Veterinary Medicine, University of Missouri, by PHS Grants RR07004 and RR04568, and a grant from Charles River Laboratories.

P02 Sensitivity and Specificity of a PCR System for the Detection of Mycoplasma pulmonis

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The polymerase chain reaction (PCR) is a rapid, reproducible and reliable method of detecting specific sequences of DNA. The sensitivity of the PCR to various strains of Mycoplasma pulmonis should depend on the degree to which the target sequence is conserved by the organism. The specificity depends on the extent to which the target sequence is unique to the organism. Using published primer sequence for M. pulmonis, we tested purified DNA from different isolates of M. pulmonis (n=36), other murine mycoplasma species (M. collagen, M. muri, M. arthritidis, and M. pneumonitis) and several bacteria. The system uniformly detected all isolates of M. pulmonis by amplifying a 711-base-pair segment of the genome. This system also appeared to amplify the same size segment in all other mycoplasma species tested, but much less efficiently. DNA from bacteria or murine host cells did not produce visible PCR products. These results indicate that the target sequence is highly conserved in M. pulmonis, and at least partially in other mycoplasma species, but not in other bacteria. This system appears to be useful for detecting murine mycoplasma.

Supported by NIH grant RR00890

P03 Identification of Intestinal Parasites in Laboratory Animals Using Formalin-ethyl Acetate Concentrations and Permanent Trichrome Stains

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Accurate identification and speciation of intestinal parasites can be the most difficult part of diagnostic pathology in laboratory animal facilities housing both nonhuman primates and rodent species. Proper preservation and staining procedures are essential for identifying all parasitic stages and to distinguish between nonpathogenic and pathogenic organisms. Conventional practices often rely solely on fecal flotation of fresh samples. Delays in submitting samples for analysis often impair the effectiveness of this technique. To ensure that all diagnostic stages of parasites are properly preserved, specimens can be collected using a two-vial method with formalin and polyvinyl alcohol (PVA) fixatives. Formalin is satisfactory for preserving cysts, ova, and larva. When formalin-fixed specimens are concentrated using the formalin-ethyl acetate method, helminth eggs and larvae, including schistosomes and operculated eggs can be identified. In addition, the likelihood of detecting protozoal cysts is increased. Specimens fixed in formalin do not adhere well to glass slides and stain poorly; therefore, PVA fixation followed by trichrome stain is recommended for making permanent slides of protozoal trophozoites and cysts. This method is especially useful for speciation of protozoans and often reveals small organisms not discernible by other techniques. The Wheatley trichrome stain is most widely used. With minor variations, this technique can accommodate staining large batches of slides. This two-vial method of sample collection provides an accurate and economical means for detecting and identifying intestinal parasites in laboratory animal facilities. It also allows specimens to be collected at the animal facility and shipped to remote laboratories without compromising diagnosis.

Supported in part by grant NIH-NCI CA-16672

P04 Transmission of Minute Virus of Mice into a Rodent Colony by a Research Technician

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An outbreak of minute virus of mice (MVM) was detected in a breeding colony of transgenic mice. The cause of the outbreak was eventually traced to a research technician's rodent colony kept at home as a food source for his snake collection. MVM was initially detected during a routine serology screen (ELISA) of sentinel animals. Once containment measures were implemented, an investigation to learn how the virus was introduced into the colony was instituted. The most likely source of infection was the vendor from which the animals had been obtained. Health profiles were obtained from the vendor, and a procedure requiring that serology be done on a sample of all incoming animals was implemented.

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Blood samples were taken from each shipment of animals having different birthdates to attempt to retrospectively identify and isolate the problem. We continued to monitor sentinel animals for MVM on a regular basis. The results of serology testing of the vendor’s animals were negative for MVM. Further investigation disclosed that the principal research technician who worked in the transgenic colony was an avid herpetologist who maintained a colony of rodents at home as a food source for his snake collection. The research technician supplied us with samples from his rodent colony for a serology screen. Serology indicated that his rodents were positive for MVM. We felt that this was the most probable source of the MVM outbreak in our colony. The research technician had served as a vector, carrying MVM on skin or clothing from home to our colony where he worked. The source of this outbreak raised a major concern for all personnel working in the laboratory animal facility. The need for proper personal hygiene (showering), clean clothing, frequent handwashing, and of course, grooming over street clothing is imperative to minimize the potential spread of infection from one’s home into the workplace.

**P05 Postsurgical Hemorrhage and Death in Cr: NIH-bg-nu-Xid Mice**

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Cr: NIH-bg-nu-Xid mice have three genetic immunologic defects including the beige (bg), nude (nu), and X-linked immune deficient (Xid) mutations. Because of their marked immunodeficiency, they have become increasingly popular for studying and propagating human cancer cells. As part of a mammary cancer research project, an investigator ovariec-tomized 45 female, 4 to 6 week-old, triple-defect mice. Although various degrees of bleeding at the surgical sites were noted during the operations, no other anesthetic or surgical complications were observed. Fifteen of the animals died within 24 hours of surgery and an additional five died during the next 2 days (postsurgical mortality of 44%). Postmortem examination revealed clotted blood at the incisions, massive hemorrhage at the ovariec-tomy sites, and pale organs including the kidneys and liver. Bleeding times in otherwise normal triple defect mice averaged over 9.4 minutes (n=5) compared with a mean bleeding time of 3.7 minutes for C57B1/6J mice (n=5). Triple-defect mice had normal platelet counts, prothrombin times, and partial thromboplastin times. We concluded that the postoperative hemorrhage and deaths were caused by a platelet storage pool deficiency which has been associated with the bg defect. Investigators who intend to perform surgery on Cr: NIH-bg-nu-Xid mice must be aware of this significant postoperative complication.

**P06 Ivermectin Treatment of Gerbils for Dentostomella translucida and Syphacia obvelata**

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*Dentostomella translucida* and *Syphacia obvelata* were initially diagnosed by necropsy in a breeding colony of approximately 320 Mongolian gerbils. To determine incidence of infection, a survey of approximately 40% of these animals was done by perianal cellophane tape test, direct microscopic examination of feces, and fecal flotation. Of the cages tested, 53% were positive for *D. translucida* and 25% were positive for *Syphacia*. The colony was treated for these infections by adding ivermectin to the drinking water. Based on average measured water consumption, gerbils were treated with ivermectin at 0.8 mg/kg body weight per day for 1 week, the drug was withheld for 1 week, and then retreatment with the same dose for an additional week. After the second treatment period, no *Syphacia* infections were detected and the infection rate for *D. translucida* had decreased to 27%. In an attempt to eliminate the remaining *D. translucida* infected animals, the dose of ivermectin was doubled to 1.6 mg/kg body weight. Again the treatment regimen consisted of two 1-week treatment periods separated by 1 week without treatment. This higher dose did not significantly reduce the incidence of *D. translucida* infection, which remained at 25%. Gerbils were again negative for *Syphacia* after the second treatment period.

**P07 Identification of a Novel Demodex sp. Mite from Armenian Hamsters (Cricetulus migratorius)**

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A 2-year-old female Armenian hamster presented with erythema and hair loss over its dorsum that extended from the face of the lumbar region. There were various degrees of crusting, scaling, and alopecia with the head, neck, and thorax most severely affected. Pruritus did not appear to be excessive but scratching was occasionally observed. A skin scraping revealed numerous *Demodex* sp. mites. They were approximately 172 μm by 33 μm and morphologically distinct form *Demodex auratus* and *Demodex criceti* which are known to infest Syrian hamsters (*Mesocricetus auratus*). Preliminary anatomic examination of the palpal setae and genital openings using light and scanning electron microscopy, also support the uniqueness of this parasite. Histologic examination of skin from the affected hamster revealed hyperkeratosis, epidermal hyperplasia, focal areas of supplicative inflammation and adult mites in the hair follicles. Subsequent identification of similar lesions on other Armenian hamsters disclosed identical mites. Elucidation of all the life stages and of the natural history of this novel ectoparasite is currently underway. Supported in part by grants RR01046 and RR07036.

**P08 Strain Variation and Susceptibility of Syrian Hamsters to Amyloidosis and its Interference with Research**

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Syrian hamsters being used for serum cholesterol and triglyceride measurement were found to have an increasingly wide variation of these parameters among individuals. We sought to ascertain
the cause of this variation so as to minimize its source, e.g., environment, nutrition, and/or infection. A review of the health records and genetic background of the hamsters indicated that the main cause of morbidity was clinical amyloidosis and that the pool of hamsters being used was not genetically defined. Confining the hamster supply to one strain (preferably inbred) with low susceptibility to amyloidosis was required. Twelve hamsters from each of the three strains available to us were maintained under routine conditions at this facility. At 6 months of age, four animals from each group were euthanized and subjected to gross necropsy and histologic examination of liver and kidney. Three months later, the remainder underwent the same procedure. The DSNI strain showed no evidence of amyloid deposition by 9 months. In both the DSNO and MBO strains, 50% of animals were affected at both time points. Since then, we have used only the DSNI strain for studies; morbidity has been low and there has been little variation in cholesterol and triglyceride levels.

P09 Cutaneous Papillomatosis with Cutaneous Horns in a Guinea Pig (Cavia porcellus)

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An adult female guinea pig (Cavia porcellus) was presented with multiple integumentary tumors. Verrucous papillomas and subcutaneous cysts were scattered over the entire body. Large cutaneous horns protruded from several papillomas. The papillomas ranged from 2 mm to 3 cm in diameter and protruded from the skin surface as warty masses with dry, friable surfaces. Several of these tumors had produced hard cutaneous horns which were up to 2.5 cm long. Histologically, the papillomas were composed of papillae with connective tissue cores which were lined with acanthotic stratified squamous epithelium. Tall columns of keratin and parakeratotic epithelial cells extended from the tips of the papillae and the interpapillary regions, respectively. The cutaneous horns were composed of mature keratin. The cysts ranged from 5 mm to 1.5 cm in diameter and were filled with white caseous material. Histologically, they appeared to arise from follicular epithelium and were filled with keratin and parakeratotic epithelial cells. The lining of the cysts consisted of stratified squamous epithelium which had similar papillae and columns of keratin and parakeratotic cells as the surface papillomas. There were no adnexal structures associated with the cyst walls. The presence of cutaneous horns has not been reported in papillomatosis of guinea pigs. Although cutaneous papillary horns are frequently caused by viruses, no viral inclusions were apparent histologically and electron microscopy of negatively-stained homogenates was negative for virus particles. Genomic DNA extracted from the papillary horns was analyzed for the presence of papillomavirus DNA by Southern blot using random primer-labeled whole genome of Cottontail rabbit papillomavirus (CRPV) probe under high, medium, and low stringency conditions. There was no hybridization of the CRPV probe to DNA of the cutaneous horns under these conditions.

Supported by NIH grant CA25462

P10 Ameloblastoma in a Guinea Pig

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A 4.5-year-old spayed female pet guinea pig (Cavia porcellus) was presented for general inappetence. While initial oral examination was unremarkable, follow-up examination revealed an ulcerated nodular mass of the mucosal surface of the palate, with grossly normal teeth. Cytologic examination of a needle aspirate of this mass suggested neoplasia of connective tissue origin. Radiographs revealed invasion and lysis of adjacent bony tissue. The animal received nursing care and slurry feedings for the following 2 months until its deteriorating quality of life indicated that euthanasia was necessary. On gross postmortem examination, a large ulcerated mass surrounding a spherical, white translucent core was removed. Histologic examination revealed this to be an ameloblastoma with cartilaginous metaplasia, an uncommon tumor in guinea pigs. No evidence of metastasis was noted.

P11 Effect of Blood Collection Volumes on the Hemograms of Laboratory Rabbits

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There has been disagreement concerning what percentage of a rabbit's total blood volume can be collected at frequent intervals without causing abnormal hematologic responses. Adult (3.5 to 4.1 kg), female, conventional New Zealand White rabbits were anesthetized and underwent weekly blood collection from the central ear artery. Experimental groups (five/group) consisted of animals subjected to weekly collection of 10, 15, 20, 25, or an incrementally increasing (10 to 25) percentage of their calculated total blood volume for a period of 4 weeks. Two milliliters of blood were collected at weekly intervals from the five control rabbits. Samples were evaluated for red blood cell count, white blood cell count, hemoglobin concentration, and hematocrit. White blood cell differential counts were determined by light microscopic examination. Mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration were calculated. Significant decreases in white blood cells occurred in the 25% and 10 to 25% groups by week 4. Red blood cells were decreased in the 20% and 25% groups at week 2, but recovered by week 4. Sustained hemoglobin decreases occurred by week 2 in the 10%, 15%, and 25% groups. There were no significant differences in any other measured parameters. Repeated blood sampling of 25% of the total blood volume resulted in alterations in the hemogram.

continued ▶
P12  Imperforate Anus with Rectovaginal Fistula in a Dwarf Rabbit
CE Hotchkiss, BR Collins
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A juvenile female dwarf rabbit presented with imperforate anus. The rabbit urinated and defecated via the vestibule. The anal sphincter appeared to have normal muscular tone and responded normally to mechanical stimuli; however, the anus was not patent. The rabbit appeared to be healthy otherwise. Contrast vaginography showed a rectovaginal fistula at the level of the ischial arch, with no rectum visible caudal to that point. Reports of imperforate anus in rabbits are very rare. In other species it is common to have urinary tract anomalies in conjunction with imperforate anus; an intravaginal urogram of this rabbit disclosed no abnormalities. This rabbit had no evidence of cystitis, despite the presence of feces in the vagina. Several months after initial presentation, the rabbit developed endometritis. Ovariohysterectomy was curative. More than 1 year after initial presentation, the rabbit is healthy.

P13  Disseminated Histoplasmosis in New Zealand White Rabbits
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Histoplasmosis in rabbits has been reported only rarely, and lesions of disseminated infection have not been previously described. The occurrence of unexpected deaths in 2 of 16 rabbits that were inoculated subcutaneously with the yeast form of Histoplasma capsulatum for antibody production prompted us to closely examine this group. Necropsies were performed, histologic sections were examined (lung, liver, spleen, kidney, lesioned organs), and cultures were obtained (spleen, liver, kidney). Gross lesions present included pale swollen livers with prominent reticular patterns, splenomegaly, papular skin eruptions on the chin and oral mucosa, prominence of cecal lymphoid tissue, and renal abscesses. Striking accumulations of macrophages packaged with H. capsulatum were noted within splenic red pulp, lung, subepidermal tissue, and intestinal propria-submucosa. Yeast forms were present in at least one organ in all rabbits, and eight rabbits had yeasts in multiple organs. Cultures of spleen and liver were positive for H. capsulatum. We concluded that disseminated histoplasmosis can be induced experimentally in rabbits, with multiple organ infection including skin, urinary, and gastrointestinal systems.

