
ABSTRACTS OF SCIENTIFIC PAPERS

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Platform Sessions

PS01 Detection of *Pasteurella multocida* Antibodies in Rabbits by Monoclonal Antibody Capture Enzyme Immunoassay

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Infection with *Pasteurella multocida* is commonly encountered in rabbits. Although culture is frequently performed to detect infection, negative culture results in infected rabbits or those with occult infections prompted development of serologic tests for antibodies. However, in general, these bacterial lysate enzyme-linked immunosorbent assays lack specificity. Thus, a murine monoclonal antibody that reacts with a 37-kDa polypeptide of *P. multocida* serotype A:12 was used in an IgG capture enzyme immunoassay (EIA) to detect *P. multocida* infection. The 37 kDa antigen was selected because it was identified as immunodominant during *P. multocida* infection in rabbits. The sensitivity of the P37 EIA determined using sera from 56 rabbits infected with *P. multocida*, was 98%. Specificity, evaluated with sera from 62 rabbits from colonies free of *P. multocida*, was 92%. Titration curves of sera from rabbits immunized with *P. multocida* serotypes A:3 or A:12 coincided, indicating that the P37 EIA was equally efficient in detecting antibodies to the two major serotypes of the organism. Comparison of the P37 EIA with the current serodiagnostic test, a bacterial lysate EIA, revealed relatively good correlation ($r=0.68$). However, specificity was greatly improved; 34% of uninfected rabbits had false-positive results of the lysate EIA, whereas only 3% of uninfected rabbits had false-positive results of the P37 EIA. The coefficient of variation for within-day tests was 10%, and was 14% for between-days tests, indicating good reproducibility. The greater sensitivity and specificity of the P37 EIA should appreciably enhance diagnostic capability to identify rabbits infected with *P. multocida*.

PS02 Phenotypic and Genotypic Relationship of *Pasteurella pneumotropica* Biotypes and Additional Selected Rodent *Pasteurellaceae*

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Bacteria belonging to the family Pasteurellaceae have a widespread distribution in colonies of laboratory rodents throughout the world. *Pasteurella pneumotropica*, as well as other species found in rodents, have not yet been fully characterized. Their phenotypic characteris-

tics and taxonomic position are presently unclear. We previously determined up to 40 biochemical criteria for 2,000 isolates and formed groups of bacteria with similar phenotypic properties by exploratory statistical methods. As a second step, 16S rRNA sequences were determined for typical isolates of major phenotypic groups to get a taxonomic classification. A 1,530 base-pair fragment of the 16S rRNA gene was amplified by polymerase chain reaction and directly sequenced, using the dideoxy method of Sanger. A computer-assisted multiple alignment of all available Pasteurellaceae sequences was performed and used for computation of a phylogenetic tree according to the neighbor-joining method of Saitou and Nei (1987). Our data indicate that members of different phenotypic groups of *P. pneumotropica* form a cluster together with V factor-dependent rat strains (yet unclassified *Taxon B*). Sequence differences between reference strains of *P. pneumotropica* type Jawetz and *P. pneumotropica* type Hevl indicate that they might belong to different genera. Another cluster is formed by *Actinobacillus muris* together with *Haemophilus influenzae* and additional rodent isolates. Another isolate representing a rat-specific phenotypic group belongs to a separate cluster. Our phenotypic and genotypic studies indicate that various *Pasteurellaceae* exist in colonies of laboratory rodents. The *P. pneumotropica* complex even contains V factor-dependent bacteria that were formerly classified as *Haemophilus* sp. Members of the family have low pathogenic potential, but all species are obligate parasites of vertebrates. As a consequence of the presently unclear taxonomy it seems necessary that all rodent Pasteurellaceae should be classified as far as possible and mentioned in health reports. Additional information is necessary about transmission between species and their influence on animal physiology.

PS03 Molecular Epidemiologic Evaluation of *Pasteurella pneumotropica* in a Laboratory Rodent Colony via Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) Analysis

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Pasteurella pneumotropica is a common opportunistic pathogen of mice, rats, hamsters, guinea pigs, and other rodents. Although this organism is widely considered to be of low clinical significance, episodes of overt disease involving principally the respiratory system, ocular adnexa, uterus, and subcutaneous tissues have been reported. Recent evidence suggests that variability among lipopolysaccharide antigens may correlate with virulence factors and host specificity for this agent. Biochemical differences among isolates have led to the recognition of three principal biotypes of *P. pneumotropica*, along with several in-

intermediate strains, which further complicates efforts to accurately describe field isolates of *P. pneumotropica* obtained from routine surveillance or disease outbreak investigations. We developed a randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) assay for *P. pneumotropica* that provides a fast and resource-efficient method of classifying individual field isolates with high specificity. Using three commercial 10-mer deoxynucleotide primers of arbitrary sequence (OPL-7, OPL-11, and OPL-12) under low-stringency PCR reaction conditions (1 min, 94°C; 1 min, 34°C; and 2 min, 72°C), sets of highly consistent RAPD marker profiles were generated that provided genetic differentiation of laboratory reference strains (n = 7) of all three *P. pneumotropica* biotypes and of *Actinbacillus muris*. Through a pilot survey conducted as follow-up to a uterine abscess attributed to the agent in an adult female C57BL/6NCrIBR mouse, we then isolated *P. pneumotropica* from oropharyngeal and other tissues of mice (n = 30), hamsters (n = 6), and rats (n = 3) housed in nearby rooms of the same animal facility. The RAPD-PCR technique was used to document presence of four genotypes of *P. pneumotropica* in the facility, two of which were confined to rats and hamsters. This technique quickly and unambiguously discriminated among two biotypes of *P. pneumotropica* recovered from mice, identified two additional genetic groups for the rat and hamster isolates, and clearly distinguished each strain of *P. pneumotropica* from other related bacteria. This novel molecular technology may significantly help advance studies involving the epidemiology, pathogenesis, and taxonomy of *P. pneumotropica* and other infective agents of laboratory animals.

PS04 Genetic Quality Testing of Cell Lines Derived From Experimental Animals

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Genetic quality of in vitro cultured cell lines derived from experimental animals should be considered, because genetic quality can be destroyed by the following three factors: changes at chromosomal and DNA levels, errors, and contamination between different cell lines. The first change is inevitable, but the second and the third changes can be prevented, using genetic techniques, because these errors will lead to contamination and spread of contaminated cell lines to other facilities. In this study, we found that cell lines can be certificated, using polymerase chain reaction (PCR) assay. Twenty-three in vitro cultured cell lines derived from the BALB/c mouse strain according to the records were introduced from JCRB. They were cultured in a CO₂ incubator, and genomic DNA was purified from each line according to the standard method. The PCR technique was applied to identify the animal species and strain. Primers were used to amplify part of the 28S ribosome DNA and simple sequence repeats (also called microsatellites). The PCR products were electrophoresed, using NuSieve 3:1 agarose to detect their molecular size. The 28S rDNA PCR products of all the cell lines were clearly differentiated from those of other animal species according to the molecular sizes and band patterns. Simple sequence length polymorphisms (SSLPs) of twelve microsatellites on different chromosomes were detected, and genetic profiles of the cell lines were compiled. By comparing their genotypes and the standard genotype of the BALB/c strain, 22 lines were identified as the BALB/c strain, but one line was not identified because it had genetic heterozygosities at several microsatellites. The PCR products of 28S rDNA were useful for differentiation of animal species, and microsatellites were useful for identification of the mouse strain.

PS05 Development of a Reverse Transcription Polymerase Chain Reaction Method for the Detection of Lactate Dehydrogenase-Elevating Virus in Mice

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The standard method used to diagnose lactate dehydrogenase-elevating virus (LDV) is detection of increased serum lactate dehydrogenase (LD) activity in infected mice. This is an indirect method of detection that can cause incorrect results due to nonspecific increases or decreases in serum LD values. We developed a novel reverse transcription polymerase chain reaction (RT-PCR) method specific for LDV in which the RT and amplification steps were carried out using a one-step method with rTth DNA polymerase. Four sets of oligonucleotide primers were chosen and synthesized on the basis of published nucleotide sequence of LDV-C. Mice were experimentally infected with a 10⁷ infective dose of three strains of LDV. Serum or plasma from LDV-infected mice was harvested, and the RNA was extracted using several methods. The LDV-specific fragments were amplified from the three strains of LDV irrespective of the nucleotide differences in the primer regions and not from four other common mouse DNA and RNA viruses. The detection level of cDNA and cRNA was 10² copies of LDV, using one primer pair, primer D. This level of sensitivity is sufficiently high to detect chronically infected mice that have a persistent viremia of about 10⁴ to 10⁵ LDV/ml. This is the first description of a one-step RT-PCR method to detect animal RNA viruses and suggests that RT-PCR is a very sensitive method for identifying different LDV strain infections in laboratory mice.

PS06 Characterization of Two Newly Recognized Rat Parvovirus Strains

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Kilham's rat virus (KRV) and H-1 virus are well-characterized parvovirus species that infect rats. Recently, additional parvoviruses have been identified in rats and designated as rat parvoviruses (RPVs). In this study, we characterized two strains of RPV (RPV1 and RPV2) and determined prevalence of infection in laboratory rats. The nucleic acid sequences of RPV1 and RPV2 were determined for 1,040 base pairs within the NS1 gene and for 560 base pairs within the VP1 capsid gene. Comparison of the inferred protein sequences between RPV1 and RPV2 revealed > 94% homology in the NS1 protein, but only a 68.1% homology within the VP1 capsid protein. These data indicated that RPV1 and RPV2 are distinct strains. Comparison of RPV1 and RPV2 with other murine parvoviruses revealed high homologies within the NS1 protein, ranging from 91.9 to 99.5%. However, homologies within the VP1 capsid protein were lower, ranging from 65.4 to 88.6%. Comparison of RPV1 capsid homology with HaPV, a newly-recognized parvovirus isolated from hamsters, revealed 99.4% homology. This high homology suggests that RPV 1 and HaPV are strain variants of each other. Evaluation of the prevalence of RPV in laboratory rats by a polymerase chain reaction (PCR) assay that detected sequences to either RPV1 and RPV2 indicated that > 90% of rats were test positive. This high percentage of PCR-positive rats suggests that RPV is prevalent in laboratory rat colonies and may pose a threat to large numbers of rat-based research investigations.

PS07 Maternal Antibody Protection Studies With Two Autonomously Replicating Parvoviruses of Laboratory Mice

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Until recently, minute virus of mice (MVM) was thought to be the only autonomously replicating parvovirus of laboratory mice. However, a recently recognized mouse parvovirus (MPV) has circulated at high prevalence in mouse colonies for at least 25 years. The MPV is not pathogenic, but induces T-cell dysregulation in murine transplantation and tumor allograft models. The predicted amino acid sequences of MVM and MPV nonstructural proteins are almost identical; however, their capsid sequences are highly divergent. The purpose of this study was to determine whether maternal antibody could protect neonatal mice against homologous or heterologous parvovirus challenge. Neonatal (< 24 h old) Swiss Webster mice born to dams that were inoculated with MVM, MPV, or vehicle (saline) were challenged oronasally with saline, 180 median infective doses (ID₅₀) of MVM or 1,800 ID₅₀ of MPV. The higher challenge dose of MPV was used because neonatal Swiss mice are relatively refractory to infection with this virus. Intestine, spleen, and kidney specimens were harvested at 6 days after challenge for *in situ* hybridization with the homologous random-prime probe. Blood samples were collected at several intervals to measure maternal antibody decay. Antibody titers in sera of pups derived from MVM- and MPV-immune dams were relatively low and decreased to undetectable values in < 6 weeks. Among MVM-challenged mice, none of nine pups born to MVM-immune dams became infected, whereas nine of nine pups of MPV-immunized dams had signal-positive tissues ($P < 0.005$). None of 21 MPV-challenged pups born to MPV-immune dams became infected, whereas seven of 16 pups of MVM immune dams were signal-positive ($P < 0.005$). Despite the low proportion of signal-positive MPV-challenged pups from saline-inoculated and MVM-immunized dams (6/12 and 7/16, respectively), protection of homotypically challenged mice was significantly greater than that of heterotypically challenged mice. These data suggest that the nonstructural proteins of MVM and MPV do not play a significant role in protection against infection.

PS08 Molecular and In Vivo Studies of a Newly Isolated Rat Parvovirus

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Historically, parvovirus infections of laboratory rats have been attributed to either rat virus (RV) or H-1 virus serotypes. Recent clinical and serologic evidence suggested that rats harbored a previously unrecognized parvovirus. Infected rats were resistant to clinical disease caused by the inoculation of RV. Serum from infected rats cross-reacted with RV and H-1 antigen by Immunofluorescent Antibody (IFA) testing, but did not inhibit agglutination of guinea pig red blood cells by RV or H-1 virus. Because attempts to isolate the agent in RV-permissive cell lines were unsuccessful, a virus amplification strategy was used that exploited the predilection of parvoviruses for mitotically active cells. A pooled spleen cell homogenate from naturally infected rats was inoculated into F344 rats bearing transmissible leukemia. Viral DNA was detected in tumor cells by *in situ* hybridization with a probe to a conserved region of the RV genome. Inoculation of rodent parvovirus-permissive 324 K cells with homogenates of infected tumor cells resulted in an amplified viral

stock. The new isolate was designated rat parvovirus-1 (RPV-1). Initial studies indicated that RPV-1 is nonpathogenic in adult and infant rats, but that it may have oncosuppressive properties *in vivo*. Approximately 97% of the RPV-1 genome was cloned and sequenced. Nucleotide sequences differed from those of other rodent parvoviruses even in regions of the genome that encode highly conserved nonstructural proteins. These results indicate that RPV is a genetically, antigenically, and biologically unique parvovirus of laboratory rats.

PS09 Surveillance of Rift Valley Fever in Egypt, Using Sentinel Animals

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Rift Valley fever (RVF), an arthropod-borne zoonotic disease, recurred in Egypt in May 1993 after a 12-year period during which there was no evidence of virus transmission. Commonly associated with sporadic epizootics in sheep and cattle, cases of usually nonfatal febrile illness had been reported in humans until 1977-78 when an extensive RVF epizootic in Egypt was unexpectedly associated with a major epidemic of severe clinical manifestations and nearly 600 deaths. To prevent similar recurrence, research personnel from Navy Medical Research Unit #3, in collaboration with the Egyptian Ministry of Agriculture, selected high-density animal population sites within the Nile delta and the Sinai peninsula to monitor RVF virus activity and to control possible spread of the disease beyond the borders of Egypt. Beginning in October 1993, sentinel groups totaling 1,363 animals (sheep, goats, and cattle) were established at 26 locations in 5 governorates (Beheira, Daqahliya, Kafr el-Sheikh, Sharqiya, and North Sinai). To identify individual study animals, metal tags with unique numbers were placed on the animals' ears. Baseline studies of anti-Rift Valley fever virus-specific IgG, as measured by enzyme-linked immunosorbent assay, ensured that all animals were seronegative. Thereafter, blood samples were collected at two-month intervals and tested at Navy Medical Research Unit #3 for seroconversion. Seroconversion in sheep was first observed in Sharqiyi (12/93-4/94). During the period from July through December 1994, seroconversion occurred in nearly 40% of sheep in the Daqahliya governorate and from August through December 1994 in 10% of sheep in Kafr el Sheikh. The RVF virus was isolated from an aborted sentinel sheep fetus in Daqahliya in August 1994. Seroconversion occurred concomitantly in goats and cattle in the respective governorates. No animals in the North Sinai, bordering Israel and the Mediterranean basin, have seroconverted. Sample collections in three governorates were terminated at the end of 1994 because some owners vaccinated their animals, and in the remaining two governorates in mid-1995. This prospective longitudinal study of RVF infections in animals provides useful data about the dissemination of Rift Valley fever throughout Egypt during the 1993-94 outbreak.

PS10 Existence of Ciliated Protozoa in Internal Organs of Transgenic Mice Bearing the Human FPS/FES Proto-Oncogene

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Ciliated protozoa have been described as intestinal parasites of rodents and other animals, but have rarely been found outside of the intestinal lumen. This report describes ciliate parasites that have been found in the brain and other internal organs of transgenic mice bearing the human fps/fes proto-oncogene. The Fps/fes-bearing mice have increased endothelial cell proliferation that leads to development of hemangiomas later in life. These mice are studied for the unique characteristics of their proliferative endothelial cells. Ciliated protozoal organisms were first identified in cultures of brain endothelial cells from these mice. The brain cell cultures in which ciliates were found were derived from infant mice ranging from 8 to 20 days of age and weanling mice ranging from 1 to 2 months of age. To investigate the parasite further, four retired breeder fps/fes mice were submitted for complete necropsy evaluation. Brain cell isolation techniques were repeated on half the brains from these mice in a different laboratory, using different reagents, including tissue culture medium, to rule out possible contamination. The other half of the brain and other tissues were collected for histologic examination, fecal pellets were submitted for flotation, and nasal washings and cecal contents were collected for direct examination for parasites. Ciliated protozoa were observed in two of four brain cell preparations from the retired breeders. Ciliated protozoa were observed in H&E-stained sections of the nodular hemangiomas in all four mice. The organisms were most frequently located in perivascular areas and were associated with localized infiltrates of eosinophils and macrophages. Ciliates were not seen in H&E-stained sections of other tissues. However, cyst forms of ciliated protozoa were found in Giemsa-stained cecal smears from three of four animals. Ciliates were not definitively identified by fecal flotation, or in nasal washings from any of the animals. An additional group of three young adult fps/fes mice was obtained from the investigator's breeding colony. Blood smears were obtained from each mouse for five consecutive days. Tissues obtained at necropsy also were submitted. Fresh brain, lung, and spleen specimens and cecal contents were inoculated into five types of medium formulated to support growth of *Ballantidium* sp. Ciliate organisms could not be definitively identified in Giemsa-stained smears of blood. The young adult mice, which did not have nodular hemangiomas had no microscopic evidence of ciliated protozoa in H&E-stained tissue sections. Ciliated protozoa did not propagate in any of the media tested. This is the first report of a ciliated protozoal organism in the internal organs of mice. The organisms appear to exist principally in the brain and in the nodular hemangiomas that develop in aged fps/fes mice. However, it is unclear what contribution the human fps/fes transgene makes to the ability of these organisms to live in their hosts. Efforts are currently underway to obtain a definitive taxonomic identification of the ciliate organism found in the fps/fes mice and to further examine the relationship between this parasite and its host.

PS11 A Murine Model of Legionnaires' Disease in Mice Genetically Deficient in Gamma Interferon

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Mice with a targeted disruption of the gamma interferon (IFN- γ) gene (i.e., gamma knock out [gko]) mice were used to develop a murine model of Legionnaires' disease in a host with compromised cell-mediated immunity. The gko and immunocompetent mice were inoculated intratracheally with virulent *Legionella pneumophila*, and bacterial clearance, histologic examination, cellular recruitment into the lung, and development of *L. pneumophila*-specific antibody were subsequently assessed. Although *L. pneumophila* is rapidly cleared from the lungs of infected immunocompetent mice, similarly infected gko mice develop persistent *L. pneumophila* lung infections, with extrapulmonary dissemination of the bacteria to the spleen. Histologic and flow cytometric analyses of lung tissue from *L. pneumophila*-infected immunocompetent mice and gko mice indicate that, although immunocompetent mice develop multifocal pneumonitis that resolves, gko mice develop diffuse pneumonitis with persistent neutrophil recruitment into the lungs. The immunocompetent and gko mice develop serum antibody to *L. pneumophila* within five days after infection, indicating that development of humoral immunity, in the absence of IFN- γ , is ineffective in eliminating *L. pneumophila* from the lungs. Intratracheal administration of IFN- γ facilitates resolution of *L. pneumophila* pulmonary infections in infected gko mice, confirming the pivotal role of IFN- γ in host resistance to *L. pneumophila* lung infections in vivo. It is anticipated that this murine model of Legionnaire's disease in gko mice will provide an important tool for future studies regarding the pathogenesis and treatment of legionellosis in the immunocompromised host.

PS12 Transgenic Animal Model for Dissecting Mechanisms Underlying B-Lymphocyte Development

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The generation of B- and T-lymphocyte populations that have an extended repertoire of antigen specificities but lack autoreactivity, is an essential characteristic of the immune system. These cells must respond to a great variety of foreign antigens without incurring self-reactivity and autoimmune disease. Understanding the signaling mechanisms that regulate development of lymphocytes is central to understanding this process, because many immunodeficiency and autoimmune diseases result from altered function of intracellular signaling proteins that would usually control these events. The low molecular weight oncoprotein p21^{ras} is one important protein that is known to regulate the differentiation and expansion of many cell types, including hematopoietic cells. To develop a whole animal model system for dissecting the role of p21^{ras} and potential downstream mediators in the differentiation and expansion of B lymphocytes, we generated transgenic mice that express a dominant interfering mutant of p21^{ras} protein (H-rasN17) in B-lineage cells under the control of a novel expression vector consisting of the immunoglobulin μ -enhancer and lymphocyte-specific *lck* proximal promoter. Seventeen founder mice were generated, and six transgenic lines were analyzed. Analysis of each line by immunoblotting and

flow cytometry revealed a transgene dose-dependent reduction in the percentage and total number of B-lymphocyte progenitors in the bone marrow. The earliest B-cell progenitor (Pro-Pre B cells) are relatively unaffected, whereas the next stages in B cell development (Pro-B cells and Pre-B cells) are significantly reduced. The development of the myeloid and erythroid lineages was grossly normal. Total splenic B-cell numbers were only moderately reduced, indicating that the limited number of progenitors that develop into immature B cells in the bone marrow are able to fill the periphery. Surprisingly, there is a moderate increase in the percentage of auto-antibody producing B-1 (Ly-1) B cells in the periphery in transgenic mice, lending support to the idea that these populations of B cells emerge from distinct precursors. These findings indicate that the development of B lymphocytes in the bone marrow requires signaling pathways mediated by $p21^{ras}$. In addition, the novel expression vector used in these experiments can be used to direct transcription of heterologous sequences in developing and mature B lymphocytes.

PS13 In Vitro Fertilization Assessment of PCB Effects in B6D2-F1 Mice

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Traditional in vivo screening of chemical or environmental contaminants in mice is time consuming and expensive. The use of in vitro fertilization (IVF) techniques, while less exacting relative to dose-response reactions, offers an opportunity to determine antifertility responses at known concentrations and to ascertain the site of action of the experimental insult to the reproductive process. The present studies were done to test the hypothesis that antifertility effects could be identified by use of a mouse IVF system. The B6D2-F1 mice were housed in a 12:12-h light:dark photoperiod, at 23°C. Feed and water were available ad libitum. Brinster's medium for oocyte culture plus 0.4% bovine serum albumin was used for spermatozoa collection and IVF. For embryo culture, Eagle's minimal essential medium supplemented with antibiotics was used. Three Aroclor mixtures (A-1221, A-1254, and A-1268) of PCBs and a co-planar congener (TCB) were tested. Statistical analysis was done by ANOVA on mean percentage of oocytes fertilized in each trial for each group. The four compounds had no effect on IVF rate at a concentration of 0.01 $\mu\text{g}/\text{ml}$, compared with controls. The A-1221 mixture caused significantly reduced IVF at a concentration of 1 $\mu\text{g}/\text{ml}$ whereas A-1254, A-1268, and TCB were effective at a concentration of 0.1 $\mu\text{g}/\text{ml}$. There were significant effects of the PCBs and TCB on the number of degenerated oocytes and number of abnormal 2-cell embryos at concentration $> 1 \mu\text{g}/\text{ml}$. These data indicate the usefulness of the mouse IVF system to assess chemical and environmental insult effects on the reproductive system.

PS14 Idiopathic Familial Megacecum and Colon in the Sprague-Dawley Rat: A New Model of Neuromuscular Gastrointestinal Tract Dysfunction

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Seven of 11 female and 1 of 6 male 32- to 36-day-old Sprague-Dawley rat littermates presented with severe abdominal distention and perineal staining. Abdominal distention was a result of severe, approximately 15 times normal, cecal and proximal colonic

enlargement. The distal portion of the colon (7 to 8 cm) was of normal size; however, the lumen was filled with misshapen dry fecal material. Light microscopy did not reveal the cause of the cecal and colonic enlargement; specifically the enteric neural plexus and muscular layers were normal. An extensive diagnostic evaluation failed to support an infective cause. Additional affected pups (14 of 56) obtained from the parents of the presenting pups, various backcrosses, and intercrosses of unaffected littermates support a congenital abnormality with an autosomal recessive pattern of inheritance. Affected pups present at 25 to 35 days, and animals die or must be sacrificed within 1 to 10 days of presentation. Gastrointestinal tract weight in affected animals was approximately 50% of total body weight, as opposed to 12% in unaffected littermates and age-matched controls. Contrast radiography and fluoroscopy of affected animals revealed severely delayed gastric emptying and transit times, and an absence of motility in the colon distal to the dilated segments. Histochemical and immunohistochemical studies of formalin-fixed and frozen sections of affected bowel for a variety of neuropeptides and chemicals, including vasoactive intestinal peptide (VIP), substance P, somatostatin, and acetylcholine; neuron-specific enolase; and synaptophysin are being developed to further define the cause of the bowel dysfunction. Results of ongoing studies and a comparison with described human neuromuscular gastrointestinal tract diseases will be presented.

PS15 Management of a NOD Mouse Colony

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The nonobese diabetic (NOD) mouse spontaneously develops type-I diabetes mellitus. With lesions nearly identical to those of the disease in humans, it has been used to study cause of and potential cures for the human disease. A small breeding colony was started in 1993 to increase availability of animals for use in experiments with xenotransplantation of islet cells. Animals are housed in sterile cages with a 12:12 light:dark cycle. The colony has produced over 950 NOD mice, with a standing population of 150 to 200 mice. Approximately 2 to 5 diabetic mice are found each week through observations of cage cleanliness and amount of water consumed. Diabetic mice are maintained on insulin once blood glucose concentration reaches $> 300 \text{ mg}/\text{dl}$. Although female rats turnover at a greater rate than males (75% vs. 35%), the average survival time on insulin is the same for either sex—22 days for females and 23 days for males. Because females begin developing diabetes as early as 3 months of age, breeding is done at 8 to 10 weeks. Diabetes onset during lactation can inhibit nursing; therefore, females are bred and housed as pairs so the non-diabetic mother can foster both litters of pups. The NOD mouse is an excellent model for the study of type-1 diabetes mellitus, but the nature of the disease requires special handling of the mice for breeding and colony management.

PS16 Stimulation of Protective Immunity to *Pasteurella multocida* Heat-Labile Toxin With a Commercial Swine Bacterin-Toxoid

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Elaboration of heat-labile toxin (PMT) is an important virulence factor in some isolates of *Pasteurella multocida* from rabbits. Previously, we reported that immunization with inactivated PMT (IPMT) stimu-

lated protective immunity to challenge with PMT. To test the hypothesis that immunization with a commercial swine vaccine containing IPMT stimulates similar protective immunity, groups of 5 rabbits were inoculated twice intramuscularly (IM), 10 days apart, with 0.5 ml of sterile saline or a commercial swine *P. multocida* bacterin-toxoid (BT) (Anchor True-Vac-2, Boehringer-Ingelheim Animal Health, St. Joseph, MO). In addition, a group was inoculated intranasally with 5 µg of IPMT. Serum and nasal lavage samples were taken at days 0, 7, 14, and 21 and assayed by ELISA for anti-PMT antibody. Serum IgG was detectable by day 7 and nasal lavage IgA was detected by day 14 in BT and IPMT-inoculated rabbits, but not in saline controls. Groups of similarly inoculated rabbits were then challenged intranasally with 28 µg of PMT 21 days after initial immunization, necropsied 7 days later along with 5 IM inoculated, nonchallenged rabbits and 5 nonimmunized, nonchallenged rabbits. Histologic lesion severity was graded on a numerical scale. Saline controls developed severe pneumonia, pleuritis, nasal turbinate atrophy, and testicular atrophy, whereas BT-and IPMT-inoculated rabbits developed significantly less severe disease. The results confirm the hypothesis that immunization with a commercial swine *P. multocida* BT confers protective immunity against challenge with PMT.

PS17 *Helicobacter mustelae*-Associated Gastric Lymphoma in Four Ferrets

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Helicobacter mustelae infection in ferrets (*Mustela putorius furo*) has been widely used as a model for *H. pylori*-induced gastritis, ulcerogenesis, and adenocarcinoma in humans. It has been documented in humans that gastric lymphomas also are associated with *H. pylori* infection. It is theorized that the lymphomas arise from a focus of chronic immunostimulation, and that metastases develop from a rapidly multiplying clone. We describe primary gastric lymphomas in four ferrets that were naturally infected with *H. mustelae*. Colonization with high numbers of *H. mustelae* was observed in the gastric antrum of all four ferrets. Each ferret developed a lymphomatous mass in the lesser curvature of the antrum of the stomach, corresponding to the predominant focus of *H. mustelae*-induced gastritis. Microscopically, two ferrets had a low-grade small-cell lymphoma, and two ferrets had a high-grade large-cell lymphoma. Although three of four ferrets had metastasis to other sites, antemortem or postmortem distribution was suggestive of a gastric origin in each ferret. Preliminary data obtained from measuring crossreactivity with anti-human kappa and lambda light immunoglobulin chain markers suggested that the gastric lesions in these ferrets were monotypic and distinct from polyclonal proliferations associated with chronic gastritis. Although the significance of *H. mustelae* infection in the development of lymphoma in these ferrets was undetermined, the ferret may provide a new model for the study of *H. pylori*-induced gastric lymphomas in humans.