P14  The Use of Cefitofur Sodium as a Treatment for Pasteurella multocida in New Zealand White Rabbits
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Pasteurella multocida continues to be a major cause of morbidity and mortality in rabbits. Based on its spectrum of activity and effectiveness in other animals, cefitofur sodium was evaluated as a potential treatment of this disease. Clinical cases of P. multocida were verified by nasopharyngeal culture and treated with cefitofur sodium in dosages ranging from 2.2 to 13.2 mg/kg. Route of administration was either subcutaneous or intramuscular, and frequency was either once or twice daily. Four animals were reassessed by culture at the end of 15 days of treatment. Eight clinically healthy New Zealand White Rabbits were used to assess the pharmacokinetics of cefitofur in the rabbits. 8.8 mg/kg cefitofur sodium was given intravenously or subcutaneously and serum samples analyzed by cefitofur concentration. Samples were obtained during a 24-hour period and analyzed by bioassay using Bacillus stearothermophilus organisms. Pharmacokinetic analysis of the data was accomplished using the commercial curve- stripping program RSTRIP. Clinical cases of P. multocida treated with all dosages of cefitofur sodium showed little to no clinical improvement. Serum half-life of cefitofur sodium in rabbits was extremely short (approximately 15 minutes) compared to cattle (approximately 6 hours). Cefitofur sodium does not appear to be a clinically useful drug in rabbits due to its extremely short serum half-life.

P15  A Randomized Trial Using Oral and Parenteral Enrofloxacin and Parenteral Cefitofur to Treat Lapine Pasteurellosis in a Biomedical Research Facility
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Pasteurellosis is a common infectious bacterial disease of rabbits caused by the nasopharyngeal commensal Pasteurella multocida. The epidemiology of 29 cases and a randomized trial of three treatments including oral Enrofloxacin (5 mg/kg BID for 10 days), parenteral Enrofloxacin (5 mg/kg BID for 10 days), and parenteral Cefitofur (2.5 mg/kg BID for 10 days) was studied. Cases were evaluated for clinical signs twice daily for 10 days after initiation of treatment (day 0) and then on days 17 and 40. Cefitofur, oral Enrofloxacin, and parenteral Enrofloxacin were successful in ameliorating clinical signs of pasteurellosis in 75%, 56%, and 60% of cases, respectively. There were no significant differences in success rates, defined as resolution of clinical signs to day 40, or mean days to clinical sign resolution between treatment groups; however, recurrence rate for the oral Enrofloxacin group was 33% compared with 0% for the two parenteral treatment groups. One rabbit treated with Cefitofur experienced posttreatment diarrhea which resolved without complication. The frequency of positive P. multocida nasal cultures in groups treated with parenteral Cefitofur and oral Enrofloxacin ranged from 33 to 75% on days 0, 10, 17, and 40 and was not affected by treatment. Treatment with parenteral Enrofloxacin decreased the frequency of positive nasal cultures from 50% on day 0 to 17% on day 10 and to 0% on day 17. However, on day 40, 75% of cultured samples from rabbits treated with parenteral Enrofloxacin were positive for P. multocida. The three regimens were free from serious side effects and treatment was not associated with elimination of P. multocida from the nasopharynx of affected rabbits. Supported by NIH grant RR 03624-05.
**P16  Toxin Production by Pasteurella multocida Isolated from Rabbits with Atrophic Rhinitis**

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Naturally occurring turbinate atrophy in rabbits was associated with *Pasteurella multocida* infection. Several in vitro and in vivo studies were conducted to demonstrate toxin production from *P. multocida* isolates and determine the relationship of toxin to atrophic rhinitis in rabbits. Ten isolates of *P. multocida* serotype A:12 were obtained from adult New Zealand White rabbits with spontaneously occurring atrophic rhinitis. Specific-pathogen-free rabbits inoculated intranasally with isolates of *P. multocida* developed rhinitis and turbinate atrophy. However, inoculation of filtrates of the same bacteria failed to induce turbinate atrophy. Toxin production by isolates of *P. multocida* was not demonstrated in agar overlay cytotoxicity assays using bovine embryonic lung or Vero cell cultures. However, toxin was detected in cytotoxicity assays using bovine embryonic turbinate cell cultures with extracts of *P. multocida*. Although turbinate atrophy was experimentally reproduced in rabbits with isolates of *P. multocida*, the presence of toxin was only confirmed by cell culture assay of *P. multocida* extracts. This is unlike the response to toxin-producing isolates of capsular type D associated with atrophic rhinitis in swine, suggesting that the toxin of capsular type A isolates from rabbits may differ.

Supported by NIH grant RR01203.

**P17  Endocrinopathies Associated with Adrenal Cortical Neoplasms in Ferrets**

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A newly recognized endocrinopathic syndrome has been observed in ferrets. The syndrome, which affects both male and female adult neutered ferrets, is characterized by alopecia, persistent vulvar enlargement, and urogenital tract abnormalities. Pruritus and behavioral changes have been noted in several affected animals, however, no consistent clinical pathologic abnormalities have been observed. Neither polydipsia nor polyuria have been noted in affected ferrets. The bilaterally symmetrical alopecia which often begins with a seasonal incidence becomes generalized and severe. Sera and/or urine have been assayed for cortisol and a variety of estrogens and androgens. Elevated serum estradiol-17β has been documented in several cases. Adrenal abnormalities were noted during exploratory laparoscopy. Histopathologic examination has disclosed that the adrenal gland, usually the left, occasionally both glands, have been irregularly enlarged and characterized by neoplastic cortical cells. Hair regrowth has been documented postadrenalectomy. Detailed clinical and pathologic descriptions of this syndrome will be presented.

Supported in part by NIH grants RR01046 and RR07036

**P18  Malignant Lymphoma and Leukemia in Cohabiting Ferrets**

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Malignant lymphoma is a very common neoplasm in ferrets, and we have recently documented clustering of lymphoma cases in several households. In this study, four groups with affected ferrets that were siblings or cohabitants for more than one year were examined. In one group, five (5/11) middle-aged ferrets had lymphomas with similar clinical and histologic presentations. In another group, two young adult ferrets had lymphoma and two had leukemia (4/15). These two leukemic ferrets had been sole cagemates for more than 1 year. In a third group of middle-aged ferrets which were sibling cohabitants, there were three (3/8) chronic lymphomas. The final group had two peripubescent siblings, which had been isolated from their littersmates, presented with lymphoma with identical clinical and histologic syndromes. Clustering of malignant lymphomas has been associated with virally-induced syndromes in other species. Statistical evaluation of these findings, specific clinicopathologic and histologic findings, and implications in research settings will be presented.

Supported in part by grants RR01046 and RR07036

**P19  Toxoplasmosis: An Avirulent Zoonosis in Hawaii**

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Serologic health surveillance of our staff and animal caretakers includes determination of *Toxoplasma gondii* antibody titers; 36 of 50 (70%) serum samples tested had positive titers. None of 60 research rabbits nor two thers monkeys had positive titers, but 1 of 6 cats (16.7%) and 3 of 40 (7.5%) *Aotus trivirgatus* monkeys had positive titers against toxoplasmosis. Since little data was available concerning prevalence in Hawaii, we assayed 139 pet cats and found 18.7% had positive titers, whereas of 191 stray cats, 62.3% had positive titers. assay of 810 human serum samples from the general population indicated that 65.6% were positive — considerably higher than the 10 to 15% prevalence in America, but within the range recorded in Melanesia and Micronesia. When the medical records since 1977 from all major hospitals were examined, only a couple of adult human cases required treatment annually, and no acute infant congenital cases during the past 8 years; no clinical outbreaks have been reported by veterinarians among chickens, pigs, or sheep.

**P20  Posterior Glottic Stenosis Repair in the Canine Model**

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Posterior glottic stenosis may result from blunt trauma or prolonged intubation. After initial injury, formation of scar tissue...
causes a drawing together and fixation of the true vocal cords in the midline, resulting in progressive, potentially life-threatening upper airway obstruction. Simply removing cicatrix leaves raw surfaces in which scar tissue will quickly reform. Using endoscopic and open surgical methods of repair, attempts have been made to address this problem by grafting skin or mucosa over the raw surface, with or without placement of a stent over the graft. However, infection and/or displacement of the graft occurs frequently; stenting delays healing, is uncomfortable, and requires a tracheostomy. A recent endoscopic technique for correcting posterior glottic stenosis has been studied in a canine model and offers an alternate solution to this problem. Using electrocautery, stenosis was created in the posterior glottis of eight canines. After scar maturation, repair was done with the use of CO₂ laser. Sutures were placed endoscopically to lateralize the true vocal cords, to act as a “stent” to prevent restenosis. Using CO₂ laser and vocal cord lateralization will greatly reduce the incidence of restenosis, which is associated with conventional endoscopic or open surgical approaches.

P21 *Trichospirura leiptostoma* Infection in *Callithrix jaccus* (Common Marmoset): Disease and Treatment

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*Trichospirura leiptostoma* is a nematode that can inhabit the pancreatic ducts of *Callithrix jaccus* (common marmoset). Since 1989, an increased prevalence of parasite infection has been noted at necropsy. Infected juvenile animals (<1yr) often have sub-par growth; some infected adults develop diarrhea and weight loss; some die. Histologically, mildly infected animals have little tissue reaction, whereas severely infected pancreases have moderate-to-severe fibrosis with occasional microabscesses. Histopathologic examinations have suggested that tissue changes may be due in part to mechanical blockage of the pancreatic ducts. Juvenile animals from infected family groups were 45 g smaller at 12 months of age (P < 0.05) than non-infected animals. Sick, infected animals were selected for treatment with Ivermectin (200 µg/kg); 9/15 were still positive on fecal sedimentation or at necropsy within 30 days of treatment. Fenbendazole (30 mg/kg) was given daily for 14 days to 12 animals. Multiple fecal samples and one necropsy within 30 days posttreatment suggest that all fenbendazole-treated animals were cleared of the parasite. This study confirms a 1990 report that *T. leiptostoma* can be pathogenic. In our small sample, fenbendazole appeared to be an effective treatment.

P22 Echocardiography as a Noninvasive Diagnostic Technique in Owl Monkeys (*Aotus sp.*)

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Cardiopathic disease was diagnosed in 40% of owl monkeys necropsied at Battelle, Northwest Laboratories during a 5-year period. The results of a preliminary study showed that echocardiography was superior to electrocardiography or thoracic radiography for discerning between normal and affected animals. Based on those results, we then proceeded to obtain echocardiograms of 314 owl monkeys maintained in a breeding colony. Echocardiograms were performed on sedated animals, and, from a parasternal short axis view, we obtained a two-dimensional view with an M-mode tracing of the left ventricle, aortic root, and left atrium. All M-mode tracings were analyzed according to American Society of Echocardiography criteria. Measured variables included left ventricular free wall thickness at end-diastole, interventricular septal thickness at end-diastole, left ventricular chamber diameter at end-diastole, and left ventricular chamber diameter at end-systole. Of the 314 animals examined, 24 were diagnosed as having dilative disease, 26 as having hypertrophic disease, and 95 were considered to be completely normal. The remaining 169 monkeys had one or more abnormal criteria, but not a preponderance that would place them clearly in one of the above groups. We concluded that echocardiography is an effective technique for diagnosing cardiac disease in owl monkeys, and is applicable to other neotropical primates.

P23 Diagnostic Ultrasonogram Findings on a Colony of Owl Monkeys (*Aotus trivirgatus*)

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With the increasing costs and efforts to limit animals used in research, it has become essential to obtain the healthiest specimens possible. Subclinical health problems can lead to deaths, project abortion, or compromise the validity of results. The unexpected deaths of several owl monkeys led to the proposal that all animals within the colony be examined by ultrasonography. Ultrasonography provides a noninvasive means to detect many diseases, to determine need for therapy, and to determine which animals are satisfactory subjects for research. Thirty-five monkeys (23 males and 12 females), aged 3 months to 13 years, were examined while they were anesthetized with ketamine-xylazine. Data about their hearts, liver, kidneys, and uteruses were recorded. Measurement of kidneys and hearts were made from two-dimensional sonograms and from cardiac M-mode sonograms. Concurrently an electrocardiogram was recorded. Postmortem examination of animals dying of natural causes during the subsequent 2 years aided in verifying the results. A 2-year follow-up examination of nine animals considered at higher risk of health problems was completed. Findings included animals with dilative and hypertropic cardiomyopathy, cardiac valvular endocardiosus, arrhythmias, changes consistent with chronic kidney disease, kidney cysts, and gall stones.
P24 Evaluation of Prepartum Measurement of Biparietal Diameter to Assess Fetal Growth and Development in Squirrel Monkeys (Saimiri sp.)
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Biparietal diameter (BPD) is one of the most reliable measures of fetal growth in primates, but the conventional ultrasound method of obtaining this measurement has many disadvantages. We evaluated simple caliper measurement of biparietal diameter through the abdominal wall of the dam (transabdominal BPD; trans-BPD) for: comparison with postnatal BPD and to predict delivery complications in the final third of pregnancy of squirrel monkeys. Twenty pregnant Saimiri known to be in the last third of pregnancy were measured weekly by hand measurement of uterine diameter and caliper measurement of biparietal diameter until delivery. All animals delivered during a 7-week period. Postpartum BPDs were consistently 1 to 3 mm smaller than trans-BPDs performed during the week before delivery (postpartum: 30.2 mm ± 0.4, mean ± SEM). Trans-BPDs for weeks before delivery were: week of delivery, 32.4 ± 0.5 (N = 17); 1 week, 32.9 ± 0.7 (N = 11); 2 weeks, 32.1 ± 0.5 (N = 12); 3 weeks, 31.9 ± 0.6 (N = 6); 4 weeks, 30.6 ± 0.4 (N = 7); 5 weeks, 30.4 ± 0.5 (N = 7); 6 weeks, 28.0 (N = 1); 7 weeks, 25.4 (N = 2); 8 weeks, 24.3 (N = 2). Two of the six animals with trans-BPDs > 33.0 mm had complicated deliveries which ended in the deaths of the infants. There were no delivery-related deaths in animals with Trans-BPDs < 33.0. Transabdominal BPD may have prognostic significance for clinical management of squirrel monkey pregnancy.
Supported by NIH grant 2P40 RR01234.

P25 Urinary Catheterization of Nonhuman Primates
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Studies in our laboratory have required using urinary catheterization to collect uncontaminated urine from baboons (Papio cynocephalus anubis) and macaques (Macaca fascicularis). Existing literature describes this technique used in female macaques. There is a paucity of information about urinary catheterization of male monkeys, and the unique problems that arise. Using 8-French Foley catheters, we were able to catheterize females only. When using a comparable-gauge infant feeding tube, we successfully catheterized both genders; however, this was impossible for extended periods of up to 6 hours. The results of our initial work demonstrated that the 8-French Foley catheter was too large and it lacked the rigidity necessary to catheterize male monkeys. The 8-French infant feeding tube provided rigidity, but lacked a balloon tip to maintain catheter placement and assure patency. We subsequently chose to use either 4 or 5 French Swan-Ganz® catheters. This design possesses a necessary, balloon tip, rigidity, and appropriate external diameters for this application. We review the methods and materials necessary to successfully perform urinary catheterizations using Swan-Ganz® catheters, and present a summary of various urine, blood chemistry, and hematolgy parameters monitored while developing this procedure.

P26 Incidence of Antibody Against Filovirus Infection in Cynomolgus Monkeys Imported from Mauritius
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With a 1989 epizootic outbreak of filovirus infection in the United States, the Centers for Disease Control published regulations for quarantining imported nonhuman primates. These regulations included testing nonhuman primates for evidence of recent filovirus infection. During the past year we have imported 1134 Macaca fascicularis from Mauritius. Paired serum samples, 31 days apart, were tested by the indirect immunofluorescence method. No filovirus antibody was detected in 1060 of the 1134 paired samples. In the remaining 74 cases, antibodies were detected in either one or both samples. In 29 cases there was no change in antibody titer from the first to the second sample, while in 20 cases the antibody titers decreased from the first to the second sample. In the remaining 25 cases, the antibody titers were higher in the second sample, but the increase was not significant to suggest filovirus infection. Our data suggest that the incidence of filovirus infection in cynomolgus monkeys imported from Mauritius is very low.

P27 Schistosomiasis in Newly Imported Cynomolgus Macaques
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Schistosomiasis, caused by three different blood flukes, infects more than 250 million people worldwide and is one of the most widespread human parasitic diseases. Asiatic intestinal schistosomiasis, due to Schistosoma japonicum, is endemic to China, the Philippines, and Indonesia. Mammals are an important reservoir for S. japonicum, but natural infection in nonhuman primates is infrequently reported. In June 1991, 48 wild-caught cynomolgus macaques were imported from the Philippines. After quarantine, the animals were evaluated by abdominal ultrasonography and physical examination. Ultrasound examination disclosed that two animals had increased hepatic echogenicity in a diffuse, heterogenous pattern. One of these animals had a palpable hepatosplenomegaly and a mild hyperbilirubinemia. In both animals, pre- and postprandial bile acids were unremarkable. Ultrasound-guided percutaneous liver biopsy was performed and histopathology showed a moderate, chronic, perportal hepatitis with multinucleated giant cells, and an intraclesional parasite ova consistent with schistosomiasis.
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Direct fecal smears did not reveal the trematode infection, but serial fecal concentrations confirmed the presence of S. japonicum in both animals. Schistosomiasis, which is most likely contracted in the animals' natural habitat, should be considered when evaluating imported cynomolgus macaques. This research was supported by NIH grant RR00169.

P28 Red Blood Cell Parameter Evaluation in Macaca mulatta Blood Donors

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The California Regional Primate Research Center provides blood samples to investigators with approved research protocols. The average volume of blood provided by our resource is approximately 8,000 milliliters annually. To meet these research requests, a blood donor protocol was instituted which has allowed us to draw from 16 clinically defined and monitored animals. Fourteen males and two females ranging from 8 to 25 years old were selected for the program based on physical examination, complete blood count, weight history, and viral screening. Animals accepted to the program are bled up to 20 percent of their body weight monthly. The blood donor colony is managed as follows: animals receive daily multivitamins with iron, red blood cell indices and weights are evaluated monthly, and viral screens are repeated biannually. Red blood cell indices of 10 of the blood donors, who had been in the group for longer than 2 years, were evaluated in comparison to colony reference values. No significant difference was found between the two groups. The results of this evaluation indicate that animal health, in particular, red blood cell indices, can be maintained concurrent sustained phlebotomy. This research was supported by NIH grant RR00169.

P30 Evaluation of Pseudomonas aeruginosa Vaccines in Animals

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Despite many years of research, a safe and effective vaccine for the prevention and treatment of P. aeruginosa infections has not been developed. Proteins unique to P. aeruginosa have been discovered by two groups of investigators who are testing for antibody-induced protection in animals challenged with homologous and heterologous strains. Traditionally, P. aeruginosa vaccines have been evaluated in the mouse or rat burn sepsis model, or by simply injecting bacteria i.v. or i.p. to cause sepsis. In a few studies, cystic fibrosis-type pneumonia has been induced in mice, rats, or guinea pigs by intratracheal instillation of bacteria. Since these models may require large numbers of bacteria or lack clinical relevance, and the burn model is not allowed by many IACUCs, another model introduced by Cryz was adapted for a study. Leukopenia, a common feature in high-risk patients, was induced in mice by injections of cyclophosphamide (100 mg/kg) i.p. on days 0, 2, 4, and 6 and verified by subsequent leukocyte counts. Animals were anesthetized with ketamine/xylazine (35/10 mg/kg) to make a 0.5 cm incision in the dorsal skin, and then P. aeruginosa (2 x 10^7 cfu) are injected into the wound. Predictable lesions of ethyma gangrenosum and sepsis occur within 3 to 4 days of inoculation. This model is simple and causes minimal pain and distress in animals.

P31 Histologic and Ultrastructural Characterization of Hepatic Injury during Expression of a Reversible Growth Hormone Transgene in Mice

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A common undesirable sequilium of growth hormone therapy is the induction of hepatocellular changes featuring megalocytosis and eventual degeneration of hyperplastic hepatocytes. These changes have also been noted in transgenic mice which express growth hormone in an uncontrolled fashion from poorly regulated transgene sequences. The transgenic mouse model which places the ovine growth hormone gene under the control of an ovine
metallothionine promoter region (oMtlα-oGH) provides the opportunity, by virtue of its reversibility, to more thoroughly examine cellular and subcellular changes of the hepatocyte in response to increased growth hormone. Male oMtlα-oGH mice were challenged with 23 mM zinc sulfate for 21 days and humanely euthanized. Immediately after death, livers were alternatively placed into 10% formalin (carbonate buffered to pH 7.4) or perfused via the portal vein with 1% glutaraldehyde. Following routine processing, tissues were examined by both light and electron microscopy and compared with similarly prepared tissues from male oMtlα-oGH mice which had not received zinc as an induction agent. Mice in which the gene had been induced exhibited predictable cirrhotic hepatocellular malacia, with an increase in cellular ploidy, hyperplasia of both rough and smooth endoplasmic reticulum as well as Golgi apparatus, and prominent intranuclear cytoplasmic evagination. Distortion and disruption of both hepatic sinusoids and bile canaliculi were noted and interpreted as being secondary to cellular changes. Histologic and ultrastructural changes in the liver due to induced growth hormone expression in this model are highly predictable and accurately resemble lesions associated with hepatic injury due to therapeutic growth hormone administration.

P32 Reversible Liver Pathology in Transgenic Mice with Regulated Growth Hormone Overexpression

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Constitutive expression of growth hormone in transgenic mice causes well documented pathologic changes in hepatocytes. Inducible transgenic growth hormone models and the dynamics of hepatic pathology have not been described. Polymerase chain reaction verified ovine-metallothionine/ovine growth hormone (oMtlα-oGH) transgenic male mice, and nontransgenic littermate controls were placed in seven groups of four to seven mice each. Growth hormone overexpression was induced at 21 or 28 days of age by adding 25 mM zinc sulfate to the drinking water for 14 to 49 days. Groups were killed at 0, 14, or 28 days after discontinuing supplemental zinc. Histopathology was performed on formalin preserved, hematoxylin and eosin stained liver sections. Characteristic hepatocellular pathology, as described in the literature, was observed in all of our transgenic groups, while no pathology was observed in controls. Morphologic change was restricted to megacystosis of parenchymal cells and was centered around central hepatic veins. The degree of hepatocellular pathology was correlated with time off zinc supplementation (duration of nonexpression). Maximum megacystosis was observed at 0 days off zinc and minimum megacystosis at 28 days off zinc, regardless of length of time on zinc or age of animal at sacrifice. Based on these results, we conclude that the hepatocellular pathology induced by the overproduction of growth hormone in these mice is reversible. This transgenic mouse model may be used to help delineate normal and abnormal growth hormone physiology and function.

P33 Anatomic and Physiologic Characterization of the WF/pmWp-`fz` Rat

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The WF/pmWp-`fz` rat is a hypotrichotic mutant inbred homozygous strain. The original goal of this breeding program was to produce a genetically homozygous strain of inbred rats for use in dermal toxicology research. A rodent model lacking a full coat of hair is a distinct advantage when conducting skin penetration studies involving potentially toxic chemicals. In addition to dermal toxicology research, these rats would also serve as useful models for wound healing, and immunologic studies involving the skin. This strain of rat, known as the “fuzzy rat,” appears to possess a functional and intact immune system, allowing it to be housed and raised using conventional techniques. We present anatomic and physiologic parameters collected for each sex at ages 6 to 8 weeks, 19 to 21 weeks, 32 to 34 weeks, and 50 to 52 weeks. Parameters include hematology and clinical chemistry profiles, organ weights and body weights, and a characterization of histologic findings, including morphometric analysis of dermal adnexal structures. These findings and certain clinical presentations observed during inbreeding of the fuzzy rat are discussed and briefly compared to other hypotrichotic rodent strains.

P34 Evaluation of Butorphanol Tartrate and Buprenorphine Hydrochloride in the Inflammatory Reaction of the Sereny Test

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The ability of virulent Shigella organisms to invade the ocular epithelia of guinea pigs and elicit a keratoconjunctivitis is the basis of the Sereny test. This test has been used to assess the virulence of Shigella strains and more recently to screen candidate vaccines. Effects of systemic analgesics on the inflammatory response have never been documented. A double-blind study was conducted to evaluate the effects of two systemic analgesics on the Sereny test in outbred Hartley guinea pigs. Groups consisted of guinea pigs treated with butorphanol tartrate (n = 16) or buprenorphine hydrochloride (n = 16), and untreated controls (n = 5). All animals were inoculated with Shigella flexneri, strain 2a 2457T, onto the conjunctiva of each eye. At the onset of clinical signs (epiphora, scleral injection), analgesics were administered. Degree of keratoconjunctivitis was evaluated per standard procedure and animals were weighed daily. After 7 days, animals were humanely euthanized and the eyes fixed in 10% buffered formalin. Clinical observations were analyzed by using the Mann-Whitney test; histologic morphometry was analyzed by using the Student's t-test. The results indicate that neither systemic analgesic interfered with the inflammatory response.

continued ▶
P35 Sleep Alterations During Infectious Disease: Immunologic Influences

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Altered sleep after microbial infection is a behavioral manifestation of the acute phase response. We hypothesized that immune modulation would alter this response. Sleep was monitored during a 24-hour baseline period and a 48 to 72 hour experimental period in adult male rabbits chronically implanted with EEG electrodes. Three experimental paradigms were used. First, treatment with an immunosuppressive dose of cortisone attenuated the somnogenic effects of inoculation with either S. aureus or E. coli. A second study demonstrated that rabbits have fever and enhanced sleep in temporal correlation with increases in serum interferon after the initial intravenous administration of influenza virus, but are tolerant of all of these effects if the viral inoculation is repeated. Finally, an evaluation of the relationship between specific temporal patterns of sleep changes after microbial challenge and the clinical response to the infectious condition indicated that sleep patterns characterized by a prolonged period of excess sleep after microbial challenge are associated with a more favorable prognosis than are patterns characterized by a relatively short phase of enhanced sleep. These data support the hypothesis that microbially-induced alterations in immune function induce sleep alterations during infections disease and suggest that sleep may provide a prognostic indicator during infectious disease.

Supported by NINDS grants NS-25378 and NS-26429, and ONR contract N00014-85-K-0073.

P36 Effects of Thrombolytic and Anticoagulant Therapy on Experimental Cerebral Ischemia in the Rabbit Embolization Model

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Tissue plasminogen activator (tPA) reduces the eventual volume of brain ischemia after an experimental embolic stroke. Simultaneous administration of tPA and heparin may further reduce the volume of ischemic brain by preventing secondary thrombosis. New Zealand White rabbits weighing 2 to 3 kg (n = 33) underwent embolization of the middle cerebral artery via the surgically exposed internal carotid artery. Five animals received no treatment. Of the remainder, five each received tissue plasminogen activator (tPA), heparin, and both drugs together 1 hour, and five each 2 hours, postembolization. All animals were killed 5 hours after treatment began. Despite quite large variations in the resulting volumes of ischemic brain, within, as well as between groups, analysis of variance showed that treatment with tPA significantly reduced the extent of resulting ischemia (P = 0.001). Heparin had no effect (P = 0.114), nor were there any significant interactions. In these acute experiments heparinization caused no hemorrhagic transformation within the ischemic areas, this being observed in only one control animal. In this rabbit model, administration of 2 mg/kg of tPA within 2 hours significantly diminished the volume of brain rendered acutely ischemic by embolic stroke. Simultaneous administration of heparin within the same period did not further diminish the ultimate size of the stroke nor did it result in any instances of hemorrhagic transformation.