PS18 Immunohistochemical Assessment of Clonality of Ferret Gastrointestinal Lymphoproliferative Disease

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The ferret (*Mustela putorius furo*) has been used extensively as a model of human gastrointestinal lymphoproliferative disease. The ferret

syndrome parallels the clinical and pathologic syndrome exhibited by humans with chronic gastrointestinal lymphoproliferation and lymphoma. It is speculated that chronic lymphoproliferative disease and lymphoma represent a continuum of pathology secondary to chronic antigenic stimulation. The distinction between lymphoproliferative disease and lymphoma is important prognostically, yet can be a diagnostic challenge. Immunophenotypic analysis of human lesions has been performed to determine the clonality of the lesions. Generally, lymphoproliferative diseases are polyclonal in nature, whereas lymphomas are monoclonal. In this study, immunoglobulin expression was evaluated by immunohistochemistry to determine the clonality of various intestinal and *Helicobacter mustelae*-associated gastric lesions in the ferret. Commercially available rabbit anti-human kappa light chain immunoglobulins and rabbit anti-human lambda light chain immunoglobulins were used in conjunction with goat anti-ferret surface immunoglobulins and rabbit anti-human CD3 on formalin-fixed sections of stomach and small intestine to begin to characterize the progression of disease in several clinical syndromes seen in the ferret. Chronic gastric lymphoid hyperplasia and chronic lymphocytic/plasmacytic enteritis in the ferret was characterized by expression of kappa and lambda immunoglobulins. Selected ferrets had monotypic expression of kappa and lambda immunoglobulin labeling, which supports histologic evidence of lymphoma. These preliminary results correlate with the kappa and lambda chain distribution seen in human lymphoproliferative diseases. Because clonal determination is critical for further understanding of the progression of lymphoproliferative disease in ferrets, these markers will enable us to further elucidate these syndromes, thereby allowing continued development of the ferret as a model for relevant human diseases.

PS19 Development of Acute Tolerance to Fentanyl in Swine After Experimentally Induced Traumatic Brain Injury

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Swine are a common model for neurologic, cardiovascular, and transplantation research because of the physiologic and anatomic similarities of swine to humans. In recent years, swine have also been used in studies of traumatic brain injury (TBI) because of similarities to humans in cerebrovascular responses to injury. Success in use of swine in TBI research has allowed one technical limitation to surface that affects the duration within which these animals can be adequately sedated to enable observation of the pathophysiologic evolution over extended periods. Because many anesthetic and sedative agents directly effect cerebral metabolism, blood flow, and intracranial pressure, investigators are limited to the use of narcotics and benzodiazepines to induce anesthesia. Recently, the unique pharmacokinetics of fentanyl, a commonly used synthetic opiate agonist, has been reported in swine models. We have recently observed that, in addition to the relatively high concentrations of fentanyl required to provide adequate anesthesia in swine (200 to 250 µg/kg of body weight/h, IV), the effect of severe mechanical brain injury results in development of acute tolerance manifested in fentanyl requirements that exceed 400 µg/kg/h by 24 to 48 h after TBI. To elucidate whether this phenomenon was related to increased clearance and elimination of the drug or whether true increases in serum concentration were necessary to maintain anesthesia, we obtained serial serum samples from swine subjected to experimental TBI and anesthetized with continuous infusion of fentanyl. Serum concentrations of this

narcotic were then determined by reverse-phase high performance liquid chromatography on a C-18 affinity column. Results from this study indicate that increasing requirements for fentanyl were not accompanied by increased clearance from serum, which would be expected if increased hepatic microsomal enzymatic metabolism was induced by continuous exposure to intravenously administered agent. Serum concentrations were linearly related to dose, even in the range of 400 to 450 µg/kg/h required after TBI. These results suggest that increased tolerance to fentanyl after TBI is related to alterations in neuronal cell receptor kinetics or to impaired delivery of the agent to the injured brain. Further studies are underway to determine whether TBI alters drug delivery across the blood-brain barrier, bioavailability, or the neuronal pharmacokinetics of fentanyl.

PS20 Primary Degenerative Spinal Cord Disease in Rhesus Macaques

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Primary spinal cord disease has not been widely reported in macaques except for some instances of disease caused by lead intoxication. A 3-year-old, nonpregnant female rhesus macaque was reported to have decreased use of the hind limbs. On neurologic examination, the animal had a crouched pelvic posture, bilateral posterior paresis, decreased extensor postural thrust, and decreased muscle mass in the quadriceps and anterior tibial muscles. The animal's mental status, cranial nerves, and forelimb function were normal. The animal was also found to have a swollen right stifle. Radiographic views of the spine were normal, but those of the stifle indicated an incomplete fracture of the patella with degenerative change. Serum biochemical analytes and blood lead concentration were within normal limits. Shortly after the animal became affected with bilateral paraparesis, it developed diarrhea and anorexia. The diarrhea responded to administration of antibiotics and supportive therapy. After the animal's condition had stabilized, CSF was collected by cisternal puncture and electrophysiology studies were on the anesthetized animal. Clinical values for CSF were within normal limits. Results of electrophysiologic studies indicated that the animal had primary spinal cord disease affecting the white matter and beginning in the thoracic part of the spine. The animal is currently healthy, but still does not use the hind limbs. A 16-year-old male rhesus macaque that presented with bilateral posterior paresis in 1989 was examined concurrently with the female. Neurologic examination revealed paresis and intention tremor in the forelimbs as well as bilateral hind limb paresis and contracted toes. The CSF collected by lumbar puncture was contaminated with blood. Results of electrophysiologic studies indicated that the male also had degenerative spinal cord disease affecting white matter, but that the affected region had progressed more cranially in the cervicothoracic part of the spine, compared with the female. Locomotory behavior of these animals has been documented on videotape. In addition to the aforementioned two cases, seven cases of idiopathic spinal cord disease have been identified from clinical and necropsy records. Cases of hind limb paralysis or paresis were only included if: the animal had not undergone recent neurosurgical procedures, the abnormality was bilateral, and there were no other causes of neurologic disease, such as bacterial meningitis or encephalomyelitis. The animals in this case series were affected as adults. The age at onset of the disease ranged from 4 to 21 years with a mean of 9.1 years. The retrospective case series included three females and four males. Mean survival time with the condition

was 372 days and death was usually due to sacrifice because of deteriorating general health or because the animal was no longer useful to the project. The case series includes two animals that were of feral origin and five animals that were born in the colony. A genetic prediction for the disease could not be identified by analysis of the pedigrees of the affected animals. Chronic, progressive degenerative spinal cord disease in rhesus macaques is a unique clinical entity and may be an interesting animal model for the study of degenerative spinal cord disease in human beings.

PS21 Dual Emission X-Ray Absorptiometry of the Beagle Dog for Bone Densitometry and Whole Body Composition Measurements

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Dual emission X-ray absorptiometry (DEXA) is a relatively new radiologic scanning technique that has clinical and research applications for noninvasive measurement of bone mineral density. In addition, the technique has research applications for the determination of total body composition, including fat and lean muscle mass. Whole body or regional analyses can be performed. Radiation exposure to research personnel and experimental subjects is exceedingly low. In our laboratory, DEXA scanning has been used to generate morphometric data for the Beagle dog. Use of DEXA has provided a rapid, precise and accurate measurement of canine bone mineral density and relative fat and lean muscle mass composition. Sixteen adult Beagle dogs (8 female, 8 male), ranging in age from 2 to 6 years, were analyzed by DEXA scanning technique. Bone density (g/cm²) and fat composition (%) were determined to be: 0.790 ± 0.033 and 22.4 ± 5.2 for female dogs, and 0.818 ± 0.034 and 21.0 ± 6.7 for male dogs, respectively. Advantages of DEXA scanning include rapid scan time, low radiation exposure, ease of operation, and relatively low equipment cost. Instrument calibration and operation, as well as data analysis, will be reviewed for canine bone densitometry and whole body composition measurements. Experimental factors affecting precision and accuracy will be discussed.

PS22 Correlation of Body Composition of Rhesus Macaques With Insulin Sensitivity

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Twelve, 7- to 10-year-old, male rhesus macaques were evaluated for percentage of bone, lean, soft tissue, and adipose mass by use of dual-energy x-ray absorptiometry (DEXA) scans. The monkeys were also evaluated for insulin sensitivity, glucose effectiveness, and glucose clearance rates, using frequently sampled glucose tolerance tests (FSIVGTT) in a modified minimal model protocol. Anesthesia was induced with Telazol (5 mg/kg of body weight) and maintained with isoflurane to ensure proper positioning and restraint during the DEXA scanning procedure. The DEXA scans were analyzed for each limb and region to determine total and regional percentage of body fat and total grams of tissue, fat, lean, and bone mineral composition. For each animal, the FSIVGTT included placement of catheters, collection of 4 pretreatment blood samples, and administration of a 300 mg/kg glucose bolus followed by collection of 27 two-milliliter blood samples drawn over the subsequent 2 h to determine serum glucose and insulin concentrations. Tolbutamide (5 mg/kg) was infused 20 min after glucose administration to induce an additional

increment in insulin response. Results of the DEXA scan indicated that percentage of body fat ranged widely, from 4 to 29.9%. Baseline serum insulin concentration and insulin sensitivity ranged from 6.2 to 59.8 uU/ml and 0.41 to $7.3 \times 10^4 \text{ min}^{-1}/\text{uU}/\text{ml}$, respectively. There was direct correlation between percentage of body fat and baseline serum insulin concentration ($r = 0.84$, $P < 0.01$) and between percentage of body fat and insulin sensitivity ($r = -0.75$, $P < 0.01$). The correlation between total body weight and baseline serum insulin concentration ($r = 0.64$, $P < 0.03$) and between total body weight and insulin sensitivity ($r = 0.68$, $P < 0.01$) is also statistically significant, but not as strong as the correlation between percentage of body fat and the insulin measurements. These findings are similar to those in humans with type-II diabetes mellitus, in which obesity is associated with reduced insulin sensitivity. The noninvasive DEXA and FSIVGTT methods allow accurate and rapid analyses of body composition and glucose tolerance, and is useful for assessing drug-induced alterations in these parameters.

PS23 Digital Fluoroscopy: An Important New Tool for Developing Novel Gene Delivery Techniques in Mice

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It is difficult and time-consuming to visualize and document routes of delivery of potentially therapeutic agents in rodents, especially mice. Modern radiographic equipment, including fluoroscopy units, often uses cineradiography (motion picture) film for image acquisition. This is an imperfect method of capturing images, particularly for small rodents, given the inherent limitations imposed by animal size, image sampling speed, and resolution of the film. In addition, the necessity for processing film provides added expense, while also making it impossible to rapidly review and enhance images. In contrast, use of direct digital acquisition of images through digital fluoroscopy (DF) has substantial advantages: real-time images can be enlarged up to 8X original size (a 3-in mouse can become a 24-in mouse); image acquisitions can be obtained up to 30 images/s for up to 90 sec and saved to a hard drive or disk for rereview of the entire event or manipulated for photographic enhancement or experimental manipulation on a PC; digital images can be immediately manipulated at the operating table to enhance visualization, such as by converting to a positive or negative image, or using "digital subtraction" or "road-mapping" techniques to track infusions as they are being administered. In this way, conventional and new drug/vector delivery methods can be evaluated, even in mice. We used all of these DF techniques to successfully develop and validate a new system for selectively delivering gene transfer vectors to specific target organs in the murine hepatobiliary and pancreatic systems. With a catheter in the gallbladder, CD-1 male mice were administered a isotonic radiographic contrast dye (diluted 1:10 in 1X PBS). Infusions were directed to specific tissue targets by selectively applying microvascular clips to different communicating ducts. Real-time DF helped us to define two important parameters: the infusion pathway and the infusate administration rate and volume required to illuminate a particular target organ. This allowed us to model the organ-specific delivery of viral and nonviral gene transfer vectors delivered in similar manner, as well as indicating the maximal volume and rate of infusion that should be used with these vectors for each specific target tissue. Digital fluoroscopy is a important tool for developing and validating novel techniques for delivery of gene transfer vectors and other potentially therapeutic agents in vivo.

PS24 Gallbladder Catheterization: A Common Portal for Selectively Delivering Therapeutic Agents to Murine Hepatobiliary and Pancreatic Tissues

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It has been traditionally difficult to selectively target the delivery of potential therapeutic agents to individual tissues associated with the hepatobiliary and pancreatic (HAP) systems. Similar to many laboratory species, the mouse synthesizes bile in the liver then stores it in the gallbladder (GB). During the intermeal period, bile flows from the GB to the duodenum via the common bile duct (CBD). Unlike that in many laboratory species, the murine pancreas also drains through the CBD into the duodenum. Hence, all of the tissues within the HAP systems of a mouse are contiguous. We took advantage of this unique anatomic relationship to develop a catheter-based system that permits selective delivery of potential therapeutic agents to individual HAP target tissues through one convenient, common portal, the GB. Using an operating microscope and microsurgical techniques, anesthetized, 20–40 gm CD-1 male mice underwent ventral laparotomy (xiphoid to umbilicus). The GB and falciform ligament (FL) were identified. A curved microvascular clip was used to grasp the GB at its cranial FL attachment. The GB was then rotated counterclockwise to approximately 10 o'clock and secured to the body wall with 7-0 silk. Bile was then drained from the GB into the cystic duct (CD), using gentle pressure and a microvascular clip. A gelfoam sponge was placed under the GB to absorb any potentially leaking bile. An opening was made in the GB 2 to 3 mm from where it was attached to the body wall. A custom-made silastic catheter was then secured within the GB lumen. An infusion was administered through this catheter and selectively directed to single or multiple designated target tissue(s). For example, when the CBD was occluded proximal to its junction with the cranial pancreatic duct, infusate sequentially traveled through the GB, CD, and CBD until it reached the MC. Flow then traveled retrograde, moving through the extra- and intrahepatic bile ducts into the hepatic parenchyma. Placing the microvascular clip below the junction of the caudal pancreatic duct with the CBD permitted flow to also travel to the pancreas. Similar techniques were developed to permit selective delivery to each of the tissues associated with the HAP systems (GB, CBD, liver, pancreas, and duodenum). Organ-selective delivery through a GB catheter provides a useful technique for evaluating potential in vivo efficacy of different therapeutic agents when selectively delivered to single or multiple HAP target tissues. We have successfully used this technique to deliver pharmacologic agents to selective HAP tissues in over 500 mice. Catheterization of the GB permits use of murine models of human disease in developing treatments that may be ultimately used in human patients, with analogous techniques of administration.

PS25 Use of Fluorescent Latex Microspheres for Visual and Histologic Evaluation of Vector/Drug Delivery in Mice

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Determining the tissue distribution of viral and nonviral gene transfer vectors after administration has been traditionally difficult and

time consuming, usually necessitating the removal and subsequent analysis of a large number of tissue samples to indirectly search for the presence of either the vector DNA or its expressed protein product. We have developed an alternative strategy, using latex microspheres, that rapidly provides useful predictive information on the comparative distribution of viral or nonviral vectors after different routes of administration. Latex microspheres are available in a range of diameters and colors, allowing direct visualization intraoperatively of vector delivery and subsequent fluorescent microscopic evaluation of vector distribution in tissues as a function of vector size, concentration, and route of administration. This strategy permits qualitative and quantitative modeling of the delivery of different-size viral and nonviral gene transfer vectors *in vivo*. For example, adeno-associated virus is approximately 20 nm in diameter, adenovirus is approximately 80 nm in diameter, and liposomes range in size from 200 to 500 nm in diameter. We have modeled the catheter-mediated, organ-selective delivery of these vectors to hepatobiliary and pancreatic tissues in mice, using fluorescent latex microspheres of corresponding sizes. Sphere sizes were verified by transmission electron microscopy. Spheres were diluted to different concentrations in 1X PBS, sonicated, then infused through a catheter surgically placed within the gallbladder of a mouse. Selective application of microvascular clips directed the infusion to specific tissue targets. These experiments visually demonstrated selective delivery of microspheres to the gallbladder, cystic duct, common bile duct, liver, pancreas, and duodenum. Fluorescent microscopy of fresh-frozen tissue sections confirmed these results at a microscopic level and provided information on likely vector distribution within a tissue as a function of vector diameter and route of administration. These results indicate that fluorescent latex microspheres are useful in the development and verification of *in vivo* systems for selectively delivering viral and nonviral vectors.

PS26 Organ-Specific Gene Transfer to the Hepatobiliary Tree of Mice.

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The selective delivery of a gene transfer vector to only its intended site of action would maximize the potential for therapeutic benefit and minimize the possibility of adverse systemic side effects. We have developed microsurgical techniques that permit selective delivery of viral or nonviral gene transfer vectors to each of the tissues associated with the murine hepatobiliary and pancreatic systems. This catheter-mediated system was initially developed and verified, using fluorescent latex microspheres and digital fluoroscopy. We subsequently evaluated this system, using a recombinant adenoviral vector encoding the nuclear localized *LacZ* gene under the control of a Rous sarcoma virus promoter (AdRSVLacZ). Tissue electron microscopy indicated that selective delivery of AdRSVLacZ resulted in viral uptake in targeted tissues shortly after vector delivery. Three days after administration of AdRSVLacZ, tissues were evaluated by *in situ* Blue-0-Gal perfusion and β -galactosidase immunohistochemistry for evidence of recombinant gene expression. Organ-specific gene transfer occurred in the liver, gallbladder, cystic duct, exocrine and endocrine portions of the pancreas, and duodenum. Gallbladder-specific gene transfer occurred at absolute viral doses multiple logs below that commonly reported for other sites and routes of administration. This indicated that focal vector delivery of low viral dosages resulted in an effectively high *in vivo* multiplicity of infection (ratio between the number of vector particles and number of target cells).

These experiments indicate the feasibility of using catheter-mediated techniques for organ-selective vector delivery in mice with techniques analogous to those that may be ultimately used in human patients.

PS27 Evaluation of Six Anesthesia Regimens in Neonatal Rats

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There are virtually no published guidelines for anesthetizing neonatal rats for surgery. Immobilization is most often achieved by chilling, which has been questioned on humane grounds. The safety and effectiveness of methoxyflurane (N = 10), ketamine (100 mg/kg of body weight, IP; N = 10), Innovar-vet (0.2 ml/kg; N = 9), pentobarbital (30 to 45 mg/kg IP; N = 3 to 7/dose), and chilling in ice water (N = 7 in a protective latex sleeve, N = 9 unprotected) were studied in 1- to 3-day-old rat pups from 7 litters. Positive controls (N = 12) were given saline IP and negative controls (N = 13) were not manipulated. Parameters measured included: loss and return of righting reflex; responses to toe pinch and minor surgery (a 1-cm skin incision over the lateral aspect of the thigh of pups that ceased to respond to toe pinch); heart rate, respiratory rate, oxygen saturation, body weight, and postprocedure (4 weeks) weight gain. Chilling (both methods) and methoxyflurane proved to be safe (0% mortality) and effective (adequate analgesia for surgery), whereas ketamine, pentobarbital (all doses), and Innovar-vet proved unsafe (> 50% mortality) and/or ineffective (inadequate analgesia for surgery). Otherwise, significant differences (ANOVA, $P < 0.05$) were found only in recovery time, which was fastest with chilling (both methods) and methoxyflurane. Methoxyflurane or chilling is recommended as safe and effective for analgesia and immobilization of neonatal rats for surgery. Previous human studies and the responses of the neonates in this study suggest that use of a protective sleeve significantly diminishes the discomfort associated with rapid chilling.

PS28 A Modified Human Pulpotomy Technique for Disarming Canine Teeth of Nonhuman Primates

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The canine tooth disarming techniques that have been described in literature often result in nonvital pulps or malocclusion, and/or are time consuming. The purpose of this project was to evaluate a modified human pulpotomy technique as a method of canine tooth disarming. The canine teeth of seven male rhesus monkeys were cut at the level of the occlusal surface, using a high-speed burr to expose the pulp (pulpotomy). Vital pulps were filled with calcium hydroxide followed by amalgam. Semimonthly oral examination and monthly radiography were performed to evaluate pulp vitality, bone integrity in the periapical areas, and the amalgam surface. Nineteen weeks after disarming, the animals were anesthetized and perfused transcardially with 0.9% saline and 4% paraformaldehyde in 0.1M phosphate buffer. Twenty-eight teeth were collected for histologic evaluation. No changes were found in the pulp and bone associated with the periapical regions on oral and radiographic examinations. Histologic sections indicated viable pulps, no evidence of inflammation, and variable degrees of secondary dentin formation beneath the amalgam. These results indicate that the modified human pulpotomy technique has two major advantages over previously published methods: maintenance of a viable pulp and the time required to perform the procedure.

PS29 A Method for Using a Pole Housing Apparatus to Establish Compatible Pairs Among Squirrel Monkeys

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To pair singly housed adult male *Saimiri sciureus*, a pole housing system was used to identify compatible animals. Pole housing allows several primates to interact or retreat to safety. First the animals are habituated to collar, leash, and pole. During this time, the animals cannot physically interact with others on the pole housing. When animals have adapted to the pole system, they are moved closer to one another. The animals are observed for aggression or fighting at frequent time intervals. If animals exhibit aggression, they are moved to another position. When two animals exhibit compatibility, having been observed interacting positively for one week, they are pair housed. Eight animals are currently housed as pairs. Pair housing the animals has not interfered with research requirements. During nine treatments with an identical test compound, singly housed animals ($n = 4$) lost significantly more weight on average than did pair housed animals ($n = 4$). Primates can be greatly enriched by being given the opportunity to choose a companion, using this approach.

PS30 Centralized Mechanism for Meeting the Information Requirements of the Animal Welfare Act

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The Animal Welfare Act requires investigators to provide assurances that alternatives have been examined for painful or stressful procedures. Despite the complexity of searching for alternatives, particularly for the nonscientist or the inexperienced searcher, many institutions require either reference librarians or principal investigators to accomplish these searches. At our institution, searches are provided to investigators through the Department of Comparative Medicine by a departmental scientist who has received specific training in alternatives searching from the Animal Welfare Information Center. Searches are performed during preparation of the IACUC Animal Care and Use Protocol, and are generated from 29 veterinary, science and medical literature databases. A search request includes a draft copy of the Animal Care and Use Protocol and a basic literature search. Search results are provided on alternatives to stressful procedures, study design, use of animals, and unnecessary duplication of research. The departmental scientist edits the results for applicable content. Investigators then provide a written interpretive statement of the search on the Animal Care and Use Protocol, along with a copy of the search results. This method is simple for the investigator and assures the IACUC of a complete and relevant search for alternatives in compliance with the Animal Welfare Act.

PS31 Development of Institutional Guidelines on Scientific and Instructional Merit Review

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The Public Health Service Policy on Humane Care and Use of Laboratory Animals requires Institutional Animal Care and Use Committees (IACUC) to ensure the scientific merit of projects using animals. This responsibility stems from Principle II of the U.S. Government Principles on Humane Care and Use of Laboratory Animals, which states that procedures performed on animals must be conducted with consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society. At our institution, peer review and funding of a project by an external agency is taken as evidence of scientific merit. However, approximately 30% of our approved research projects are not subjected to external peer review because they are funded through gifts, contracts, or internal allocations. Similarly, courses that use animals do not undergo external peer review for instructional merit. In both instances, our IACUC requires the unit head (dean, chairperson, or director) of the administering unit to ensure the scientific or instructional merit of any project or course that will be undertaken prior to or without external peer review. Some IACUC members have expressed concern over the likely variability among administering units in their review procedures and the criteria for evaluating scientific or instructional merit. These concerns led the IACUC to survey administering units to gather information on their merit review methods and criteria. A subcommittee was appointed to review the results, advise the IACUC on a course of action, and, if deemed necessary, develop recommendations for administering units on scientific and instructional merit review. Forty-five units responded to the survey. Merit review was conducted by the unit head (29% of cases), by a faculty member or consultant designated by the unit head (22%), or by a unit review committee (29%). Twenty percent of units reported no established merit review process. Only 22% of units with established procedures had written merit review guidelines. Because most units already had evolved some form of appropriate review process, the subcommittee declined to recommend the development of standardized review procedures. However, the subcommittee recommended that merit review guidelines be developed to assist units lacking written guidelines and to better communicate the expectations of the IACUC to administering units. After reviewing the literature on scientific merit review and existing unit guidelines, the subcommittee developed a document that explained the mandate for scientific merit review and proposed a series of questions that reviewers should ask to evaluate the scientific merit of projects. The questions addressed long-term scientific goals, short-term objectives, appropriateness of the methods, and the probability of obtaining usable data. Supplemental questions on the potential for publication or for generation of a grant proposal were suggested. Similar questions were proposed to evaluate the instructional merit of animal use in courses, namely, long-term instructional goals, specific learning objectives, appropriateness of animal use to illustrate the principles, and the likelihood of providing a meaningful learning experience. Use of merit review guidelines is a valuable strategy for guaranteeing appropriate scientific or instructional merit review in a large, complex, and decentralized organization.

PS32 Persistent Hepatitis and Enterocolitis in Germfree Mice Infected With *Helicobacter hepaticus*

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Helicobacter hepaticus has been associated with naturally acquired hepatitis in certain inbred strains of mice and, in A/JCr mice, has been linked to development of hepatic adenocarcinoma. *Helicobacter hepaticus* was orally inoculated into axenic outbred female mice and studied longitudinally to fulfill Koch's postulates and to ascertain the pathogenic potential of the organism under defined germfree conditions. Cage contact mice were also housed in the same germfree isolator to study transmission patterns, and germfree mice were maintained in separate isolators as controls. Mice serially euthanized from postinoculation (PI) week 3 through PI month 24 were surveyed by culture and polymerase chain reaction (PCR) for *H. hepaticus* in liver and intestinal tissue. Tissues were analyzed for histopathologic changes, and serum was assayed for IgG antibody to *H. hepaticus* and changes in activity of the liver enzyme alanine transaminase (ALT). Inoculated mice and cage contact mice were persistently infected with *H. hepaticus*, as identified by culture and PCR, in the intestine and, less frequently, the liver for the duration of the 2-year study. Animals developed persistent chronic hepatitis and, in selected animals, enterocolitis was observed. Hepatocellular carcinoma was diagnosed in one *H. hepaticus*-infected mouse. Titer of *H. hepaticus* serum antibody was highest in experimentally infected mice at PI weeks 12 to 18, which in general corresponded to the time interval when the highest activity of ALT was recorded. Although cage contact mice became persistently infected with *H. hepaticus*, lesions were less severe as were the amounts of serologic biomarkers measured in the study. The *H. hepaticus*-infected mouse will provide an ideal model to study putative bacterial virulence determinants and how they interact with the host to induce chronic inflammation and tumorigenesis.

PS33 Evidence for the Presence of a Cytotoxin Associated Gene in *Helicobacter hepaticus*

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Helicobacter pylori is recognized as a human pathogen and is associated with chronic gastritis, peptic ulcer disease, and gastric carcinoma. About half of all *H. pylori* strains produce a vacuolating cytotoxin in vitro. This vacuolating cytotoxin is known to be an 87-kDa protein encoded by the *vacA* gene. Another *H. pylori* gene, known as the cytotoxin-associated gene (*cagA*), has also been identified and is closely associated with the presence of the *vacA* gene. The *cagA* gene codes for the production of a 128-kDa protein. Patients infected with *H. pylori* CagA-positive strains are at higher risk of developing peptic ulcer disease as well as gastric carcinoma. *Helicobacter hepaticus* has been associated with hepatitis and hepatic adenocarcinoma in A/JCr mice. Polymerase chain reaction analysis of *H. hepaticus*, using *cagA* *H. pylori*-specific primers, has detected the presence of the *cagA* homologue in selected strains of *H. hepaticus*, as well as *H. mustelae*. Western blot analysis using *H. pylori* Cag+ IgG antiserum was performed on strains of *H. hepaticus* and *H. mustelae*. The presence of a high molecular weight antigen was detected in *H. hepaticus* comigrating with the CagA protein from *H. pylori* that is not present in *H. pylori* CagA-negative strains. Further study is needed to determine the potential pathogenic potential of the presence of such a protein in *H. hepaticus*-induced liver disease.

PS34 Serodiagnosis of *Helicobacter hepaticus* in Mice by Enzyme-Linked Immunosorbent Assay (ELISA)

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Helicobacter hepaticus is a newly recognized bacterium that has been associated with chronic active hepatitis, hepatic carcinoma, and inflammatory bowel disease in mice. Currently, polymerase chain reaction (PCR), fecal culture, or histologic examination of silver-stained liver sections are used to diagnose *H. hepaticus* infection. We describe an enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of *H. hepaticus* in mice, using a membrane digest preparation of *H. hepaticus* as the antigen. Sera from mice positive for *H. hepaticus* by PCR or histologic examination (n = 89) or negative for *Helicobacter* sp. by PCR (n = 161) were used to evaluate the ELISA. Results indicated that ELISA provided 92.1% sensitivity and 97.5% specificity. To determine the potential for cross-reactivity with other murine *Helicobacter* sp., groups of 10 C57BL/6 mice were inoculated orally with *H. hepaticus*, *H. muridarum*, or *H. bilis*. Sera were collected and examined by use of the ELISA at 2-week intervals. *Helicobacter hepaticus*-infected mice seroconverted by 2 weeks and maintained detectable antibody throughout the 8-week study whereas mice infected with *H. muridarum* and *H. bilis* did not seroconvert. These results indicate the ELISA is highly sensitive and specific for serodiagnosis of *H. hepaticus* infection in mice.