P37 An Animal Model for Evaluating Novel Therapeutics Targeted for Acute Septic Toxemia

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Developing new compounds targeted for alleviating septic toxemia requires the application of an animal model predictive of therapeutic effectiveness. The onset of acute septic toxemia leads to a release of phospholipase A2 (PLA2) into the blood stream. A conscious rabbit hypnotensive model was developed to respond to the sensitive changes in blood pressure, lactate, glucose, platelets, and WBC levels induced by the toxin. Indwelling catheters were surgically placed in the carotid artery 1 day before the study. After complete recovery and before dosing, direct blood pressure measurements were taken and blood samples obtained to attain baseline values. Four to six rabbits were studied simultaneously. Acute septic toxemia was induced by intravenous administration of endotoxin. Within as soon as 48 minutes, a decrease in mean arterial pressure occurred. At the earliest time point of 30 minutes, there were notable increases in lactate and glucose levels and decreases were observed in platelets and WBC levels. PLA2 began to increase after a lag time of approximately 2 hours. Detailed written criteria for monitoring the health and comfort of the rabbits were incorporated into the protocol. This conscious animal model for evaluating novel therapeutics against acute septic toxemia provided reliable and reproducible results.

P38 A Canine Model of Intimal Hyperplasia in Autologous Vein Grafting

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Relatively high failure rates of autologous vein grafts used in peripheral bypass surgery due to progressive intimal hyperplasia (IH) has prompted investigators to search for an animal model that develops IH in a relatively short time. We have attempted to develop such a model. Eight to ten centimeters of both external jugular veins were exposed and gently ligated in 40 adult mongrel dogs. The vein sections were divided and stored in a room-temperature papaverine-saline solution. The segments were pre-randomized to determine whether or not they were anastomosed in an end-to-end fashion into the carotid and femoral circulation sites. Six weeks postoperatively, the grafts were perfusion fixed,
harvested, histologically processed, and the amount of IH occupying the lumen of the midgraft sections was determined by using image analysis. In all dogs, the graft segments harvested from the femoral circulation had a significantly greater degree of IH than those residing in the carotid circulation. In all graft segments, a significant degree of IH was detected. We conclude that our protocol is effective in inducing IH in a canine model in a short postoperative time. Our model will be effective in assessing pharmacologic modulations of autologous vein graft IH.

**P39 Testicular Function after Total Body Irradiation and Bone Marrow Transplantation in the Feline Model of Mucopolysaccharidosis**

C Just, M Thrall, P Gasper, M Haskins, J Howard


Domestic cats are a model for numerous human genetic diseases including mucopolysaccharidosis VI (MPS-VI; Maroteaux-Lamy Syndrome). MPS-VI is an autosomal recessive lysosomal storage disease characterized by arylsulfatase B deficiency, urinary glycosaminoglycans, facial dysmorphism, and skeletal defects. Marrow ablation by total body irradiation (TBI; 10 Gy total dose divided into six doses, 2 days for 3 days via linear accelerator), in conjunction with bone marrow transplantation, has been a successful combination for correcting feline MPS-VI. However, the detrimental effects of irradiation on male fertility are unknown. In this study testicular function and sperm fertilizing ability were assessed in irradiated/transplanted MPS-VI cats (TBI) for comparison with nonirradiated/non-transplanted MPS-VI male cats (NON-TBI). Sperm motility, progressive motility (0 to 5, 5=best), concentration, morphology, and serum testosterone were evaluated. Artificial insemination was used to determine the ability of males to produce offspring. Males ranged from 1 to 4 years of age. Following ketamine anesthesia (15 mg/kg), 33 electroejaculates were collected from three TBI males (n=5 ejaculates; 10 to 40 months post TBI) and 9 NON-TBI males (n=28 ejaculates). Females (n=20) were hormonally stimulated and inseminated in utero with TBI (n=3) or NON-TBI (n=17) sperm. Spermic ejaculates were obtained from all males. In TBI males no difference (P > 0.05) existed in mean sperm motility (63.0 ± 5.4%), progressive motility (3.4 ± 0.4%), concentration (206 ± 94×10^6/ml) and normal sperm/ejaculate (30.1 ± 9.4%) compared to NON-TBI males (61.7 ± 3.3%, 37 ± 0.1, 363 ± 78×10^6/ml, 40.8 ± 5.1%, respectively). Serum testosterone levels were greater (P<0.05) in TBI-treated cats (1.1 ± 0.3 mg/ml) than non-TBI cats (0.4 ± 0.1 mg/ml). Pregnancies were achieved in both groups and pregnancy rates were similar (P > 0.05) between the TBI and NON-TBI sperm donors. One of three females (33.3%) inseminated with TBI sperm produced a pregnancy compared with 5 of 17 (29.4%) females inseminated with NON-TBI sperm. The results of this study indicated that there was no difference in ejaculate traits or sperm fertilizing ability between irradiated and unirradiated cats; and irradiation and bone marrow transplantation performed between 3 and 6 months of age to treat feline MPS-VI had no detrimental effect on male fertility.

NIH grants RR00045, 2RO1 37095-06A1

**P40 Concentration, Source, and Role of Plant Hormones (Phytoestrogens) in Laboratory Animal Diets**

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Rodents' diets significantly differ in estrogen activity. A study was conducted to determine the concentration, source, and role the phytoestrogens play in the total estrogenic activity observed in rodent diets. Seven rodent diets and six dietary ingredients (wheat, oats, soybean meal, corn, wheat middlings, alfalfa meal) were assayed for the phytoestrogens (Diadzein, Genistein, Formonentin, biochanin A, and Coumestrol) using high performance liquid chromatography coupled with mass spectrometry. Estrogenic activity was assayed in each diet by using the mouse uterine growth bioassay. Diadzein and Genistein concentrations (μg/g) and their combined dihydrolstibestrol equivalent estrogenic activity (μg/g) for each diet are listed respectively: Purina Chow #5001 [277, 214 (4.3)], #5002 [75, 61 (1.2)], #5015 [130, 97 (2.0)], NIH-31 [35, 28 (.5)], NTP-88 [38, 31 (.6)], NIH-07 [124, 104 (2.0)], AIN-76A [<1, <1(0)], soybean meal [1133, 954 (18.4)], and control soy-flakes [695, 785 (13.4)]. Formonentin, biochanin A, and Coumestrol were not detected. Soybean meal was the major source of the Diadzein and Genistein and their content was directly correlated with the soybean meal content. We concluded that phytoestrogens were present in rodent diets. The effect of the phytoestrogens on total dietary estrogen activity is unclear, since the diet with the highest concentration of Diadzein and Genistein had the lowest level of measured estrogen activity.

**P41 The Concentration, Source, and Significance of the Fumonisins, a New Class of Recently Recognized Mycotoxins, in Laboratory Animal Diets**

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The fumonisins (B1, B2, B3, B4) are primarily produced by the fusarium fungi growing on corn. *Fusarium moniliforme* is the most common fungus associated with corn. Dietary fumonisins cause equine leukoencephalomalacia, porcine pulmonary edema syndrome, hepatic neoplasia in rats, lowered productivity in poultry, and have been associated with human esophageal cancer. The carcinogenic potential of the mycotoxins have long been recognized, and experimental rodent diets are routinely assayed for the aflatoxins. The effects of the fumonisins on laboratory animals are not clearly understood. A survey was conducted to determine the concentration, source, and significance of the fumonisins in rodent diets. Seven different rodent diets and major continued ▶
dietary ingredients from four feed vendors were assayed for the
fumonisins by using high performance liquid chromatography.
The fumonisins were detected in four of seven rodent diets (range
1-3 ppm), in 6 of 8 samples of corn (1 to 3.2 ppm), and in one
of two samples of oats (3.0 ppm). The major source of the
fumonisins was corn. These data confirm that the fumonisins
are present in rodent diets. Corn is a major dietary ingredient
and since safe levels of the fumonisins for laboratory animals are
unknown, we concluded that rodent diets used in long-term
carcinogenicity studies should routinely be assayed for the
fumonisins.

P42 Laboratory Management of Capybara
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The large body size range within the order, Rodentia, makes
rodents well suited for allometric studies of resting and exercise
metabolism. The world’s largest rodent, the capybara (Hydrochoerus hydrochaeris), was imported from Brazil to obtain the
broader range of body size for these studies. Capybaras are a
semi-aquatic species of the family Hystricognathi. They require
a water source allowing full submersion to maintain healthy skin.
They prefer to defecate in water and use it for protection when
frightened. Capybara can be group-housed if adequate space
is provided. Sexually homogenous groups are suggested. Visual
barriers are helpful to minimize fighting. Capybara require warm
temperatures and should be acclimated to temperature changes.
Their webbed feet may become abraded on concrete flooring; therefore, bedding or thick rubber mats should be
provided in the frequently used areas. The nutritional
requirements of capybara are poorly defined, but these animals
can be successfully maintained by offering a varied diet of monkey
chow, alfalfa hay, and yams or carrots. These animals are very
shy, but acclimate to familiar people and can be maneuvered
easily by using basic pig handling techniques such as a pig board.
With consistent training, they quickly learn to move from their
pens on to a treadmill in the study area. With proper attention
to their physical and behavioral needs, capybara can be
successfully managed in a laboratory environment.

P43 Cephalopods as Model Organisms for Biomedical and
Basic Research: Scope of Current Research and Animal
Availability
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Cephalopods (squid, cuttlefish, and octopus) have been used for
a wide range of scientific research. Three Nobel prizes have been
awarded to individuals whose research was based on cephalopod
nervous system physiology. The squid giant axon's use in
biomedical research continues, with emphasis now focusing on
the subcellular physiology of ion channel formation and function.
The visual and equilibrium receptor systems have been active
areas of basic research in receptor physiology and morphology.
Less well-known areas producing medically significant results
are: skin pigment synthesis and physiology, cellular aspects of
aging, cataract formation, regulation of reproduction and
development, respiratory and cardiac physiology, and
biochemistry of gene repair. The Marine Biomedical Institute
has established a cephalopod culture program to provide a
consistent supply of these unique animals for the research
community. The squid, Sepioteuthis lessoniana, (500/yr) and the
cuttlefish, Sepia Officinalis, (2,000/yr) are cultured through the
life cycle in the laboratory using standard methods, resulting in
animals of known age and excellent physical condition. Both
species can survive overnight freight to anywhere in North
America. This research is supported by NIH grants RR 01024 and RR
01279.

P44 Xenopus Laevis Housing DD A Labor and Water
Conserving Method
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Many Xenopus housing and husbandry methods currently in
use require frequent, complete water changes which greatly
increase water use and labor. At Neurex, we had a custom tank
designed and built, which uses a canister filtration system, has
partitioning capabilities, and secure top. The tank measures 30"
× 16.5" × 20" and can house up to 22 frogs. At initial setup,
water is poured in directly from the tap and then treated. Instead
of the usual practice of removing the frogs from the tank and
draining it completely, a siphon is used to remove excess food
and waste from the bottom of the tank. This procedure usually
removes 90 to 95% of the debris but only 15 to 30% of the water.
Fresh water is added to Tank and then treated. The canister
system filters the smaller debris and maintains the purifying
bacteria which control ammonia and nitrite levels in the water.
Feeding and maintenance takes 15 to 20 minutes twice weekly
and the filter canister maintenance takes about 45 minutes every
14 to 16 weeks. The canister filter system is easily adaptable to
other types of plastic or acrylic tanks. Our colony has been
established for over 2 years and there have been no health
problems to date.

P45 Use of High Efficiency Filtration Systems to Minimize
Amphibian Disease Problems
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Xenopus laevis are maintained as a source for fresh ova. In the past,
there were recurring problems with postoperative animals
becoming infected. We thought that a large part of this problem
was due to inadequate husbandry efforts. To minimize infections,
we decided to change the housing conditions of the animals and use more efficient filtration systems. Postoperative animals are individually housed in multitank continuous filtration systems. The high efficiency filters use diatomaceous earth to filter particles down to 1 micron in size. Since initiating this system, there have been no deaths due to infections in the preoperative or postoperative animals.

**P46 Cage Enrichment for Hamsters Housed in Suspended Wire Cages**

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Golden Syrian hamsters (n=99) were housed individually in suspended wire cages so that spilled food and excreta could be removed. After 8 days, the hamsters developed bizarre aggressive behavior which consisted of growling, hissing, aggressive posturing toward humans, destruction of water bottle rubber stoppers, and attacking objects introduced into the cage. Many developed inappetence which progressed to anorexia, depression, and unresponsiveness. Initially, we evaluated the following cage additions: a small pan with shavings, a small pan with 4.5 cm cotton nestlets (Ancare Corp.), nestlets alone, a small pan alone, and nesting boards with nestlets. Nestlets alone were selected as the best choice since the pans and boards were not used by the animals and the shavings did not remain in the cage. When nestlets were provided to all of the hamsters, their appetite and responsiveness improved, but the aggressive behavior remained unchanged. The nestlets were replaced by a 13-cm length of 5.5-cm-diameter polyvinyl chloride pipe (PVC). The water bottles were replaced by an automatic watering system. After adding the PVC, the aggressive behavior diminished in 3 days and was unnoticeable in 14 days. In conclusion, when these hamsters were provided with nesting material their well-being was improved as indicated by resolution of inappetence and depression. Providing the PVC apparently resolved the aggressive behavior problem by providing a means for seclusion in addition to functioning as a burrow and as a toy.

**P47 An Economical Nesting Box for Callithricidae**

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In compliance with the USDA Animal Welfare regulations implemented on August 14, 1991, we developed a plan for the environmental enrichment of nonhuman primates in our institution. A part of that plan called for the use of nesting boxes for all marmosets and tamarins as a means of cage enhancement. We developed an economical and easily constructed nesting box consisting of standard 4-inch PVC tubing. PVC components consist of a drainage tee connector fitted with a threaded plug as the top and a leaf screen as the floor. The tee side arm is modified to accept a stainless steel guillotine door so that the animal can be restrained within the nest box. Nest box design allows for mounting within the cage, or modified for attachment through the cage front to allow for the normal function of a squeeze-back cage.