PS35 Use of Pulsed-field Gel Electrophoresis to Determine Genomic Diversity in Strains of *Helicobacter hepaticus* Isolated From Geographically Distant Locations

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In 1992, a helical microorganism in the hepatic parenchyma of A/JCr mice was associated with chronic active hepatitis and high incidence of hepatocellular tumors. By use of biochemical tests, phenotypic characteristics and 16S rRNA analysis, the organism was classified as a novel *Helicobacter* sp. and was named *Helicobacter hepaticus*. Recent surveys completed in our laboratory indicate that *H. hepaticus* is widespread in academic and commercial mouse colonies. The aim of this study was to examine the *H. hepaticus* genome, using pulsed-field gel electrophoresis (PFGE) to determine the degree of genomic variation and genomic size. This technique has been used to document genomic diversity among strains of *H. pylori* and genomic conservation among strains of *H. mustelae*. Genomic DNA from seven strains of *H. hepaticus* from the United States, Australia, and The Netherlands was subjected to PFGE after digestion with *Sma* I. Strains from the United States appeared to have similar PFGE patterns, suggesting that the genomic DNA of these strains is conserved. Strains obtained from Australia and The Netherlands had PFGE patterns that differed markedly from the U.S. strains and from one another. This indicates diversity among strains geographically distant from one another. Use of DNA fingerprinting may be valuable in subsequent epidemiologic studies of *H. hepaticus*, when source and method of spread of *H. hepaticus* need to be ascertained. Using PFGE patterns, the genomic size of *H. hepaticus* is approximately 1.34 Mb, which compares to 1.67 Mb for *H. pylori*.

PS36 Isolation of a Novel *Helicobacter* sp. from the Gallbladder of Syrian Hamsters with Cholangiofibrosis

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Cholangiofibrosis is a syndrome of unknown etiopathogenesis that has been sporadically reported in Syrian hamsters. In this study, we examined the biliary flora of 3-, 4-, 5-, and 8-week-old hamsters from a colony known to have a high incidence of cholangiofibrosis. A filamentous gram-negative motile bacterium with a single polar sheathed flagellum was isolated from the gallbladder of 20 of 22 hamsters with cholangiofibrosis. Bacteria grew under microaerophilic conditions at 37 and 42°C, were oxidase, catalase, arginine aminopeptidase, L-arginine arylamidase, and esterase positive, reduced nitrate to nitrite, hydrolyzed indoxyl acetate, were resistant to cephalothin, and had intermediate susceptibility to naladixic acid. Sequence analysis of the 16S rRNA gene indicated that the bacterium was a novel member of the *Helicobacter* genus most closely related to *H. pametensis*. We have proposed to name this bacterium *H. cholecystus*. In epidemiologic studies, isolation of this novel helicobacter correlated strongly ($P < 0.0001$) with the presence of cholangiofibrosis, indicating that this bacterium may play a role in the disease syndrome.

PS37 Infection of the Ferret Stomach by Isogenic Flagellar Mutant Strains of *Helicobacter mustelae*

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Helicobacter mustelae, similar to *H. pylori*, has two flagellin molecules, FlaA and FlaB. Isogenic mutant strains of *H. mustelae* were constructed by disruption of the *flaA* or *flaB* genes with a kanamycin resistance cassette or by introduction of both a kanamycin-resistance cassette and a chloramphenicol-resistance gene to produce a double mutant. The objectives of these studies were to determine whether one or both flagellin proteins were necessary for colonization and persistence of infection of *H. mustelae* in the ferret. Nineteen ferrets, specific pathogen free for *H. mustelae*, were given either the FlaA (moderately motile), FlaB (weakly motile) or FlaA/B (nonmotile) mutant strain, the wild-type parent strain, or sterile broth. Gastric tissue specimens were obtained during sequential gastric biopsies and during necropsy at 3 months after infection. *Helicobacter mustelae* infection status was determined by culture and histologic examination. The wild-type parent strain of *H. mustelae* infected all six ferrets at all times (10^7 colony-forming units [cfu]/g). The FlaA mutant strain colonized at low amounts (10^2 to 10^3 cfu/g) in all three ferrets at 3 weeks; however, at 7 and 11 weeks, only one ferret in this group was culture positive. At 12 weeks, two ferrets were positive for infection, but still had reduced numbers of organisms (10^4 to 10^5 cfu/g). The FlaB mutant strain infected all four ferrets at all times. At 3 weeks, numbers of organisms were reduced, whereas at 8 and 11 weeks, numbers of organisms were comparable to those in the positive-control group (10^7 cfu/g). The FlaA/B double mutant strain was unable to colonize the ferret stomach at any time. Infection with the wild-type parent strains of *H. mustelae* induced mild lymphocytic gastritis with lymphoid follicle development. Infection with the FlaB mutant strain

resulted in lesions similar to those induced by the wild-type parent strain, whereas infection with the FlaA mutant strain induced only minimal to mild mononuclear cell infiltration of the gastric mucosa. These results indicate that, although the double mutant strain was unable to colonize, the FlaA and FlaB single mutant strains were able to initially colonize the ferret stomach at a low level and establish persistent infection with increasing numbers of organisms over time. The severity of gastritis induced by infection with these strains of *H. mustelae* correlated with the number of organisms in the gastric mucosa. Therefore, flagellar motility is an important virulence factor for colonization and pathogenesis in the *H. mustelae* ferret model.

PS38 *Helicobacter canis* Isolated From the Liver of a Dog With Multifocal Necrotizing Hepatitis

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On the basis of biochemical, phenotypic, and 16S rRNA analysis, a novel gram-negative bacterium, isolated from normal and diarrheic dogs as well as humans with gastroenteritis, has been recently named *Helicobacter canis*. A 2-month-old female mixed-breed pup was submitted for necropsy because of a history of weakness and vomiting for several h prior to death. The liver had multiple and slightly irregular yellowish foci up to 1.5 cm in diameter. Microscopically, the liver parenchyma contained randomly distributed, occasionally coalescing hepatocellular necrosis, often accompanied by large numbers of mononuclear cells and neutrophils. Sections of liver stained by the Warthin-Starry silver impregnation technique revealed spiral-to-curved-shape bacteria predominantly located in bile canaliculi and occasionally in bile ducts. Results of aerobic bacterial culture of liver were negative, whereas small colonies were noted on *Campylobacter*-selective media after 5 days of microaerobic incubation. The bacteria were gram negative and oxidase positive, but were negative for catalase, urease, and indoxyl acetate; nitrate was not reduced to nitrite, and the organism did not hydrolyze hippurate. The bacteria were also resistant to 1% bile. Electron microscopy revealed spiral-shape bacteria with bipolar sheathed flagella. By 16S rRNA analysis, the organism was determined to be *H. canis*. This is the first observation of *H. canis* in a dog with active hepatitis and correlates with recent findings of *H. hepaticus*-related hepatic disease in mice. Further studies are clearly warranted to ascertain whether *H. canis*-associated hepatitis is more widespread in dogs as well as a cause of previously classified idiopathic liver disease in humans.

PS39 Proliferative Enterocolitis Associated With Dual Infection of *Lawsonia intracellularis* and an Attaching and Effacing *Escherichia coli* in a Colony of New Zealand White Rabbits

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Proliferative enterocolitis is an intestinal disease of many animals including swine, ferrets, and hamsters. Recently, the intracellular *Campylobacter*-like organism (ICLO) in the ferret and hamster has been identified as being closely related to a *Desulfovibrio* sp. An ICLO organism was identified in swine as being related to *Desulfovibrio* sp., but had sufficient phenotypic differences to warrant a new genus and was named *Lawsonia intracellularis*. In the rabbit, enterocolitis

has been associated with several organisms, including *Escherichia coli* and an ICLO. A dual infection of *L. intracellularis* and an attaching and effacing *E. coli* (AEEC) was diagnosed in a colony of New Zealand White rabbits. This co-infection was associated with morbidity and mortality of up to 70% in 2- to 4-month-old rabbits. Clinically, the rabbits had acute diarrhea, dehydration, and deterioration, followed by death. Affected animals had grossly thick ileum and colon, as well as mesenteric lymphadenopathy. Warthin-Starry staining of ileum and colon indicated intracellular organisms consistent with *L. intracellularis* in areas of intestinal epithelial hyperplasia. Organisms intimately associated with the intestinal surface were consistent in their appearance with AEEC colonization. *Lawsonia intracellularis* infection was confirmed by a 16S rRNA-specific polymerase chain reaction (PCR) analysis of colon scrapings. *Escherichia coli* was recovered from five rabbits, and AEEC strain identity was confirmed by colony blot analysis and fluorescent actin staining. This is the first documented outbreak of co-infection with these organisms in rabbits. This novel co-infection, which may be acting synergistically, appears to have increased severity of the diarrheal disease outbreak in this rabbit colony.

Poster Sessions

P01 Age-Related Changes in Small Intestinal Epithelial Enzymes and Hepatic Antioxidant Enzymes in Wistar Rats

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Age-associated factors in laboratory animals may be important in the assessment of some chemicals, including drugs, toxicants, and nutrients. To support this hypothesis, the present study was designed to determine age- and site-related differences in intestinal epithelial enzymes and age-associated alterations in hepatic antioxidant enzymes and lipid peroxidation. Eight male Wistar rats per group were sacrificed at the age of 2 (preweanling), 5, 10, 20, and 40 weeks to examine specific activities of alkaline phosphatase (ALP), acid phosphatase (ACP), Na⁺/K⁺ATPase, r-glutamyltransferase (GGT), sucrose, maltase, and lactase in the small intestine and superoxide dismutase (SOD), glutathione peroxidase (GSII-PX), glutathione-S-transferase (GST), and malondialdehyde (MDA) in the liver. The kinetics of all intestinal enzymes and hepatic antioxidant enzymes were assayed using a computer-controlled ELISA and a spectrophotometer, respectively. The electron microscope was used to examine morphologic changes in microvilli of the small intestine. The most significant increases in weight, length, and mucosal protein contents of the small intestine were observed in rats between 2 and 5 weeks of age. Specific activity of ALP, except in 2-week-old rats, was not influenced by age; however, regional gradient along the length of the intestine was remarkable ($P < 0.001$; duodenum > jejunum > ileum). The mucosal tissues from 5-week-old rats had significantly higher ($P < 0.05$) specific activity of GGT than did tissues from rats of other ages. Also, regional distribution of this enzyme was similar to the pattern of ALP. There were no significant effects of age and site on the specific activities of ACP and Na⁺/K⁺ATPase. It was apparent that the epithelial tissues from preweanling rats had the highest lactase activity among all age groups. Conversely, for sucrase and maltase, 2-week-old rats had lower ($P < 0.05$) activities of these enzymes than did any postweanling rats. There was a significant ($P < 0.001$) effect of site in postweanling rats, with activities higher in the jejunum than duodenum and ileum for the three enzymes associated with carbohydrate digestion. By electron microscopy, there were no remarkable changes in the structure of microvilli by age. The activity of hepatic cytosolic SOD was not affected by age, whereas the activities of GSH-PX and GSH-ST in 40-week-old rats were enhanced ($P < 0.05$), compared

with rats aged 2, 5, 10, and 20 weeks. The concentration of MDA in 10- and 20-week-old rats was increased ($P < 0.05$), compared with that in 2-, 5-, and 40-week-old rats. It is concluded from these results that development of intestinal enzymes is influenced by age and site; hepatic antioxidant enzymes are also affected by age in rats.

P02 Evaluation of Proparacaine Hydrochloride as a Topical Anesthetic for the Collection of Blood From the Orbital Sinus of Mice

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Collection of small volumes of blood from mice is frequently accomplished by insertion of a glass capillary tube into the orbital venous sinus. This procedure, even when performed by skilled personnel, usually requires general anesthesia of the mouse. Protracted recovery time and hypothermia are major disadvantages to this method. This study was done to compare the effects of general anesthesia, topical anesthesia, and lack of anesthesia on the ease of blood collection, cellular content of the blood sample, and behavioral response of the mice to the procedure. Balb/c mice of both sexes were randomly assigned to three treatment groups. One group was anesthetized with methoxyflurane until a toe pinch did not elicit a pain response; group 2 had one drop of 0.5% ophthalmic proparacaine applied to the eye 5 min before blood collection; the third group had blood samples collected without the use of anesthetics. Mice were monitored for 48 h, and a second blood sample was collected before euthanasia. Unanesthetized mice objected vigorously to the procedure and were stoic for many min afterward, whereas the mice that received topical anesthesia struggled less and resumed normal behaviors when returned to their home cage. The mice that received general anesthesia recovered within min to h and were stoic for some time after that. Little significant difference was observed when samples were compared for packed cell volume and differential white blood cell counts (neutrophils, basophils, eosinophils, lymphocytes, and macrophages). No differences were observed between beginning and ending body weights. Under conditions of this study, the use of topical proparacaine hydrochloride for the collection of blood from the orbital sinus of mice is an acceptable procedure without the risks of general anesthesia.

P03 Clinical, Hematologic and Clinicochemical Assessment of Serial Blood Sample Collection in Sprague-Dawley Rats

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Blood withdrawal is an experimental procedure that may impact the health of laboratory animals. Pharmacokinetic studies are particularly problematic due to multiple, frequent withdrawals necessary to characterize drug concentrations over time. Well defined guidelines for definitive safe maximal blood withdrawal volumes are lacking. Two sequential studies, designed to mimic pharmacokinetic experimentation, were conducted to address this issue. Parameters included body weight, clinical signs, hematologic and serum biochemical values, organ weight, and histopathologic findings. Groups consisted of 6 to 8 male, viral antibody free, Sprague-Dawley rats. Included were controls, and blood sample collection groups (expressed as percentage of total blood volume) of 10, 15, 20, 30, and 40%. Blood withdrawal volumes were based on an estimate of 6 ml of blood/100 g of body weight. Blood was withdrawn from a lateral tail vein, using

a 23-gauge butterfly catheter. Volume collection per time point was based on the percentage group assignment divided by the 13 sample collection episodes as follows: 0, 15, 30, and 45 min, and 1, 1.5, 2, 3, 4, 5, 7, 9, and 24 h. Samples were taken for clinical pathologic testing at 0, 24, 48, and 72 h, during days 7 through 9. After a two-week rest period, the regimen was repeated. Approximately two weeks after the second blood sample collection regimen, animals were euthanized following terminal blood collection. Animals in the first study were necropsied and selected tissues taken for histopathologic examination. Analysis of variance was used to assess group differences. Results indicated no significant differences in body weight gain and no adverse clinical signs in blood sample groups, compared with controls. Group differences in the erythrogram were significant; changes were typical of acute blood loss anemia and were proportional to the volume of blood removed. In addition, these changes were characterized by generally rapid and complete recovery to control values within 14 days. No significant differences were seen in serum biochemical values. Although organ weights were similar to those of controls, minimal to mild splenic hematopoiesis was evident in all blood sample groups, compared with controls. These data indicate that removal of up to 40% of a rat's total blood volume over two 24-h periods, two weeks apart, caused no gross ill effects. Although statistically significant differences between control and blood sample rats were observed for a number of hematologic parameters, the data *in toto* suggest that true biological significance, with respect to the health of the animal, should not be inferred.