**P48 Inexpensive Food Puzzles for Primate Enrichment**

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Providing an adequate stimulus to enrich the lives of singly housed primates can be both frustrating and costly. Frequently, the durability of the device is dependent on the destructive nature of the intended species. The expense of the item neither guarantees its durability nor a prolonged interest in the item. One solution to this problem is addressed by the construction of an inexpensive shaker style food puzzle. The required materials can be obtained in the plumbing section of any hardware store. The skill, time, and cost required are minimal (usually under $4.00). This version is constructed by cementing a male 1-1/2 inch PVC slip joint adapter into a female adapter of the same size, forming a single pipe with threaded ends. (These adapters are the same as those used in sink drain traps). Discard the washer included in the assembly, and replace the cardboard insert with end plates cut from a rubber feed container lid (or other suitable material). Drill a 5/8 inch hole in one or both end pieces and firmly screw the assembly in place. These puzzles are sanitary and easy to fill with a wide variety of textured treats. It has been our experience that changing the contents of the puzzle and varying the frequency of presentation aids in holding the interest of the primates.

**P49 A Social Housing Strategy for Rhesus Monkeys Used Frequently in Biomedical Research**

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Generally, nonhuman primates used in research are adult males which are accessed frequently and are participants in studies requiring prolonged manipulation. Acts of aggression resulting from animal removal have precluded nonhuman primates used in these conditions from being group-housed. To examine the feasibility of group-housing monkeys frequently used, a management study was conducted with 30 male and female rhesus monkeys placed in groups of 3 to 8 animals. Five of the groups were comprised of adult males. The sixth group consisted of seven subadult and adult females, and one adult male. Use of the animals and colony stability was maintained for 14 months. Ten percent of the monkeys were removed weekly from the colony for a period of 6 to 12 hours for experimental manipulation. Surgical manipulation of cerebrospinal fluid and venous catheters continued.
during the 14-month period necessitated the removal of 30% of the animals from the colony for a period of 14 to 20 days. Within this 30% cohort, 20% were removed twice for the same duration. In both the experimental and postsurgical situations the animals were returned with minor incidences of aggression. The frequency and duration of removal, necessitated by our daily research needs, did not affect our ability to socially house these animals in small groups. Therefore, when possible, group housing should be considered for frequently accessed monkeys in lieu of pair or single caging.

**P50 A Systematic Approach for Estimating Feed Requirements for Individually Housed Rhesus Monkeys in a Primate Colony**

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Feeding macaques in a controlled environment presents a problem if their body weight is to be maintained. When monkeys are fed *ad libitum*, excessive weight gain often occurs. On the other hand, weight loss or even malnutrition may occur if insufficient calories are provided relative to animal energy requirements. The purpose of this project was to evaluate a recently developed feeding regimen for individually-housed rhesus monkeys at the National Center for Toxicological Research Primate Research Facility. In calculating the number of biscuits to be provided daily (Purina Monkey Chow, Purina Mills, Inc., St. Louis, MO), we took into consideration not only the basal metabolic rates for the primates but also their levels of activity. We applied the feeding regimen to 12 adult females of average body weight 7.4 ± 1.2 kg (mean ± SD) remaining in our breeding program. Initially, the animals had been fed based on routine practice in the colony a total of 8 to 14 large biscuits (Purina 5047) daily. Implementing the new feeding regimen, they received only 7 to 10 biscuits depending on their body weight. Their diets continued to be supplemented with fruit and chewable multivitamins and they had free access to water. The animals were weighed weekly. Fed according to this regimen, the monkeys were able to maintain their initial body weight. Therefore, implementing this new feeding system reduced feed waste and maintained body weights for each monkey.

**P51 Bacterial IgA Proteases: A Comparative Study in Great Apes**

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IgA proteases produced by several species of pathogenic bacteria are putative virulence factors enabling these organisms to evade IgA-mediated immune functions including colonization and invasion of mucosa. The results of many studies have shown that these enzymes specifically cleave the hinge region of human IgA1. Other human immunoglobulins including IgA2 and serum IgA from several animal species such as Old World and New World monkeys are resistant to cleavage. Recent reports of hominoid Cα1 genes suggest that IgA from ape species would be useful substrates for further studies of the specificity of IgA proteases. We purified serum IgA from gorilla, chimpanzee, orangutan, and gibbon by affinity chromatography and incubated these substrates with each of seven species of IgA protease-producing bacteria. IgA cleavage products in supernatant fluid were detected by Western blots. In most cases ape IgA was cleaved whenever the hinge region amino acid sequence of ape and human IgA was identical at the cleavage site. Exceptions were evident in the case of streptococcal IgA proteases which cleave proline-threonine in human IgA1 at position 227-228. Gorilla and orangutan IgA, which is identical to human IgA1 at this site was cleaved by *S. pneumoniae* IgA protease. Conversely, the IgA protease produced by *S. sanguis* cleaved human but not gorilla IgA yet the IgA gene from *S. sanguis* cloned and expressed in *E. coli* produced a protease that cleaved both human and gorilla IgA. Thus, primary structure of the hinge region alone appears not to be the only factor that determines the ability of these proteases to cleave IgA.

**P52 Normal Electrocardiographic Parameters in Sedated Owl Monkeys (Aotus nancymai)**

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Although the owl monkey is widely used in biomedical research, normative physiologic data is lacking. Such information is of particular importance given the high prevalence of spontaneous cardiomyopathic disease in the species. Standard six-lead electrocardiograms (EKGs) were obtained for 43 animals on a single channel electrocardiograph. Normalcy was determined by an echocardiographic procedure. All EKGs were obtained, following sedation, with the monkey in dorsal recumbancy. Tracings were recorded for leads I, II, III, aVF, aVL, and aVF. The paper speed was set at 50 mm/sec and amplitude calibrated at 1 cm = 1 mv. All EKG leads were analyzed by a veterinary cardiologist. The lead II tracing was used for all variables measured with the exception of mean electrical axis in the frontal plane. Measured variables included heart rate, P wave duration; P-Q interval; QRS complex duration; Q-T interval; P wave amplitude; R wave amplitude in leads I, II, and aVF; Q and S wave amplitudes in leads I and aVL; and mean electrical axis. Electrocardiographic parameters in owl monkeys were significantly different from those in squirrel monkeys and macaques.
A Summary of Normal Organ and Brain Region Weights in Mice and Rats

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As a by-product of ongoing receptor studies in our laboratory, we have accumulated a large amount of data on the weights of organs/brain regions in rats and mice. These weights will be presented in tabular form, with mean, SD, SEM, and confidence levels given for each organ/region. Organ weights for CF-1 mice (female, mean weight = 30 gm., n = 150+) are: heart, lungs, liver, kidneys, spleen, and brain. Brain regions from S-D rats (male, mean weight = 300 gm., n = 200+) are frontal cortex, cortex, striatum, and cerebellum. (Mesencephalon, olfactory bulb, hippocampus, etc. with n = 15 to 50 will also be included). Structures were identified using anatomical landmarks, some autoradiographic analyses, and known radioactive ligand affinities for specific areas. While these weights may be somewhat dependent on body size, this data could be useful for others interested in what “normal” values should be and provide a guide for calculating dose response, receptor density, and other parameters involving these organs/regions.


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Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly & Company, Greenfield, IN 46140-0708

The Fischer 344 rat has been used in this laboratory since 1976 for evaluating the oncogenic potential of test compounds. Control animal data from 23 oncogenic studies conducted during the past 15 years were analyzed for temporal changes in survival, growth, food consumption, and the causes of early death. Over time, survival at the end of 2-year studies has decreased. A comparison between studies started from 1976 to 1982 and those initiated from 1983 to 1989 indicates that mean survival in the more recent studies was 23% lower for males and 6% lower for females. Decreased survival in males has become problematic, with rates decreasing to less than 50% at the end of 2 years in several recent studies. Although there was no cumulative increase in maximum body weight, as reported by the National Toxicology Program, it appeared that males achieved their maximum body weight at a younger age, which does agree with the National Toxicological Program findings. Food consumption values at the age when maximum body weight was achieved also increased over time. Considering all the major causes of early death, only the incidence of mononuclear cell leukemia (MCL) in males showed a significant increase over time. During the same time periods examined for survival, the percentage of males that died early due to MCL increased from 10% to 24%. Continued monitoring of these trends is necessary to verify that the Fischer 344 rat remains a suitable animal model for oncogenic studies.

A Per diem Rate Comparison, Interrelating Budget Size, Percent Recovery, Facility Size, Accreditation, and Cost Accounting Factors

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Per diem rate schedules vary greatly from institution to institution. To compare the per diem rate structures, 25 medical institutions or institutions affiliated with medical schools were surveyed. Four hundred and fifty-eight data points were collected from a possible 550 points. One hundred fifty of these data points allowed a profile of the institutions surveyed to be formed. The institutions’ animal facilities in the survey have an annual budget of one million or less (61.9%), recover 30 to 50% of their budget (40%), maintain greater than 30,000 square feet of animal space (66.7%), are AAALAC accredited (71.4%), and use a cost accounting system to determine rate structures (76.2%). The remaining 308 data points allowed the extrapolation of the average per diem rates for 16 species commonly found in the research environment. Of the institutions surveyed, 80% maintained at least 12 of the species in the survey. A statistical analysis will be presented interrelating per diem rates with total budget, percent of funds recovered, square footage of facility, utility recovery, and cost accounting factors.

Cost Comparison of Random-source Conditioned Versus Laboratory-raised Dogs

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A cost analysis was done comparing random-source conditioned dogs from four vendors with dogs from two vendors that were specifically bred and raised for research purposes. These two groups of dogs were compared with regard to initial purchase price and additional costs incurred as a result of disease, heartworm infections, and pregnancies. During a 2-year period, 1,877 random-source conditioned dogs were compared with 962 laboratory-raised dogs. Of the random-source conditioned dogs, 329 (17.5%) needed treatment for diseases, 175 (9.3%) were positive for heartworm, and 15 (0.8%) were pregnant. In comparison, only 17 (1.8%) of the laboratory-raised dogs needed treatment for disease, and no dogs were positive for heartworm (0%) or pregnant (0%). The average purchase price of a random-source conditioned dog was $227 as opposed to $437 for a laboratory-raised dog. However, the added incremental cost incurred because of disease, heartworm, and/or pregnancy, to make the dog acceptable for a long-term protocol, was $48.51 per dog for the random-source conditioned group but was only $2.73 per dog for the laboratory-raised group. Although the initial purchase price of the laboratory-raised dog was 101% higher than the random-source conditioned dog, after the additional incremental costs were calculated and added for both groups, the actual cost difference was 66%. Also, indirect costs including loss of time because of an unacceptable animal, primarily attributed to random-source conditioned dogs, are not reflected in this cost difference.

continued ➤
P57 Development of a Just-in-Time Inventory Control System for Daily Delivery of Animal Husbandry Supplies in a Multi-building Animal Resources Program

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Construction of a new animal research building mandated the relocation of a centralized warehouse for animal husbandry supplies. As space was limited, the development of a daily system of delivery, Just-in-Time (JIT), or stockless stockroom was initiated to reduce required storage space while gaining efficiencies related to manpower and centralized purchasing of supplies. A task force was formed with personnel from all areas that would be affected by the JIT system. This task force began by translating the broad objectives into specific actions and target dates while focusing on internal requirements and constraints. The system design parameters included ordering system integration, relative location, and overall quality of service for outside vendor selection. When vendor selection was completed, an incremental approach was used to add additional supplies from third party sources other than the major outside vendor selected until all major animal husbandry items were available. The JIT system now provides deliveries twice daily to the end user. Based on over a year of operation, we have concluded that the numerous advantages of the JIT system have greatly offset any disadvantages such as slightly increased costs.

P58 Development of a Laboratory Animal Sciences Departmental Newsletter

DM Stark

Bristol-Myers Squibb Veterinary Sciences, Princeton, NJ 08543-4000

Communication is essential to any human interaction. When managing a large group of diverse employees, frequent person-to-person communication is often not a realistic goal. Many laboratory animal facilities are growing in size beyond the point where direct interactions among staff members are feasible on a regular basis. The creation of satellite facilities or dispersed geographic sites with centralized management adds to problems of maintaining good communication. The Bristol-Myers Squibb Veterinary Sciences is a laboratory animal science program, with seven facilities in four states and more than 150 employees. As one of many efforts to enhance communication among the sites and staff, a quarterly newsletter was developed. The presentation will outline the results of national readership surveys, copyright problems, readability levels, use of clip art, design, editing, and ideas for making the communication more effective. Cost and production schedules will also be covered. Feature articles designed to help laboratory animal facility employees improve their understanding of biomedical research and their job-related effectiveness will be described in detail. The application of these techniques can also improve interfacility communication through local branch AALAS newsletters.

P59 Prototype Program for Educational Resource Materials for Grammar Schools

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Antivivisectionist groups have targeted the nation's schools for propagation of their viewpoint. Our Institutional Animal Care and Use Committee feels that the research community must encourage and assist presentations at public forums to discuss the contribution of animals to the health of humans and animals. Our facility formed a partnership with a neighboring grammar school to develop and foster interest in science and technical subjects. Through this partnership, our facility provided a Science Day for the school. Each class, pre-K through grade 6, visited our facility auditorium where educational, interactive displays promoted research and science. The Division of Veterinary Medicine developed and manned a display for the Science Day. The evolution of ideas, decisions made (and why), and the resultant display will be discussed. Many of the staff volunteered to assist with the exhibit, yet despite a common work background, strong differences in opinion emerged as to what was (is) appropriate for such a venture. This exercise became a challenge with few parameters to guide us in our decisions. Ideas that worked and didn't work will be presented as well as outlines for developing lesson plans to take into the classroom.