P04 Use of Chlorine Dioxide for Antimicrobial Prophylactic Maintenance of Cephalic Recording Devices in Rhesus Macaques

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Microbial contamination of cephalic recording cylinders enclosing craniotomy sites potentially exposes the underlying CNS tissues to potential pathogens. Typically, prophylactic maintenance of these devices involves instillation of broad-spectrum antibiotic solutions at predetermined regular intervals in conjunction with routine cleaning of the entire cranial implant. Under this type of treatment regimen, a variety of bacterial and mycotic organisms have been isolated from the recording cylinders of five instrumented, asymptomatic Rhesus macaques within a study colony. In an attempt to develop a more effective maintenance protocol, a preparation of chlorine dioxide was tested to evaluate its effectiveness in controlling the microbial contamination. The chlorine dioxide solution was infused in lieu of antibiotic within the cylinders and specimens for culture were routinely taken over several months to evaluate efficacy. Results indicate that although it was effective against bacterial isolates *in vitro*, the chlorine dioxide solution fails to control these organisms *in vivo*. It does tend, however, to eliminate the mycotic organisms within one to two treatment applications. In contrast, bacterial growth can be eliminated with antibiotics alone, but mycotic organisms are subsequently and consistently recovered. A treatment schedule entailing the use of antibiotic solutions with chlorine dioxide solution interspersed at set intervals has proven to be an effective means of controlling the microbial contamination of recording cylinders in this rhesus colony.

P05 Normal Cardiac Ultrasonographic Measurements in the Rhesus Macaque (*Macaca mulatta*)

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Ultrasound is a noninvasive tool that has clinical and experimental applications. Many parameters can be quantitatively assessed, using ultrasound to provide information about the anatomic and physiologic status of the heart. The amount of information in literature concerning normal echocardiographic measurements in rhesus macaques is limited; therefore, echocardiograms were obtained from 12 clinically normal adult male rhesus macaques ranging from 7 to 10 years of age and 9 to 20 kg. The echocardiographic examination consisted of 2-dimensional imaging, time motion mode (M-mode), pulse wave Doppler and color flow mapping. Anesthesia was induced with Telazol[®] (5 mg/kg of body weight) and maintained with isoflurane (1 to 2%), then positioned in left lateral to dorsal recumbancy. Parasternal views, using a 3.5-MHz transducer angled medially in the second intercostal space (near the sternum) allowed measurements (mean \pm SD) of mitral valve slope (94.08 \pm 14.53 mm/s), E-point septal separation (0.83 \pm 1.04 mm), left atrial size (18.49 \pm 2.72 mm), aortic valve leaflet separation (9.66 \pm 0.89 mm), left ventricular diastolic dimension (28.95 \pm 2.84 mm), percentage of fractional shortening (50.08 \pm 7.38%), interventricular septal diastolic dimension (4.70 \pm 0.65 mm), caudal wall diastolic dimension (4.80 \pm 0.52 mm), and percentage of ejection fraction (82.08 \pm 7.04%). Further information using 2-dimensional imaging and pulse wave Doppler were taken with the transducer at the apex of the heart at a point of maximal impulse near the cranial axillary line, which allowed apical 4- and 2-chamber long-axis views. A pulse wave Doppler cursor was placed just below the mitral valve to measure "early" ventricular filling velocity (0.67 \pm 0.13 m/s) and "atrial" component of ventricular filling velocity (0.42 \pm 0.10 m/s). An E-to-A (1.67 \pm 0.38) ratio was calculated, and color flow imaging was used to quantitatively assess valvular flow. These normal values will provide a foundation for assessing future cardiac ultrasonographic findings in rhesus macaques.

P06 Retrospective Clinical Study of Computer-Detected Electro Cardiogram Abnormalities in a Random-Source Dog Population

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A retrospective clinical study was conducted on computer-based analyses of three-lead electro cardiograms (ECG). Seven-hundred random-source dogs (16 to 37.5 kg) were examined during routine quarantine procedures encompassing a 22-month period. Dogs were determined to be normal on the basis of results of physical examination, heartworm ELISA, and CBC, and creatine, BUN, and ALT determinations. All animals were positioned in right lateral recumbency with standard ECG leads on the limbs. A commercially available computerized ECG analyzer and software program were used to record and analyze the ECGs. The majority of dogs (93.0%) had normal ECGs or slight sinus arrhythmias (considered normal in dogs). The remaining dogs (7%) had the following abnormalities: sinus arrest (3.43%), first-degree heart block (2.43%), premature ventricular contractions (0.43%), left cranial fascicular block (0.29%), atrial

fibrillation (0.29%), and sinus bradycardia (0.14%). Veterinary computer-based ECG analysis is an emerging technology. The literature is relatively void concerning canine ECG data, and to the authors' knowledge, previous studies of computer-generated frequencies of canine cardiac diagnoses have not been conducted. Research personnel need to be aware of population frequencies of canine cardiac diagnoses to avoid the confounding of research data.

P07 Evaluation of Analgesic Effects of Buprenorphine in Bile Fistulated Rats

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The analgesic properties of buprenorphine and efficacy of route of administration were evaluated in male Wistar rats (n = 4 to 7/group) after bile fistula surgery. Behavior, food and fluid intake, and body weight were recorded for seven days prior to treatment and for 72 h after anesthesia/surgery. Bile flow rates were monitored for 72 h after surgery. Three groups of rats were prepared with bile fistulas, fitted with Velcro vests attached to tether/swivel assemblies and were treated with vehicle or buprenorphine (0.5 mg/kg of body weight, PO, or 0.05 mg/kg, SC) after surgery. Two anesthetic control groups were fitted with Velcro vests and received buprenorphine (0.5 mg/kg, PO, or 0.05 mg/kg, SC) after surgery; the third anesthetic control group received vehicle alone. Locomotion and grooming were depressed in all fistulated animals after surgery. Food intake and body weight were significantly reduced in all bile fistulated animals and in control animals fitted with vests, compared with unvested vehicle controls ($P < 0.001$). Buprenorphine administration, by either route, had no effect on surgical recovery, bile flow rate, food or fluid intake or body weight. Reductions in food intake and body weight were attributed principally to partial restraint with Velcro vests.

P08 Results of Microbiological Monitoring of Mice and Rats in Experimental Facilities in Japan (1992-1995)

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This is an outline of the results of microbiological monitoring in mouse and rat experimental facilities of pharmaceutical companies (PC) and universities/institutes (U/I) in Japan from 1992 to 1995. A total of 437 mouse facilities (3,686 samples) and 537 rat facilities (4,193 samples) from PC and a total of 633 mouse facilities (9,362 samples) and 233 rat facilities (2,120 samples) from U/I were tested for 27 variables by serologic, cultural, and parasitologic methods. Positive findings in mouse facilities were mouse hepatitis virus (MHV), *Syphacia* spp., *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, Sendai virus (SV), *Spironucleus muris*, *Giardia muris*, mouse adenovirus, *Clostridium piriforme*, and *Corynebacterium kutscheri*. The highest detection rate involved MHV in PC (10.1X) and U/I (25.8X). The number of positive findings were lower in PC than in U/I. Positive findings in rat facilities were *Syphacia* spp., *M. pulmonis*, CAR bacillus, Sialodacryoadenitis virus (SDAV), *C. piriforme*, pneumonia virus of mice, *S. muris*, SV, and *G. muris*. Also in rat facilities, the number of positive findings and detection rates were lower in PC than in U/I. Among these positive results, *Syphacia* spp., *M. pulmonis*, SDAV, and *C. piriforme* had high positive rates in U/I. The most prevalent pathogen in mouse experimental facilities in Japan was MHV. Examination by restriction analysis of PCR product patterns indicated that almost

all MHV isolates were low-virulence types that were pathogenic for immunodeficient mice only. It is difficult to detect this MHV infection on the basis of clinical signs of disease. Therefore, we suspect that high prevalence of MHV is caused by its low virulence and exchanges of infected mice between facilities. The difference in microbiological status between PC and U/I may reflect whether the facilities comply with good laboratory practice.

P09 Tidal Volume and Inspiratory Pressure in the Mechanically Ventilated Anesthetized Dogs

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Dogs undergoing prolonged or complicated surgical procedures often are underventilated, as measured by blood gas and end-tidal CO₂ (CO₂) values when published ventilatory guidelines are used. We investigated the relationship between body weight, tidal volume, and inspiratory pressure in 59 anesthetized dogs (19 to 33 kg). Animals were ventilated under pressure control and noninvasively instrumented to monitor blood pressure, ECG, oxygen saturation, CO₂ and tidal volume. Body weight, sex, and thorax measurements were recorded. All dogs were monitored at inspiratory pressures of 10, 14, and 18 cmH₂O, with measurements recorded once CO₂ stabilized. Veterinary guidelines recommend tidal volumes of 10 to 15 ml/kg and inspiratory pressures of 15 to 25 cmH₂O. When pressure was below guidelines (10), tidal volume was "normal" (10 to 15 ml/kg), but dogs were underventilated. When pressure was "normal" (14 or 18 cmH₂O), tidal volume was above guidelines. Physiologic variables were normal only when pressure was 14 cmH₂O. Weight and thorax depth were associated with tidal volume (32 and 6%, respectively), yet tidal volume varied by 1,250 ml at any given weight and pressure. None of the measured physical variables accurately predicted tidal volume. These data suggest the inconsistency in tidal volume is due to a previously undescribed variability in respiratory compliance in the anesthetized dog, and that the guidelines for ventilation during surgery need further investigation.

P10 Nasal Swab Specimens as Alternative Samples for PCR Detection of the Cilia-Associated Respiratory (CAR) Bacillus

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The cilia-associated respiratory (CAR) bacillus is an unclassified, gram-negative, motile bacterium that has been implicated as an etiologic agent of respiratory tract disease in laboratory rodents. Traditional methods of CAR bacillus diagnosis include histologic examination and serologic testing, but these methods may lack sensitivity or specificity. Recently a highly sensitive and specific polymerase chain reaction (PCR) assay that detected CAR bacillus DNA in respiratory tract tissue was developed. One disadvantage to this test was the need to euthanize animals for sample collection. In the present study, we evaluated the use of nasal swab specimens as alternative samples for CAR bacillus PCR analysis. Nasal swab specimens were collected from 3-, 5-, 7-, 9-, and 11-week-old anesthetized rats that were naturally infected with CAR bacillus. At each time point, a group of rats was euthanized and examined for CAR bacillus by tissue PCR assay and histologic examination. The CAR bacillus DNA was detected in

nasal swab specimens from 53% of 3-week-old rats, 94% of 5-week-old rats, and 100% of 7-, 9-, and 11-week-old rats. With the exception of one 3-week-old rat, CAR bacillus DNA was detected in all nasal swab specimens from rats that were test-positive by tissue PCR assay. In contrast, four 3-week-old rats that were test-positive by tissue PCR assay were test-negative for CAR bacillus by histologic examination. These results indicate that nasal swab specimens provide viable alternative samples to tissues for CAR bacillus PCR analysis.

P11 Incidence of Peritoneal Adhesions After Administration of Tribromoethanol

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Tribromoethanol (TBE) is widely used as a general anesthetic for rodents; nonetheless, its use has been questioned because of reported postoperative fatalities associated with chemical-induced peritonitis. To examine the association between TBE and peritonitis, this drug was evaluated in an ongoing, subcutaneous hormonal implant study. One-hundred juvenile BALB/c female mice, weighing between 11 and 21 g, were randomly assigned to one of 5 treatment groups. All mice received intraperitoneal injections (IP; 0.2 to 0.4 ml) of either TBE dissolved in water (25 mg/ml; group 1), TBE dissolved in tertiary amyl alcohol [1 g/ml; diluted with 0.9% saline (25 mg/ml; group 2)], deionized distilled water (group 3), tertiary amyl alcohol (2.5% diluted with 0.9% saline; group 4) or 0.9% saline alone (group 5). Tribromoethanol was maintained at pH \geq 5.2 and was administered at a mean \pm SEM rate of 359 \pm 9.4 mg/kg of body weight. Total doses of TBE were similar ($P > 0.05$) between treated mice in groups 1 and 2. Mice not receiving TBE were anesthetized with halothane for subsequent subcutaneous implantation. All mice recovered from anesthesia and were returned to their respective cages. After nine days, mice were euthanized with carbon dioxide followed by cervical dislocation and the bodies were submitted for necropsy. Small localized adhesions were found in all IP injection groups. Focal adhesions were observed in 15% of the mice in each of groups 1, 2, and 5 and in 10% of the mice in groups 3 and 4. No differences in gross lesions were observed among the treatment groups ($P > 0.05$). These findings indicate that the incidence of peritoneal adhesions after IP injections is similar among mice receiving either of two formulations of TBE, distilled water or saline.

P12 *Balantidium coli* Infection in a Colony of Naked Mole Rats (*Heterocephalus glaber*)

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Balantidium coli is an intestinal protozoa common to swine, that has been isolated from a number of animals, including humans, nonhuman primates, guinea pigs, and rarely, dogs and rats. The organism has a direct life cycle, is transmitted by the fecal-oral route, and has a zoonotic potential, with swine the usual source of infection for humans. Infection in animals, including humans, is often asymptomatic, with passage of cysts in feces, but acute or chronic intestinal disease may be induced. Although rats are considered a possible host for this parasite, reports of infection in rats are rare. Detection of *B. coli* infection in a colony of naked mole rats, (*Heterocephalus glaber*) permitted description of the clinical and pathologic features of disease associated with this organism. Over a 4-month period, 9 adult rats from a

closed colony of 31 animals became clinically ill, manifesting abdominal distention, diarrhea, and respiratory distress, or died acutely. Necropsy revealed gas-distended intestines. Histologic examination revealed mild to moderate enteritis and colitis; the small and large intestines were markedly dilated with numerous *B. coli*. Colonic mucosal changes varied from mild necrosis to organisms penetrating the mucosa. Analysis of fecal samples from the colony by use of wet mount and fecal flotation revealed absence of pathogenic protozoa and endoparasites. The source of *B. coli* includes contaminated feed or spread from animal technicians. Alternatively, chronically infected rats may have developed clinical disease secondary to environmental stress and changes in normal intestinal flora. The absence of subclinically infected rats and the detection of large numbers of parasites in diseased rats indicate that *B. coli* is pathogenic in *H. glaber*. Given the susceptibility of this species to *B. coli*, it may serve as an animal model for other protozoal infections, such as cryptosporidiosis, for which current rodent models are dependent on immunosuppression.