P60 A Rat Simulator Alternative for Beginners Handling Laboratory Animals

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The development of alternatives to laboratory animals is one of the most important activities in laboratory animal science. Although the development of alternatives to animal testing is active, the development of a simulator for animal handling and experimentation is relatively inactive. We developed a rat simulator to educate beginners who are going to handle laboratory animals. The silicone cast of skull, mandibula, laryngo-pharynx, trachea, esophagus, and stomach were taken from a male SD rat carcass. Various materials including silicone were used to imitate these organs, as well as the skin and tail vein. The ventral part of the chest was made with transparent silicone allowing the upper digestive tract and laryngo-pharynx to be observed. Rat simulators made from various materials were evaluated by several experts in laboratory animal science. The simulator was improved several times after their comments. The rat simulator is considered to be a useful educational tool for students, technicians, and others who are going to handle laboratory animals but who have not experienced laboratory animal handling, including oral catheter feeding, intravenous injection to a tail vein, and other techniques.
Supported by Grant-in-Aid for Co-operative Research by the Japanese Ministry of Education, Science and Culture

P61 Construction of a Biosafety Level 3-Facility in a Research Animal Facility

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Special consideration must be given to the construction of facilities designated for biohazard experiments in research animal facilities. At Stanford University we have recently completed the construction of three self-contained biosafety level 3 (BL-3) suites in the main centralized animal holding facility. These renovated facilities provide researchers with animal holding rooms equipped with power for centrifuges, incubators, freezers, animal caging system, bedding dump stations, and other necessary handbundsry and research equipment. In addition, emergency power circuits provide protection for equipment which must remain operational in the event of a power failure. The ventilation system was expanded and designed with ceiling ports which allow direct exhaust of flexible film isolators and biological safety cabinets. A photo hdic air pressure monitoring system was installed to ensure proper air balance. Prevention of unauthorized entry of personnel into the biohazard suites was ensured by the installation of a retinal eye scanning security system at the entrance to the BL-3 facility. Our biohazard facilities provide an optimum environment allowing researchers to work with infectious agents with minimal risk of contamination to the remainder of the animal facility.

Supported in part by NIH Grant 1G20RR05228-01.

P62 Medical Alert Card for Macaque Handlers

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Despite heightened awareness of the potential for *Herpesvirus simiae* infection in macaque handlers and the fact that most institutions strictly adhere to the guidelines established by the Centers for Disease Control for minimizing the risk of exposure to macaque secretions, another person subsequently died from this occupational disease in 1991. In an effort to respond to possible deficiencies in the overall occupational health program at the University of Texas Medical School and to prevent a similar episode at this institution, additional precautions have been instituted at every level of the program from the initial primate training classes to the interaction with the attending physician treating a person exposed to macaque secretions. Due to the worldwide mobility of those with occupational exposure to macaques (the natural host for *Herpesvirus simiae*) and the possibility that a physician removed from the primate centers might not be fully aware of this infrequent but potentially fatal disease, a medical alert card was developed to be carried by all personnel with potential exposure to macaques or their secretions. The card clearly states the source of occupational exposure, the presenting symptoms and progression of the disease, the possibility of successful treatment if the disease is identified early, and the persons to contact at the Centers of Disease Control and Southwest Foundation for Biomedical Research if *Herpesvirus simiae* infection is suspected in the patient.

P63 Effect of Irradiation with 2.5 and 4.5 Mrad and Autoclaving on the Vitamin Levels in Guinea Pig Chow (5026)

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Irradiation (I) and autoclaving (A) are methods used for microbial disinfection of diets. Both I and A influence vitamin stability, and therefore, the effect of A (121°C/30 min) and I (2.5 and 4.5 Mrad) on vitamin stability was evaluated using Guinea Pig Chow. In addition, the effect of storage (2PC) for 90 and 270 days on vitamin stability following I was evaluated. Three replicates (manufactured lots) were used and the data analyzed using analysis of variance. Levels of vitamins A, C, B₁, B₉, B₁₂, pantothenic acid, and folic acid were lower (P < 0.05) after A than after I at 2.5 or 4.5 Mrad. There was no effect of either A or I on vitamin D, choline, niacin, and biotin. Vitamins A, B₁₂, riboflavin, and folic acid were reduced (P < 0.05) at 4.5 Mrad but not at 2.5 Mrad. Vitamins E, C, and B₉ were reduced at 2.5 and 4.5 Mrad. Storage had no effect on vitamins D, E, B₁₂, niacin, pantothenic acid, and folic acid. The effect of storage on vitamin A was less severe in I diets than in the nonirradiated control. The reverse was true for B₁ and choline. These results indicate that vitamin stability was less affected by I than by A. The effects of I on stability were vitamin-dependent. Shelf life of certain vitamins was altered by I.

P64 Are You Exercising Your Dogs Yet?—A Survey of Dog Exercise Plans

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As of August 14, 1991, all research facilities housing dogs were required to have an appropriate plan, approved by the attending veterinarian, to provide them an opportunity to exercise. The plan must contain written standard procedures to be followed which comply with the “performance” standards listed in 9 CFR, Part 3, Subpart A, section 3.8. The use of “performance” standards as compared with more rigid “engineering” standards provides each research facility the opportunity to tailor their exercise plan to the specific needs of the institution and the associated research program(s). It also means that there may be significant differences in how facilities develop and implement their plan. This seemed an opportune time to compile data concerning the new dog exercise plans just as they are beginning to be implemented. A survey questionnaire containing specific continued
questions about the number of dogs the facility maintains, the amount of exercise that is provided, and the costs associated with implementing this plan was sent to 300 different research institutions. Responses were received from approximately 35% of the recipients. Results were graphed based on a variety of parameters including number of dogs housed, exercise periods offered per week, length of exercise periods, labor hours, and costs associated with implementing the plan. Correlations were drawn between related parameters.

P65 A Comparison of Four Methods for Sterilizing Surgical Instruments for Rodent Surgery
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The Guide for the Care and Use of Laboratory Animals states “survival surgery on rodents does not require a special facility, but should be performed using sterile instruments, surgical gloves and aseptic procedures to prevent clinical infections.” This recommendation presents obvious problems for researchers performing multiple rodent surgeries each day. To identify practical suggestions for sterilization of instruments between animals and to ensure that researchers follow guidelines set by the Guide and internal procedures, we evaluated four commercially available systems. Seventy percent ethyl alcohol solution, Cidex® 7-day solution, 10% povidone iodine solution, and the Inotec® Glass Bead Sterilizer were evaluated for bacteriocidal capabilities after inoculation of sterile surgical instruments with a mixture of Escherichia coli, Staphylococcus aureus, and Bacillus subtilis. All four methods met or exceeded manufacturers’ specifications. However, the Inotec® Glass Bead Sterilizer was found to have the quickest effect. In addition, this system is easy to use, keeps the instruments dry and clean, and requires minimal bench space.

P66 An Institutional Research Animal Tracking Program
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Tracking animal research programs has become an extremely important aspect of the animal research process. To ensure compliance with regulatory agencies and Institutional Animal Care are Use Committees (IACUC) mandates, an efficient, practical, and flexible program for tracking research animal use has been developed using Claris FileMaker Pro software. The primary file or Animal Procedure Statement (APS) spreadsheet with which all other files communicate, summarizes each research program submitted to the IACUC. It allows close monitoring of the IACUC review process and animal research programs. Using sorting tasks and secondary layouts, this file creates monthly summaries of IACUC-approved programs and creates memos alerting investigators to pending expirations of programs. It also enumerates programs by animal species or strain, by the associated USDA pain/distress category, by study location, by IACUC-approved exemptions, and more. The APS IACUC-approval number in the APS spreadsheet file is the common link to several other FileMaker Pro files. These linking files include an Animal Delivery file which provides daily animal delivery information. A secondary layout in this file tracks the number of animals ordered for use in a program against the maximum number of animals approved by the IACUC for use. A tertiary file communicates with the Animal Delivery schedule file to automate the production of animal cage identification cards. A second linking file to the APS file monitors the annual renewal activity of animal research programs, a third file tracks annual animal use including the USDA pain/distress category distribution for reporting to regulatory agencies, and a final linking file tracks research personnel to determine appropriate enrollment in occupational health programs. Claris FileMaker Pro is one of several commercially available programs that can facilitate the efficient and accurate tracking of animal research programs and animal use in research facilities.

P67 A Simple Record Keeping System for Small Animals
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A system was developed to track small laboratory animals from the time they arrive at the facility, are placed on study, and termination of the study. When a consignment of animals arrives, the whole batch is entered into a computer with custom-written D-BASE software. They are assigned a group number and each ascribed a facility three-digit number. This system allows for a unique numbering system. An Individual Animal Observation Record (IAOR) sheet is assigned to each animal and pertinent information is recorded (hard file and computer). All of the IAOR are put into a stock file until the animal is placed on study. A study file is opened when a study is initiated. This D-BASE file contains IAOR, Implant Records, and Explant Records. The hardcopy or manual file also contains a protocol. The IAOR and the computer software contain the study name and number, date, length of implant, and termination date. The Implant Record contains information pertaining to the study, including complications which had occurred at the time of implant. An animal's monitor and termination dates automatically come up on the weekly surgery schedule to ensure important data points are not missed. Key information is also on each animal's cage card for a quick reference. The Explant record is used to record observations during the termination. With this arrangement one is able to keep all animal and study records in an efficient organized system.
P68 Animal Facility Database Management Using an Apple Macintosh Computer and Commercially Available Software
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Record keeping is a continuous, essential part of managing an animal research facility. Finding a system that is inexpensive and user friendly is the first step to keeping accurate records. A system that is easy to use will be readily accepted by employees, and one that is inexpensive is ideal for a small facility. Using a Macintosh LC computer and commercially available software, databases can be created to keep records on their animals, budget, and inventory. We are using Filemaker II with a Macintosh LC computer to keep records of our 35 monkeys including inventory for USDA reports, experimental histories, SRV status, and an animal's location in the building. We also have databases for our budget, companies, and supplies. The program's ease of use and adaptability to many recordkeeping requirements make this system ideal for a small facility. It is also ideal for a facility on a limited budget unable to invest in a custom-made program.

P69 Use Of A Local Area Computer Network In A Laboratory Animal Resources Department
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The rapid and substantial growth in both size and scope of the Laboratory Animal Resources Department outpaced the organization's ability to effectively process required information regarding animal health and treatment records, animal care manpower and facility management data, animal protocol development and approval, requisitioning processes, and regulatory issues. Even though custom programs were written and installed on the remote mainframe computer, limitations in speed, accessibility, processing, and output were realized. To enable storage, communication, and retrieval of this data, stand-alone personal computers with a user-friendly common local network were installed, followed by an optical document scanner to enable rapid sharing of documents while reducing filing. The network's high-speed processing and large storage capacity provides the users with rapid access to data stored on the mainframe, with the added advantage of shared software packages including data and project management, computer-aided design, scheduling, electronic mail, and access to outside computer services. The advantages of speed, ease of use, and reliability of a local computer network outweigh the disadvantages of initial cost and system management, while providing the capability to easily access, manage, and share information. The system provides a powerful tool to optimize financial, manpower, and animal facility resources critical to a large laboratory animal resources organization.

P70 Use of a Relational Database for Tracking Institutional Animal Care and Use Committee Activities and Research Animal Usage
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Research institutions are required by federal regulations to maintain and report information about research animal use and activities of the Institutional Animal Care and Use Committee (IACUC). A commercially-available relational database of moderate cost was used to track animal study proposals and animal ordering and use information. The relational database allowed multiple tables to be developed and linked without the need for multiple entries of the same information. The database enabled animal facility managers to use a look-up table linking animal study proposal information with their animal ordering table to check on the approval status of the proposal and the species, strain, and number of animals approved by the IACUC. Examples of reports generated include monthly animal orders by each investigator; animal study proposals approved for each animal facility; animal study proposals involving hazardous agents; animal usage by pain category for the USDA Annual Report; and animal study proposals requiring renewal or annual review. The database program can be run on a stand-alone personal computer or can be used on a local area network with multiple users accessing the data. It has proven to be a powerful tool with ever-expanding uses as the complexities of federal reporting requirements change in the future.

P71 Effective Computerization of Animal Resource Departments
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The increase in research using animals with a decrease in funding to pay for support personnel makes accurate record keeping increasingly difficult in the areas of census, protocol files, and billing, as well as others. A viable computer system has become an accepted method of dealing with these areas. The basic custom-designed program using the UNIFY/ACECELL database on a Unix Operating System is currently in use. Refinements to this program are in progress. This system is using custom-designed, barcoded cage card labels for recording census and calculating per diem charges. Documents necessary for billing within a university system are automatically produced on demand as well as informational letters regarding changes that are sent to investigators and departmental business administrators. Agendas for the Institutional Animal Care and Use Committee and the Committee's informational letters are published through protocol information entered in the system. The results of this development have been a smoother and more professional flow of research information for the investigators and continued
departments. Fewer people are required to process this information within the animal care department until increased expansion of the facility requires additional personnel. Prepackaged computer programs for research animal facilities have generally been limiting and unsatisfactory. Often, a change in departmental management practices are required in order to effectively use these programs. A custom-designed system allows flexibility and expansion in animal care facilities as is required in today's research.

P72 Strategies for Management of a Breeding Colony of Animals with an Inherited Genetic Defect
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In our animal facility, we maintain a breeding colony of rats characterized by clinical anophthalmia. The expression of this trait ranges from apparently normal eyes to bilateral anophthalmia with a variety of intermediate forms of expression. Such variability creates difficulty not only in record keeping, but also in selection of animals to serve as breeders for the next generation. We have designed a coded ID system and, using an inexpensive computer shareware filing program, have developed a database that allows us to maintain individual records on every animal in our anophthalmic breeding colony. These records enable us to record the degree of expression of the trait and produce summaries of the variation within a litter. We also have created a database that records mating information, litter production, and a limited genetic tree. Summaries created from these databases allow us to choose animals that will serve as the breeding stock for the next generation. This system could easily be adapted to facilities breeding animals with other heritable defects.

P73 A Novel Technique to Assess Cell Viability
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Existing methods for assessing tissue viability do not provide the possibility of simultaneous assessment of cell viability and morphology. We describe a new technique combining 3H-labeled proline absorption method with autoradiography, which enables this correlation to be made. Tissue samples were cultured overnight in 1640 RPMI complete medium with 10% fetal bovine serum to promote viable cell growth. 3H-labeled proline was added and incubated for 24 hours at 37°C. The viable cells in the tissue absorbed the 3H-labeled proline as they metabolized. The samples were then washed, embedded, sectioned, and processed for autoradiography. After developing and counterstaining, the viable cells were easily identified by numerous silver grains in their cytoplasm, while the nucleus and the extracellular structures had minimal labeling. Tissue morphology was well preserved allowing a clear correlation of cellular viability and histology. The specificity of this technique was established with identically treated nonviable cells and actively growing tumor cells as negative and positive controls respectively. This new methodology is reliable and provides a useful tool in assessing cell viability of intact tissue.

Supported by Deborah Hospital Foundation

P74 A Novel Application of a Microcarrier Culture System Technique for Harvesting Chlamydia
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Diseases caused by chlamydia species are of worldwide veterinary and human medical importance. Recently, a Chlamydia trachomatis strain FeCo was isolated from ferrets with proliferative colitis in our laboratory and another Chlamydia sp. SFPD was recovered from hamsters with proliferative ileitis. Significant amounts of purified chlamydia are needed to characterize the organism on a molecular level and elucidate the pathogenesis of disease. Our laboratory has used a microcarrier culture system technique to propagate and harvest chlamydia, in particular the C. trachomatis FeCo strain, for further molecular and biologic characterization. Briefly, the technique involves culturing McCoy cells, the preferred cell line for chlamydia infection, on microcarrier beads suspended in a glass spinner vessel. When confluent cell growth is achieved, the vessel containing cell-coated beads is inoculated with chlamydia and allowed to incubate for 2 hours. Forty-eight to 72 hours after inoculation, chlamydia is harvested from the contents of the spinner flask. The contents are sonicated and centrifuged at 500 g for 10 minutes to remove cellular debris. The supernatant is centrifuged at 30,000 g for 30 minutes. The resultant pellet is resuspended in buffer, overlayed on a 32% renografin gradient and centrifuged for 1 hour at 30,000 g. The pellet is recovered, washed with buffer, and frozen at -70°C. This technique allows for the isolation of larger amounts of the organism in a shorter period of time when compared with conventional tissue culture systems. For example, the amount of chlamydia obtained from one 250-milliliter spinner vessel is approximately equivalent to that recovered from at least fifteen 150 cm² tissue culture flasks. Yields can be easily modified by adjusting cell number, flask number and volume, and microcarrier bead concentration. Modifications and improvements of this system will further augment recovery of this organism in the laboratory.

Supported by NIH grants RR01046 and RR07036; NCI grant CA26731

P75 Ascites Production Using Severe Combined Immunodeficient Mice
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A study was conducted to determine if severe combined immunodeficient (SCID) mice are as suitable a host for the
production of ascites fluid as BALB/c mice. Twenty adult SCID mice and 20 adult BALB/c mice, primed two weeks prior, were inoculated intraperitoneally with $1 \times 10^6$ cells of a mouse × mouse hybridoma line known to produce ascitic fluid in BALB/c mice. Mice were observed daily and fluid was tapped when visibly obvious. All mice were tapped twice and then euthanized for a third collection. The ascites fluid was centrifuged, frozen, and subsequently pooled and the antibody purified using a Protein A column. The quantity of raw ascites, purified antibody, and the levels of specific and nonspecific antibodies were compared. The SCID mice yielded similar amounts of ascites with greater antibody than the BALB/c mice. These results indicated that SCID mice may be a better host choice than BALB/c mice for ascites production.

P76  In vitro Fertilization Results Between Cryopreserved Mouse Gametes (Cryopreserved Unfertilized Eggs and Cryopreserved Sperm)

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In vitro fertilization was performed using cryopreserved unfertilized eggs and sperm of mice, and birth of offspring by transfer of the embryos to female recipients was investigated. For freezing, eggs were collected from C3H/He and C57BL/6 female mice. The eggs were placed in DAP213 (2M DMSO, 1M acetamide and 3M propylene glycol in PB1) in 0.5 ml tubes and then immediately immersed in LN2. Thawing was by rapid heating in water at 37°C. For freezing, sperm from the epididymis of male DBA/2 and Jcl:ICR mice were used. A sperm suspension (raffinose and skim milk at concentrations of 18% and 3%, respectively, in distilled water) was prepared and placed in tubes. The tubes were exposed to LN2 gas for 10 minutes and then immersed in LN2. Thawing was performed by leaving the tubes at room temperature. In vitro fertilization was performed using C3H/He and C57BL/6 eggs and DBA/2 and Jcl:ICR sperm. Embryos developed to the two-cell stage were transferred to recipients. The rate of development of two-cell embryos was in the 22 to 45% range. When these two-cell embryos were transferred to recipients, offspring were produced from 23 to 35% of the embryos.

P77  Superovulation and Fertilization Efficiencies Between Two Mouse Strains (Balb/c and Balb/cBy)

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Large numbers of preimplantation mouse embryos are required to determine the optimal culture conditions and for quality control testing of media for human in vitro fertilization (IVF). In most IVF programs, hybrid mouse strains are commonly used because of their high embryo production rates. However, hybrid mouse embryos may not be ideal for measuring medium quality due to their in vitro developmental hardiness. The use of inbred mouse embryos to test culture media and conditions may provide a more sensitive assay. The objective of the present study was to determine the embryo production efficiencies of two related inbred mouse strains. Mature female BALB/c and BALB/cBy (a BALB/c substrate) mice were primed on day 1 with pregnant mare serum gonadotropin (5 IU) followed 48 hours later with human chorionic gonadotropin (5 IU; hCG). Females were bred with fertile males following hCG administration. Oocytes were recovered on the morning of day 3 and the number of unfertilized and fertilized oocytes (2-cell stage embryos) were recorded. Data were analyzed by T-test or 2X2 contingency table Chi squared. A total of 3,804 oocytes were collected from 250 BALB/cBy mice (15.2% mouse), the fertilization rate was 40.0%. A total of 2,877 oocytes were collected from 183 BALB/c mice (15.7/mouse); the fertilization rate was 47.1%. There was no significant difference in the superfertilization response between the two strains. BALB/c mice had a significantly higher fertilization rate than BALB/cBy mice ($P < 0.001$). Therefore, the BALB/c mouse strain may produce suitable numbers of embryos which may be suitable to test media and/or culture conditions. However, additional studies are required to determine the developmental potentials of the BALB/c mouse embryo.

P78  A Device for Increasing Efficiency of Rat and Mouse Tattooing

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Permanent and humane identification of laboratory animals from day-old to maturity is being done at many laboratories using tail, ear, or body tattoos employing AIMS black pigment #242. In conducting large studies, speed of tattooing is an important factor. Tattooing usually involves dipping the tattoo needle into pigment before application to the skin. To improve tattooing speed, a micropipetystempump was employed to deliver pigment via flexible plastic tubing to a stainless steel cylinder soldered on the tattoo needle. The pump was activated with a foot switch which also started the tattoo equipment. Separate rheostats regulated the speed of the pump and tattooing unit. Using this device, pigment flowed into the tattoo in microliter quantities as the needle penetrated the skin. In rats and mice the tattoos were darker than those obtained with the dipping procedure, since the tattoo site was saturated with pigment. Tattooing speed increased 20 to 50%, depending on the tattooist and the number of digits applied to the tail. The more digits, the greater improvement obtained in tattooing speed. More rapid tattooing reduced time involved in restraint and minimized animal stress. The pigment delivery device and modified needle improved efficiency of tattooing.
P79  Measurement of Intraocular Pressure by Tono-Pen® II Applanation Tonometry in Normal and Buphthalmic Rats

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Measurement of intraocular pressure in rats is typically performed by invasive techniques while an animal is anesthetised (Ophthal. Res., 9:247D253, 1977). We assessed the usefulness of the Tono-pen® II applanation tonometer (Oculab, Glendale, CA) to measure intraocular pressure in rats. Intraocular pressure was measured in both three month-old clinically normal (27 males and 24 females) rats and in buphthalmic rats (4 males and 1 female) from a Wistar-derived colony with a low (0.2%) incidence of spontaneous buphthalmia. Following administration of one drop of proparacaine (Ak-Taine® 0.5%, Akorn, Abita Springs, LA) to the eye, intraocular pressure was measured by applying the tonometer to the cornea. The tonometer determined an average reading based on individual measurements with ±5% variance. The average intraocular pressure of eyes of all normal rats was 19.89 ± 4.70 (mean ± SD). No significant difference (P < 0.01) in pressure between left (20.35 ± 4.63) and right eyes (19.43 ± 4.77) or males (19.90 ± 5.73) and females (19.75 ± 5.03) was noted. The intraocular pressure of buphthalmic eyes was significantly lower (10.6 ± 2.90) compared to eyes of normal rats. Hypotension associated with buphthalmia is likely due to damage of the ciliary body epithelium and subsequent decreased aqueous production. In summary, intraocular pressure measurements similar to those of other domestic animals were obtained with the Tono-pen® applanation tonometer of clinically normal rats. The results of this study suggest that Tono-pen® applanation tonometry is a useful technique for measuring intraocular pressure in clinically normal rats.

P80  A Modified Technique for Endotracheal Intubation in Rats

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Endotracheal intubation and ventilation is essential during intrathoracic surgical procedures. In some species, including rats, direct oral intubation is not consistently successful and tracheotomy may lead to the development of a one-way valve resulting in subsequent asphyxiation. In response to the potential for these complications, we developed a new technique. In 30 anesthetized adult Wistar rats, the larynx was exposed via a 1 cm midline vertical neck incision. The lymph nodes were reflected laterally and the peritracheal muscle split along its longitudinal fibers. Once the hypopharynx and trachea were visualized, a cannula mounted over a blunt, anteriorly curved trocar was introduced orally. The translucency of the hypopharynx allowed direct visualization of the cannula as it traversed the larynx into the trachea. The position of the catheter was confirmed by direct palpation through the tracheal rings. The trocar was withdrawn, the cannula secured to the side of the mouth in preparation for mechanical ventilation, and the neck incision closed in two layers. In the procedures performed, all 30 rats underwent successful, safe, and uneventful intubation and ventilation. The technique is a safe and reproducible method for endotracheal intubation which reduces the risk of traumatizing the structures of the hypopharynx and larynx, provided the appropriate gauge cannula is used.

P81  Cytocentrifugation for Rapid Estrous Determination in Rats

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Estrous cycles in rodents are used to determine experimental parameters in many areas of research such as neurophysiology, endocrinology, and behavior. Stages of the estrous cycle are usually determined by microscopic examination of vaginal cytologic smears. The major disadvantage of the smear technique is cell distortion leading to possible misinterpretation. Cytocentrifugation applied to vaginal cell preparation provides a monolayer of flattened cells with minimal cell distortion and folding. Small sample amounts are uniformly distributed on the slide with a high concentration of various exfoliated cell types. Ten Sprague Dawley rats were used to obtain control samples and experimental samples at various stages of the estrous cycle. The experimental samples were obtained by flushing the vaginal vault with normal saline and aspirating the exfoliated cells. The samples were prepared by cytocentrifugation and prepared with a modified Wright-Giemsa stain. The advantages of this technique are high-quality slides providing rapid information for staging of the estrous cycle, distinctive cell presentation and consistent results. Research applications include timed breeding programs, rapid evaluation of abnormal cell types and bacteria, and accurate reproducible estrous staging.

P82  Construction and Calibration of Economical Pulsed Doppler Transducers for Measuring Blood Flow Changes in Chronically Instrumented Rats

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Although pulsed Doppler transducers have been commercially available for several years, most are not designed for repeated use and are therefore prohibitively expensive for many laboratories, even in subassembly form. We describe the construction and calibration of a single-use transducer at a fraction of commercial costs. Piezoelectric crystal material was purchased in one-inch circles from Transducer Products (10 MHz; Goshen, CT) or squares from Valprey-Fischer (20 MHz; Holliston, MA). Crystals were cut into individual two to four square mm sections with a scalpel blade, and attached to pre-
tinned 36-gauge silver plated copper wires using a dissecting scope and controlled-temperature soldering station. After flux removal, the crystal and distal ends of the wire were encapsulated in degassed epoxy. Acoustic baffling was applied to the back side of the crystal to prevent a detection of Doppler shift from surrounding tissues. After overnight curing (40 to 50°C), the crystal was mounted directly on stainless steel tubing with an outside diameter equivalent to the vessel under consideration at a 45-degree angle. Suture material for positioning and tying the transducer was applied, and the crystal was covered with self-leveling silastic. Transducers were placed on the ascending aorta via a parasternal approach and calibrated two to three weeks later on anesthetized rats. Mean Doppler shift was significantly correlated with ascending aortic flow as determined with the reference microsphere technique (n = 27 observations in six rats; r = 0.96, p = 0.0001). This economical construction technique can be applied to Doppler transducers for a variety of vessels and species.

P83 Adrenal Medullectomy as an Alternative to Complete Adrenalectomy for Studying Allergic Response in Guinea Pigs

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Epinephrine and corticosteroids modulate the severity of the allergic reaction in those with asthma. Adrenalectomy has been employed in animal models of bronchoconstriction to negate these hormonal influences. However, necessary steroid replacement profoundly alters allergic reactivity. Two-stage surgeries were performed on ovalbumin (OA)-sensitized, male, Dunkin Hartley guinea pigs using isoflurane anesthesia. In an initial study, eight animals underwent complete bilateral adrenalectomies and were supplemented daily with steroids. Airway reactivity to aerosol OA was not different from nonsurgical controls. A new method of bilateral adrenal medullectomy (BAM) using a suction technique was then evaluated. Two groups of eight guinea pigs underwent either BAM or sham operations then were given brief postsurgical steroid therapy. No postoperative complications occurred. Blood chemistries were not significantly different between groups. BAM guinea pigs were five-fold more sensitive to aerosol OA than were sham-treated guinea pigs. Reactivity to acetylcholine was the same in both groups indicating normal contractile capacity. Medullectomized animals had significantly lower plasma epinephrine levels, and mild or no adrenal cortical damage was seen histologically in 12/16 adrenals in the group. Results show that adrenal medullectomy is a useful method to study the effects of catecholamines on the allergic response.