P13 Cytokine Expression in Subclinical Murine Tyzzer's Disease

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Clostridium piliforme an obligate intracellular bacterium, is the causative agent of Tyzzer's disease. Although clinical disease may result in marked morbidity and mortality, subclinical infections with *C. piliforme* are more common, particularly in rats and mice. Little is known about the physiologic effects of subclinical infections on the host and on research data obtained from infected animals. To assess effects of subclinical infections on the murine immune system, we evaluated expression of the proinflammatory cytokines, tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) in experimentally inoculated mice. Four-week-old DBA/2 and C57BL/6 mice were inoculated intravenously (IV) with 10^5 *C. piliforme* organisms propagated in the mouse hepatic cell line, BNL. Control animals were inoculated IV with either phosphate-buffered saline (PBS) or with BNL cells. Animals were necropsied and the liver from each was collected at postinoculation times ranging from 15 min to 14 days. Messenger RNA was isolated from hepatic tissues and evaluated for TNF- α and IFN- γ expression by reverse transcription polymerase chain reaction (RT-PCR). Competitive PCR, using cytokine mimics, was subsequently performed for semi-quantitative cytokine analysis. Expression of TNF- α and IFN- γ was undetectable prior to inoculation and in control animals at all times, but increase in both cytokines was evident in mice inoculated with *C. piliforme*. These data indicate that subclinical *C. piliforme* infections induce expression of proinflammatory cytokines, which may perturb the host immune system and affect results of research investigations using *C. piliforme*-infected animals.

P14 Parasitic Organisms Found in a South African Clawed Toad

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A colony of South African clawed toads (*Xenopus laevis*) was established in 1991 from adult wild-type animals purchased from a laboratory animal supplier. During a routine abdominal surgery, one healthy, approximately 18-month-old adult female was found to have a large number of small (1 mm long) mobile organisms in and around

the ovarian tissue. The frog was sacrificed, and gross necropsy was performed. Additional organisms were observed throughout the body cavity, but all major internal organisms appeared grossly normal. The clavate to spherical body of the parasite moved with a leech-like motion. Samples of the parasite were fixed, stained, and mounted on a slide. The forebody and large hindbody were continuous. The terminal oral sucker was roughly circular in outline and was flanked by small distinct lappets. The pharynx was small and longitudinally ovate. The short esophagus led to a tubular bifid gut, which extended to the posterior margin of the Brandes organ (holdfast organ). The Brandes organ was longitudinally oval with a median slit and considerably distant from the ventral sucker located at the level of the mid-body. The excretory vesicle was Y-shaped. Random fecal samples were collected from tanks containing the remaining toads in the colony. No parasite ova or other parasitic stages were observed. At least 10 subsequent surgeries performed on other toads have produced no evidence of additional infections of this or any other type. The organisms were identified as the metacercarial (immature) stage of digenetic trematodes (flukes) from the family Diplostomidae that most closely resemble *Tylodelphys xenopodis*. The metacercarial stage of this fluke has been found predominantly in the pericardial sac of wild-caught *Xenopus* sp. toads. In heavily infected hosts, metacercaria can be found in the body cavity where survival time may be over 3 years. Therefore, this toad was most likely naturally infected in South Africa. Because neither the intermediate host (snail, mollusk, or fish) nor the definitive host (bird or mammal) were present in the laboratory environment, other toads in the colony are unlikely to be infected.

P15 Evidence of an Acquired Portolymphatic Shunt in a Beagle

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Portal vein access ports are commonly used in pharmacokinetic studies of novel compounds; however, few complications of this technique have been reported. A colony-bred Beagle had a portal vein vascular access port (VAP), as well as an intraduodenal access port, implanted and was used for pharmacokinetic studies. Nine months after implantation, infection of the portal VAP was confirmed by three consecutive cultures yielding *Staphylococcus intermedius*. During that time, the port became nonpatent for blood withdrawal, and difficult to infuse into, suggesting portal vein thrombosis. The CBC results and serum biochemical profiles were normal. Bacterial culture of blood yielded no growth. Contrast radiography of the portal system confirmed portal vein thrombosis; no contrast was evident in the hepatic vasculature. However, contrast was identified in a vessel that continued into the thoracic cavity, immediately ventral to the vertebral bodies. Differential diagnosis at this time included acquired portosystemic shunt, specifically a portoazygous shunt, and portolymphatic shunt. Further contrast radiography was performed, and the contrast-filled vessel coursed from the level of the third lumbar vertebra into the thorax, dorsal and cranial to the base of the heart, terminating in the thoracic inlet area. Further study of the structure was precluded by rupture of the region adjacent to the portal vein thrombus site. Necropsy revealed multiple, tortuous portocaval shunts. The structure seen radiographically was confirmed to be the lymphatic system, including the cisterna chyli and thoracic duct. This represents the first known report of a portolymphatic shunt.

P16 Neuropathology Associated With Seizures in FVB Mice

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The FVB mouse is used extensively in transgenic research because of its well-defined inbred background, superior reproductive performance, and prominent pronuclei, which facilitates microinjection of genomic material. Seizures associated with a known mutation and seizure-susceptible inbred strains are well documented in mice; however, seizures in the FVB strain have not been evaluated, to the authors' knowledge. Affected unmanipulated FVB/N ($n = 5$) and transgenic FVB/N mice generated using 9 unrelated transgenic constructs ($n = 63$), were submitted for pathologic examination. Most cases were detected during routine observations in animal rooms; however, seizure induction by tail tattooing, fur clipping, and fire alarms has been observed. The majority of mice were female (61/68), with a mean age of 5.8 months (range, 2 to 16 months). Observations made during seizure presentation in 12 of 68 mice included facial grimace, chewing automatism, ptialism with matting of the fur of the ventral aspect of the neck and/or forelimbs, and clonic convulsions that frequently progressed to tonic convulsions and death. Two mice were dead at presentation, with matting of the fur of the neck and forelimbs. The remainder of the mice presented with non-specific signs, such as lethargy, moribundity, or matting of the fur. Vendor and in-house animal health surveillance reports indicated that mice were seronegative for antibodies to all murine pathogens. Gross pathologic findings were unremarkable. Microscopic findings were limited to the brain and liver. In all mice, acute neuronal necrosis was present in the cerebral cortex, hippocampus, and thalamus. A concurrent astrocyte hypertrophy, as evidenced by an increase in glial fibrillary acidic protein staining, was detected. Acute coagulative necrosis of centrilobular hepatocytes was present in the liver of some cases (19/68). No infective agents were detected in selected brain specimens submitted for electron microscopy or in brain and liver specimens evaluated, using a modified Steiner stain. Cytopathologic effect was not observed in 3T3 and BHK-21 cell lines inoculated with affected brain and liver specimens. Antibodies to murine pathogens were not detected in weanling mice inoculated with affected brain and liver tissues. The ischemic neuronal necrosis observed in these mice is consistent with lesions associated with status epilepticus in man. The hepatocellular changes are interpreted to be agonal and associated with terminal hypoxia in seizing animals. Our studies provide evidence of a previously unrecognized, often lethal, epileptic syndrome in FVB mice that may have a major impact on transgenic research and other disciplines that use this mouse strain.

P17 Management of an Epizootic of Tularemia in Group-Housed Rhesus Macaques

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Tularemia is a zoonotic disease caused by the bacteria *Francisella tularensis*. The organism is highly infective, and is transmissible by ingestion, inhalation, and skin contact or penetration. Potential reservoirs can include mammals, birds, insects, and standing water. An epizootic of tularemia occurred in an outdoor-housed breeding colony of *Macaca mulatta*. The at-risk population consisted of approximately 300 animals. The initial differential diagnosis, based on clinical presentation and results of histologic examination of affected animals included but was not restricted to yersiniosis, salmonellosis,

streptococcosis, and tularemia. Preliminary laboratory evaluation using various methods resulted in a positive diagnosis in nine cases, with seven cases confirmed by positive culture results. Further evaluation of the outbreak, including fluorescent antibody (FA) testing, direct isolation, mouse inoculation studies, and serologic testing is in progress. Management of this zoonosis included: identification and characterization of the etiologic agent through the use of reference laboratories, attempts to identify any reservoir and/or vector involved in the outbreak, and coordination with public health officials and public relations representatives to educate and ensure personnel safety.

P18 Lymphosarcoma in T-cell Receptor Transgenic Mice

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T-Cell receptor (TCR) transgenic mice have been valuable tools for investigating pathways of lymphocyte development. We generated independent lines of transgenic mice expressing either a transgenic α -chain gene or a transgenic β -chain gene that were derived from a TCR specific for myelin basic protein. These mice were then bred together to produce $\alpha\beta$ -chain TCR mice to study the immune response to myelin basic protein. In the course of our studies, we noted development of lymphoblastic T-cell lymphosarcoma in the α -chain transgenic mice (12%, 22/146) and β -chain transgenic mice (22%, 46/209). Lymphosarcoma was not seen in either the β -chain transgenic mice or the background strain (0/492). To determine whether lymphosarcoma developed in other TCR transgenic models, we completed a cohort study of the incidence of lymphosarcoma in two additional TCR transgenic models, using changes in thymus and spleen size as our criteria. One model was an $\alpha\beta$ -TCR transgenic line also specific for myelin basic protein; the other model was an α -TCR transgenic line of different specificity. In this study, 48% (10/21) of the mice expressing a complete transgenic $\alpha\beta$ -chain TCR developed lymphosarcoma by six months of age, whereas 20% (6/31) of the mice expressing a transgenic α -chain and endogenous β -chains developed the disease. Lymphosarcoma was not seen in the background strains. The tumors are highly metastatic and transplantable. Interestingly, expression of the transgenic α -chain alone has a profound affect on lymphocyte development, resulting in decreased cellularity of the thymus as well as thymocyte subset differences. The α -chain transgenic and the $\alpha\beta$ -chain transgenic mice have increased numbers of CD4-CD8 thymocytes, with the $\alpha\beta$ -chain transgenic mice containing twice the absolute number of these cells as the α -chain transgenic mice. These developmental abnormalities may play a role in the development of lymphosarcoma.

P19 A Unique, Labor-Saving Primate Enrichment Strategy

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Our small staff spent much time each week distributing, affixing, and/or hanging toys for 7 rhesus, 1 stump-tail, and 9 cynomolgus monkeys—with variable degrees of success. Although some animals enjoyed their playthings for a short period, most became quickly bored. The time it took to unlock each cage, throw in the toy, re-lock the cage, deal with the toy when it became lodged in the squeeze mechanism, and retrieve the toy for recycling provided the incentive to find a more acceptable, less labor-intensive form of entertainment. The result? An activity unit composed of washable, sanitizable items

of interest screwed to a board and mounted on the wall next to each animal cage. Every few days, cages are moved about the room so that each animal is presented with a new set of stimuli. No two boards are the same. Each station contains items that require various degrees of concentration and ability. A typical unit might include: large and small movable brass hinges, a bell (similar to that found at a service counter), small, brightly colored driveway reflectors, and a door knocker. A different unit might contain: a sliding latch lock apparatus, a collection of stainless steel rings, a quick connect plumbing fixture, and a plastic mirror. These are just a few of the hundreds of possible combinations of objects that can be used. A trial and error period occurs—some items are extremely popular and some are not. Using items that allow for similar mounting patterns enables easy replacement of one item with another. The units can be sanitized as part of the regular room cleaning. These stationary, wall-mounted activity centers provide an affordable, creative means of supplying primates with a variety of tactile experiences of variable degrees of complexity and interest while reducing labor and stress levels for their caretakers.

P20 Weight Loss in Rats Associated With Exposure to Infrasound

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Seventy-one 45-five-week old pretest CD rats of equal sex lost weight over several weeks' period while kept in a single room. Rats of similar age and sex kept in adjacent animal rooms of the same design and husbandry conditions gained weight during the same period. Infective agents, animal husbandry, and routine HVAC (temperature, humidity, ventilation) problems were ruled out as possible causes of this weight loss after extensive review of the problem and testing. After investigation, it was determined that changes were made to the air handler units (AHU) the air ducts of which ran through an interstitial mechanical space contiguous with the animal room in question. These changes were made just prior to the rats being moved into the room as part of an AHU rebalancing project. Vibration and sound frequency testing were conducted in the animal room, nearby unaffected rooms, and adjacent AHU. One AHU was found to be vibrating at 6.3 Hz and translating the sound to the contiguous animal room, but not into nearby control animal rooms. After a sheave misalignment in the problem AHU was corrected, the problem AHU and contiguous animal room were retested and determined to have infrasound levels similar to those in the control rooms. The identical rats were put back into the room and gained weight normally. Exposure to infrasound has been shown to cause multiple abnormalities in man, rats, and other species, and we believe it was responsible for weight loss in these rats. Monitoring of animal areas and their environment may need to be expanded to include measurement of sound frequencies outside the usually tested range of human hearing. Such frequencies potentially may be detrimental to rats and result in weight loss.

P21 Design and Use of a Transport Device for Miniature Swine

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Miniature swine are an important model for biomedical research. However, in conducting long-term safety toxicology studies, we found

that the care and handling of large numbers of swine present many unique challenges. Even routine handling for clinical examinations, cage rotations, and body weight measurements can be stressful for the animal and handler. Initially, we used a sling for cage rotations and examinations and found it difficult and cumbersome; therefore, alternative methods were explored. We designed and built a swine transport cart that would be of adequate size to accommodate each of the animals while restricting their movement to facilitate examination procedures. The frame of the cart was constructed of painted square tubular steel stock and fitted with four heavy duty wheels. The side panels and floor were constructed of 1/2-in acrylic sheets, which were fastened to the metal frame. The acrylic was slotted to aid in ventilation and the drainage of urine. The end panels were fitted into welded channels and functioned as gates. The top of the unit was left open except for three metal support beams. The floor was overlaid with a commercial plastic floor grating to provide a more secure footing for the animal. All swine entered the cart on their own without handling or restraint. Initial training required the technician to enter the pen and gently encourage the animal to get into the cart. However, with some repetitive training, most of the animals quickly learned to enter the cart when the pen door was opened. It was never necessary to actually place the animals in the cart. The cart was particularly useful for transporting the animals between pens, obtaining body weight, conducting physical examinations, and for general restraint. Use of the swine transport carts proved to be an efficient and humane device for the short-term holding of swine for various procedures. Our technicians found the cart easy to use, and the ergonomics of the cart probably prevented technician injuries. The ease of cleaning the cart in our cage and rack washer, and its low cost of construction made this a useful addition to our animal care equipment.