P84 Catheterization of the Urinary Bladder in Ferrets

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The technique of catheterization of the urinary bladder, an important clinical skill for diagnosing urinary tract disorders, has not been described for ferrets. The bladder was catheterized in 20 ferrets (18 females; 2 males) using a 3-1/2 French, red rubber urethral catheter fitted with a steel wire stylet. Ferrets were anesthetized with isoflurane or ketamine/xylazine and prepped by conventional methods. Females were positioned in ventral recumbency with the rear quarters elevated by a rolled surgical towel and the catheter was passed by direct visualization of the external urethral orifice using a vaginal speculum. The orifice was approximately 1 cm cranial to the clitoral fossa on the ventral floor of the vaginovestibule. In males, the distal end of the penis was exteriorized from the prepuce and the external urethral orifice cannulated without stylet. No difficulty was encountered in advancing the catheter past the os penis. This catheterization technique allows urinary tract access for urine collection, pneumocystography, contrast cystography, double contrast cystography, and urine output determination in pharmacological studies or in critical care of debilitated animals. Supported in part by grants RR01046 and RR07036

P85 The Isolation of Feline Natural Killer Cells from Bone Marrow Cells

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Our interests focus on the role of natural killer (NK) cells in the immunology of AIDS infection, using feline immunodeficiency virus infection in cats as a model. We developed a method for isolation and further study of feline NK cells. We defined the NK cell as cytotoxic to FL 74 feline lymphoma cells in the absence of MHC restriction, negative for CD3, CD4, and CD5, and positive for large granular lymphocyte morphology. When peripheral blood lymphocytes (PBLs) were compared with bone marrow cells as a source for cultured NK cells, we found that both are cytotoxic in a dose responsive manner and have large granular lymphocyte morphology. In contrast to mice and humans, we found that the large granular lymphocytes are nonadherent to plastic. Nine-day cultured PBLs were positive for CD3, CD4, and CD5 by flow cytometry analysis. In contrast, bone marrow cells were negative for CD3, CD4, and CD5 after nine days in culture. Since T-cell lymphocytes are capable of natural cytotoxicity when cultured with high levels of IL-2, the PBL as source of NK cells is confounded by the presence of T cells. Therefore, we conclude that bone marrow cells are the preferred source of cells for isolating and studying feline NK cells. Supported by NIH grants RR00890 and RR07031

continued ➤
P86 Influence of Flushing Technique on the Infection Rate of Chronic Vascular Access Ports in Beagles
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Maintaining patency and freedom from infection is crucial to the long-term success of chronic indwelling catheters. We investigated the effects of variation in skin preparation and frequency of flushing the vascular access port (VAP®) on catheter patency and infection rate. Sixteen beagles with venous VAPs were monitored for 12 months. Four groups were defined by type of skin preparation and flushing interval: 1) full surgical scrub — flushed weekly, 2) full surgical scrub — flushed biweekly, 3) modified surgical scrub—flushed weekly, and 4) modified surgical scrub—flushed biweekly. Monthly samples of VAP blood were obtained to assess bacteriologic contamination, complete blood counts and chemistry values. Body weights were recorded. There were no statistically significant differences in any parameter (Fisher’s Exact and contingency table Chi Square tests). Although not statistically significant, there were differences in the frequency rate of infection during this study. Group 3 dogs were twice as likely to become infected compared to Group 4 animals. Groups 1 and 2 had the lowest infection rate. Coagulase-negative Staphylococcus species was the most common contaminant cultured. VAPs of 14 dogs remained patent for the 12 month period; two ports were lost due to mechanical failure of the dome. Based on this study, it appears that any of these protocols satisfactorily maintains VAP in beagles for up to 1 year.

P87 Intratracheal Instillation of a Liquid to Neonatal Beagle Dogs
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We developed a simple technique for intratracheal instillation of liquids to neonatal dogs. Neonatal dogs were chosen as an animal model for testing drugs given intratracheally to premature infants. Using a small animal anesthesia chamber, the desired level of anesthesia was reached. The animal was then restrained in a vertical position so that the pharynx could be viewed with the aid of a laryngoscope. When the opening to the trachea was observed, a three inch long, 18-gauge, ball-tipped, slightly curved, stainless steel needle was gently placed approximately 1 cm into the trachea. Verification of needle placement was done by observing the movement of the glottis around the needle during inhalation and exhalation. Liquid was slowly administered for 2 to 3 minutes during inhalation. After the liquid was administered, a 0.3 milliliter flush of air was delivered to ensure full placement of the liquid. A dose volume of 5 milliliters per kilogram body weight could be administered repeatedly without respiratory complications. This technique allows for rapid anesthetic recovery without adverse effects. Intratracheal instillation of a liquid to neonatal dogs is a practical technique for use in the laboratory. Supported by Hazleton Wisconsin, Inc.

P88 Telemetry in Marmosets
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We describe the continuous measurement of blood pressure, heart rate, and motor activity in conscious marmosets freely moving in their home cages. Consistent diurnal variations in these parameters were observed under standard conditions that were sensitive to changes in the environment. Blood pressure values were similar to those measured by nontelemetric methods in conscious restrained marmosets. However, heart rate was significantly lower indicating that telemetry is less stressful. An acute or a prolonged treatment with converting enzyme inhibitor of marmosets maintained on a low-sodium diet induced a decrease in blood pressure as previously observed using nontelemetric methods. However with telemetry, more accurate information about the duration of response and the effects of the treatment on the diurnal rhythms was obtained. The converting enzyme inhibitor did not affect the diurnal rhythm of blood pressure but did influence the heart rate rhythm. These observations demonstrate the advantages of this telemetry system for evaluating the hemodynamic effects of drug treatments under physiologic conditions.

P89 A Novel Noninvasive Technique For Measuring Specific Airway Resistance in Conscious Squirrel Monkeys (Saimiri sciureus)
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A double plethysmograph offers a means of measuring specific airway resistance, defined as airway resistance times thoracic gas volume, in trained unanesthetized squirrel monkeys. The system consists of a leak-proof plexiglass bodybox (the thoracic plethysmograph) enclosing a seated monkey with an airtight seal at the neck and a head plethysmograph with an airtight nasal seal chamber. The noninvasive technique is based on the time delay between the thoracic and nasal volume changes, which is a function of the compressibility of air and the airway resistance. A computerized analyzer measures lag time between the two waveforms at the transition point from inspiration to expiration and derives a value for specific airway resistance. Our measurements provide consistent and reproducible baseline specific airway resistance values in monkeys that are directly comparable to those measured using standard invasive studies. One of the advantages of this method over standard invasive techniques is that it provides a sensitive, rapid, noninvasive way to measure changes in pulmonary mechanics in primates and other species. Additionally, the results obtained with the double plethysmograph technique compare well with those derived in humans using the whole-body plethysmograph.
P90 Chronic Intramuscular Cocaine Exposure in Pregnant Rhesus Monkey

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We studied the effect of chronic cocaine administration on pregnancy and fetal outcome in monkeys. Cocaine was injected intramuscularly (1.0 mg/kg) three times a day, 5 days/week to pregnant animals beginning at about gestational day 24 and continuing until parturition. Three groups of three animals each were dosed with saline, 0.3, or 1.0 mg/kg of cocaine. A 4th group of three animals was given gradually increasing doses of cocaine of up to 8.5 mg/kg. Because cocaine is a potent vasoconstrictor and the average number of injections to be given each animal during the experiment was large (mean = 290), a schedule was developed to minimize the number of times a specific site would be injected. Ten injection areas, each with three sites, were identified for all monkeys. Use of each site was rotated so that each area was used only once every 10th injection. Before the study, animals were trained to present limbs upon request to reduce stress associated with handling. The condition of injection sites was monitored daily and fetal health was monitored periodically via ultrasonography. The success of these procedures was evidenced by the absence of any abscesses, infections, tenderness, or demonstrable pain in any of the injection areas and by successful parturition in all animals.

Supported by NIDA/NCTR IAG #24-89-0003

P91 Inferior Vena Caval Catheter Placement in Yucatan Miniature Swine

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Yucatan miniature swine are used as a large animal model of autologous and allogeneic blood or bone marrow stem cell transplantation. These procedures require an easy and high volume venous access for blood samples, apheresis, administration of drugs, and transplantation. This is achieved by using a 9.6F silicone rubber catheter (Hickman) in the inferior vena cava. The pig is anesthetized by Halothane/oxygen and placed under a fluoroscope in the left lateral decubitus position. An indelible mark is made on the skin of the back 2.5 to 3 cm to the right of the midline and 4 cm above the level of the iliac crest. This is slightly caudal to the space between the transverse processes of the third and fourth lumbar vertebrae. After careful sterile preparation with alcohol and povidone iodine, a 15 cm 18 gauge thin wall needle is passed through a small stab incision, made with a No. 11 blade, and directed medially, ventrally and slightly cephalad until it advances beyond the lateral edge of the vertebral body. The needle is then advanced 3 to 4 cm and, with suction applied, withdrawn slowly until venous blood from the inferior vena cava is drawn up. A guide wire is passed through the needle into the inferior vena cava, the needle withdrawn, and a 12 French dilator passed over the guide wire. The dilator is then removed and the catheter which has been cut to length, so that the end is just below the diaphragm, is passed over the guide wire. The dacron cuff on the catheter is positioned about 1.5 cm below the skin surface and secured with 3-0 prolene suture. The catheter is flushed daily with heparin (1,000 U/ml) to maintain patency. This procedure has been very successful with no infection at the catheter site. The average catheter lifespan has been 45 days with the longest still working 130 days after placement.

Supported by State of Nebraska LB506 Funds

P92 Effects of Isoflurane Anesthesia on Glucose Tolerance and Insulin Secretion in Yucatan Minipigs

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Isoflurane's effect on intravenous glucose tolerance and insulin secretion was studied in six adult Yucatan minipigs. Unanesthetized animals, with previously placed indwelling catheters, were tested while resting comfortably in a sling. The same animals were then retested while receiving isoflurane anesthesia. Serum glucose and insulin concentrations were measured at predetermined times in response to a 0.5 ml/kg intravenous bolus of a 50% dextrose solution. The glucose disappearance rates (k) were significantly higher (1.61 ± 0.11 vs 1.132 ± 0.098; P < 0.02) in the unanesthetized animals. The baseline plasma insulin concentrations (P < 0.03), the area under the insulin response curve (P < 0.03), and the insulinogenic index (P < 0.01) were significantly lower in the anesthetized animals compared to controls. This study indicates that isoflurane anesthesia significantly alters the glucose/insulin response to an intravenous glucose tolerance test and therefore, is not suitable for studies where glucose tolerance is to be assessed.

Funded by MUSC Institutional Research Funds

P93 Tiletamine-Zolazepam Pharmacokinetics in Sheep

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A single-dose tiletamine (T)-zolazepam (Z) combination was shown to provide intermediate-duration anesthesia in sheep (AJVR 52:1441, 1991). However, a biphasic cardiopulmonary response was also noted. Similar effects have been reported for several species and the mechanism is uncertain. We examined T and Z pharmacokinetic differences as a possible explanation for the biphasic response. Twelve sheep, six sheep in each of two T-Z dose groups (12 and 24 mg/kg, i.v.), were prepared with both venous and arterial catheters. Blood samples were anaerobically withdrawn from the arterial catheter immediately before and after T-Z, and 1 minute after T-Z. Subsequently, continued ▶
samples were taken every 5 minutes for the first hour and, thereafter, every 15 minutes, up to 120 minutes. Blood T and Z concentrations were measured by capillary gas chromatography with a nitrogen-selective detector. Arterial oxygen partial pressure was measured as a marker for the biphasic response. Preliminary results suggest the biphasic response is directly related to both T and Z plasma concentrations. Moreover, T and Z concentrations are quantitatively similar at each sampling time. Further experiments are underway to elucidate this phenomenon.

**P94 A Successful System for Long-term Infusion in Cattle**

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Previous attempts to design a long-term, low maintenance predictably efficient, animal-proof infusion system for cattle have been marginally successful. The use of a vascular access device in conjunction with a unique infusion saddle or a suspended infusion system has proven to be successful for long-term infusion in cattle. Vascular access devices were surgically implanted in cattle. Tunnel infection was minimized by collagen infiltrating the percutaneous port constructed of a woven matrix fitted with a polyurethane catheter. The port and external catheter were installed dorsal and medial to the scapula with the internal catheter extending subcutaneously to the jugular vein where it accesses the vascular system. The externalized catheter is connected to a battery-powered infusion pump housed on a saddle fitted to the animal or suspended above the animal. This procedure has been performed on 72 lactating Holstein dairy cows and 24 growing Holstein steers continuously infused for 60 and 36 days, respectively. The catheter remained patent in 100% of these animals with less than 5% experiencing tunnel infection. These results demonstrate the low maintenance, efficiency, and animal inaccessibility of this long-term infusion system in cattle.

**P95 Validation of a Chicken Expired-CO_{2} Respiration Chamber**

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Chickens are used as an animal model in radiolabeled metabolism studies. Validation of an enclosed respiration chamber is necessary to determine expired carbon dioxide recoveries. The actual space inside the chamber is 14.5 in. long $\times$ 16 in. wide $\times$ 15 in. high. Air is drawn into the chamber through holes on the top of the chamber by use of a vacuum pump and is removed from the bottom through holes located in the corners. For validation, airflows through the chamber were measured by two flow meters, one for total volume (15 L/minute) and one for the percentage of air trapped (1.5 L/minute). The system was validated by measuring the recovery of $^{14}$C_{ CO_{2}} generated when 1N sulfuric acid was added to a known amount of $^{14}$C-sodium carbonate (20 to 30 $\mu$Ci) within the chamber. The carbon dioxide trapping solution consisted of a 400 mL mixture of 2-ethoxyethanol and ethanolamine (1:1). Collections of the mixture were made up to 4 hours postreaction. Radioanalysis was done by direct counting using a Packard scintillation counter. The results of this validation after three trials indicated a total mean recovery of 103%. This respiration chamber is suitable for total recovery of expired CO_{2} collections. Quantitative analysis is reasonable, but not absolute.

**P96 Allometric Scaling for Calculating Dosages of Pharmaceuticals for Reptiles and Questions of Surface to Volume Scaling**

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Metabolic scaling is used to estimate pharmaceutical dosing for reptiles, as there have been few studies to determine optimum doses in snakes. Since the surface area to volume relationship of an animal is an important portion of metabolic scale, the question arose: How does snakes' surface area to volume scale? One hundred forty-two snakes (103 Colubridae and 34 Boiidae) were weighed, volume determined by water displacement, and measured. With the snake partitioned into 4 quarters length, and circumference at the nose, one quarter, one half, and three quarters of length were measured in centimeters. Surface and volume were calculated by using the formula for a truncated cone; quarters 1 and 3 — a cylinder, quarter 2 — a cone, quarter 4. Regression yielded the equation, volumes 3 to 38,000 cc:

\[
\text{SURFACE AREA} = 14.25 \text{ cm}^2 \times \text{VOLUME}^{0.5} \quad R^2 = .99
\]

Surface area to volume of these snakes scaled isometrically, scaling exponent of 0.66, supporting the use of metabolic scale for estimating pharmaceutical doses.

Supported by UVM funds College of Veterinary Medicine