P22 ISO9000 in the Animal Facility

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ISO certification signifies a comprehensive quality system in the Animal Facility that builds on other processes required for the care and use of laboratory animals. Benefits can be realized in areas of strategic planning, customer feedback, simplified processes, reduced time and costs, training, and improved sharing and exchange of information. The ISO9000 series of standards were established in 1987, in response to the need of the European Community to establish uniform regulatory requirements. The standards provide a mechanism to ensure consistency in processes and products, eliminating the need for individual country standards. The strategy for removing technical barriers to trade was to rely on the principle of mutual recognition and product certification by a third party. The United States is a vital supplier to the European Community, which now comprises the largest market for goods and services in the world. To continue exporting into this extensive market, many American companies are required to comply with ISO standards. To date, use of ISO9000 standards has grown worldwide to include over 60 countries and 70,000 companies. Animal facilities of multinational and domestic companies have used ISO certification as a instrument to expand marketability and profitability. Fortunately, the needs of the biomedical community and the existing regulations concerning animal welfare and good laboratory practices provide a foundation on which to easily interface ISO standards. Goals of ISO9000 include: obligatory documentation systems, training, customer service, correction of nonconformances, corrective action, and continuous improvement. Standards such as the Animal Welfare Act, the Good Laboratory Prac-

tices Act, and the AAALAC approval process define criteria for success, and ISO provides a systematic approach to business management.

P23 Easy, Efficient, and Economical Rabbit Restraint: A Time Tested Method

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Recently, several new commercially available rabbit restraint devices have been reported. Although these devices appear to be quite effective, they may be cost prohibitive to a small facility or to a facility that only occasionally has a need for rabbit restraint. In 1990, a system was devised that securely restrains a rabbit while allowing free access to the ears for venous blood collection and intravenous drug administration. This method minimizes animal stress, is cost effective, and can be performed by one person. The rabbit is first wrapped in a 22 x 40-in towel, which prevents virtually all movement except for the head. The rabbit is then placed in a 7 x 28 x 4-in box (i.e., a modified stainless steel rabbit restraint box). Plastic blocks are then inserted behind the rabbit to push it forward enough to allow access to the ears and to prevent excess movement. The rabbit is then secured into the box, using an 8 x 40-in elastic band equipped with snaps or Velcro. When the rabbit is restrained in this manner, it is usually quite calm and does not resist handling. If the rabbit becomes fractious, this method prevents escape and/or injury. Since the method was initiated, it has been used for over 9,000 blood collection procedures and hundreds of intravenous injections in rabbits ranging from 6 to 16 lb. The incidence of restraint related injury is < 0.012% (one incident in approximately 9,500 procedures). This method has been quite successful in our facility over the past five years, and our rabbits adapt quickly to the procedure.

P24 Effective Hand-Rearing of Orphaned Owl Monkeys

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For the period 1987 through 1995, there were 272 live births in our research/breeding facility, and only 12 of these offspring required hand-rearing. Eleven of these orphaned infants have been successfully nurtured and reintegrated into the colony, using methods adapted in our facility. There are four situations that demand hand-rearing of an owl monkey infant: an infant is orphaned; a postpartum female has agalorrhea; a female has milk, but neglects the infant; and the parents exhibit aggressive behavior toward each other or toward the infant.

In case of any of these situations, the infant is immediately removed from the cage and placed in a modified microisolator that is maintained at a temperature of approximately 100°F. It will be fed and cared for by the staff, weighed and observed daily for 10 days, and thereafter, twice a week for 6 months. An infant animal nursing bottle is attached to a surrogate made by securing real or synthetic fur to a cylindrical roll. The infant is fed a mixture of replacement infant milk for primates, supplemented with sugar (200 ml of formula + 5 g of sugar) every 2 to 3 h for the first 48 h. Usually, the infant drinks 1 to 2 ml of the mixture. From day 3 to day 14, the infant drinks 4 to 6 ml of formula every 3 to 4 h. After day 14, the formula is changed by adding baby cereal and a small piece of a banana. Once the infant begins eating on its own (5th to 6th week), the formula is further supplemented with canned primate diet, banana, and primate vitamins. Chopped apples, oranges, and bananas are also available ad libitum. The nursing bottle is then used to administer only sugar water. By the 6th or 7th week, the infant is either reintroduced to its

parent/parents or to a surrogate family. If the infant is being given to a surrogate family, the fur surrogate is rubbed on both parents daily to transfer the baby's scent to them and vice versa. This is done during the week prior to adoption. The introduction itself occurs during working h (supervised by a staff member). If, after a few days, the infant has adjusted to its new surroundings (weight increasing, no bite wounds, and is able to grasp onto a parent), it is considered to be acclimated to its new environment and accepted into the family.

P25 Use of an Off-the-Shelf Computer Program for Animal Facility Business Management

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Computer programs specifically designed for animal facility record-keeping are expensive to buy and maintain. An alternate solution is to use prepackaged programs. We have been able to adjust an off-the-shelf accounting program to our use. We chose a moderately priced, small business accounting program that includes job costing. We set up the financial management of our animal facility as a small business. The accounting program lets us track our costs and expenses and our "income" of per diems and sales of supplies and services. Flexibility with terminology enables us to fit the program to our "business." The job costing allows breakdown into cost centers for doing a cost analysis relative to setting per diems. Reports can be generated, inventory controlled, and budgets maintained. Fiscal tracking is done in a very user-friendly atmosphere. The learning curve is relatively short. The program may be started any time during the fiscal year, but more easily at the start of a new quarter. An off-the-shelf program, if selected with the needs of the facility in mind, is capable of meeting fiscal monitoring requirements, easily adapted to this type of "business," user friendly, and inexpensive.

P26 Silicate Crystallization in Water Resulting From Autoclaving Glass Bottles

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Crystals were visible in autoclaved (standard cycle: 121°C, 17 p.s.i., 20 min) glass bottles with sipper tube and stopper filled with tap water. Crystals were observed in bottles on two occasions approximately six months apart. Analysis of crystals and crystal-laden water by transmission electron microscopy and inductively coupled plasma and energy X-ray analysis identified crystals as silica with total silicate concentration of 49 ppm. Subsequently, the procedures and processes involved in water bottle preparation were analyzed to determine the source or factors involved in silicate contamination. Analyses of silicate concentrations were performed in tap water (1.88 to 2.60 ppm); tap water autoclaved in polycarbonate (1.90 to 2.60 ppm) and glass water bottles (1.80 to 23.5 ppm), after purposeful contamination with varied amounts of alkaline washing detergent (3.2 to 3.6 ppm); and steam condensate from the autoclave (2.25 ppm) to ascertain the source of silicate. Water from autoclaved glass bottles had greater concentration of silicates, compared with unautoclaved tap water. Silicate concentration increased variably with successive 20-min autoclave cycles when glass bottles were used. Prolonged autoclave cycles (40 and 60 min) resulted in 34 and 51% higher silicate concentration, respectively, compared with the standard cycle. The increased

concentration of silicate induced was not sufficient to increase concentrations to supersaturation and induce crystallization. Detergent contamination did not influence silicate concentration. These data indicate that autoclaving glass water bottles can lead to increases in the silicate concentration. Under some circumstances crystal formation in bottles may result.

P27 Innovative Housing for African Clawed Frogs (*Xenopus laevis*)

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A unique, innovative, cost-effective aquatic nonrecirculating system (ANS) was developed in response to the need to house an increased number of aquatic frogs, while minimizing medical problems and contamination caused by poor water quality. African clawed frogs (*Xenopus laevis*) are commonly housed in polyethylene tanks or polycarbonate cages. Traditional nonrecirculating water systems are labor intensive, requiring frequent cage and water changes, in addition to the time required for water dechlorination. The system we designed uses modified polycarbonate rodent cages and a nonrecirculating water system that delivers temperature-controlled, dechlorinated, 5- μ m filtered water directly to each cage. Simultaneously, multiple slow water flushes are delivered over a 1-h period twice daily to all cages. The result has been a relatively clean, unstressful environment for *X. laevis* that lessens the potential for development of conditions associated with poor water quality while allowing adequate time for food consumption between flushes. The ANS includes two filters (charcoal and 5- μ m), a temperature-controlled 55-gallon reservoir, an inline nonrecirculating water pump, and a 2-way solenoid valve. The pH and chlorine contents are monitored weekly. The ANS is constructed of standard equipment that is readily available, producing a cost-effective alternative to expensive commercial aquatic systems. This unique housing system limits potential disease to only the cage of origin while providing quality care with minimal maintenance and cost.

P28 An Integrated Management Information System for the Laboratory Animal Facility

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Managing information in the animal facility is more important than ever. Federal requirements, such as the Office of Management and Budget circular A-21, Principles for Determining Costs Applicable to Grants, Contracts, and Other Agreements with Educational Institutions (OMB A-21), coupled with the Institutional Animal Care and Use Committee (IACUC) and other institutional requirements are making the collection, entry, and manipulation of data a crucial element in the daily operations of the facility. At the same time, increasing financial strains frequently preclude acquiring additional personnel for these important functions. These conditions led to the complete review of all major areas in the facility involving data management. The objective was to design an automated system that would: reduce paperwork, facilitate collection of essential data, integrate seamlessly with other areas within the facility, and interface with other selected database servers on campus. Five major areas were identified: protocol management; animal procurement; animal census; billing (per-diems and procurement chargebacks); and cost accounting. Next, the information collected and used in each area was carefully

reviewed to identify how information flowed throughout the facility, and where information was being duplicated. Finally, an information system was designed so that each area would have its own module designed specifically for that operation, but would share data with other areas from a centralized database server. Some of the more salient time-saving features include automatic electronic notification to investigators of animal arrivals in the facility, bar-coded transaction-based census, which eliminates the need for manual daily census counts, and the ability to interface with the Office of Research Administration at the University to extract protocol data. Since implementation, the result has been a fully integrated information system that eliminates duplicate data entry and automates the collection and manipulation of data for each functional area as well as for the organization as a whole.

P29 Dark-Phase Light Contamination Stimulates Tumor Growth and Metabolism in the "Photoperiodically" Controlled Animal Facility

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Photobiologically induced alterations occur in animal physiology and metabolism. Controlled lighting in the animal facility is an important factor in maintaining healthy laboratory animals, a protocol that may not always be rigorously applied. Previous investigations indicate that neoplasms grow more rapidly in animals maintained in constant light. Recent results indicate that tumor growth in rats is stimulated by increased blood concentrations of arachidonic acid and linoleic acid (LA). Uptake of LA and release of its putative mitogenic metabolite, 13-hydroxyoctadecadienoic acid (13-HODE), are suppressed by the circadian neurohormone melatonin (MLT). Its production is regulated in the laboratory animal by light. The hypothesis is that minimal light contamination (3 Lux) in the animal facility during the dark phase disrupts the normal circadian production of MLT and stimulates tumor growth. At five weeks of age, male Buffalo rats (BUF/NCR) were allotted to three groups of six animals each. Group-1 rats were maintained on a 12L (140 Lux):12D (0 Lux) photoperiod (lights on at 6 AM); group-2 rats were maintained in a 12L (140 Lux):12 light-contaminated dark phase (3 Lux); group-3 animals were maintained in a constant light environment (140 Lux). All animals were provided standard laboratory chow (Prolab, RMH 1000) and water ad libitum. Mean daily plasma total fatty acid/LA content (TFA/LA) for groups 1, 2, and 3 were $3.1 \pm 0.6/0.72 \pm 0.1$; $2.9 \pm 0.6/0.66 \pm 0.1$; and $2.6 \pm 0.2/0.59 \pm 0.04$ mg/ml, respectively. At ten weeks of age, all rats were implanted with the Morris hepatoma 7288CTC grown subcutaneously as "tissue-isolated" tumors. Latency to onset of palpable tumor mass was 13, 11, and 7 days, respectively. Tumor growth rates were 0.72 ± 0.3 , 1.3 ± 0.4 , and 1.5 ± 0.4 g/day, respectively. Arteriovenous difference measurements of TFA and LA uptakes for the three groups were 4.2 ± 2.2 , 8.3 ± 1.6 , and 7.1 ± 1.9 and 0.8 ± 0.4 , and 6 ± 0.3 , and 1.5 ± 0.4 mg/min/g, respectively. Glucose utilization and lactate production rates were not significantly different among groups 1, 2, and 3 ($P > 0.05$). Tumor FA/LA contents were $14.0 \pm 1.2/1.5 \pm 0.2$, $70.9 \pm 11.8/10.5 \pm 1.8$, and $107.5 \pm 10.0/18.5 \pm 1.8$ mg/g, respectively. In conclusion, light contamination with as little as 3 Lux during the dark phase of an otherwise normal diurnal photoperiod stimulates tumor growth in laboratory rats, presumably via a direct effect on host MLT secretion.

P30 Comparison of Gross and Histologic Effects of Six Vehicles for Subcutaneous Injection of Hydrophobic Steroid Mimetic Compounds in Rats

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Six vehicle preparations, successful in dissolving hydrophobic steroid mimetic hormones and reported to be nontoxic and nonirritating, were compared for evidence of pain on injection and gross and microscopic lesions at the site of injection. Oil vehicles could not be used because rapid release into tissues was required. Each test vehicle was injected (0.1 ml) once daily subcutaneously at the nape of the neck in weanling rats for three, seven, and ten days. The six vehicles were: 50% DMSO in 50% phosphate-buffered saline (PBS) (B); 90% polyethylene glycol 400 in 10% ethanol (C); 50% dimethyl acetamide in 50% PBS (D); 100% 2,2 dimethyl-1,3 dioxolane-4-methanol (E); 10% polyoxyethylene sorbitan monooleate with 10% ethanol in 80% PBS (G); and 100% propylene glycol (H). Phosphate-buffered saline (A) and 50% ethanol in 50% PBS (F) were injected as the negative and positive controls, respectively. Results were analyzed by the Student-Newman-Keuls comparison method. All vehicles were associated with a significant ($P < 0.001$) increase in microscopic lesions, compared with PBS. Vehicles D and E and control vehicle F induced extensive cellulitis, myositis, and dermatitis. Vehicles D and E induced a pain reaction when injected. Vehicles B, C, G, and H did not elicit a pain reaction, but there were mild gross and histologic lesions. However, the viscosity of vehicles C and H made them difficult to inject through a reasonably sized needle (23 to 25 gauge). They were rejected from further study. On the basis of information obtained, vehicles B and G were chosen for further study of subcutaneous injection of hydrophobic steroid mimetic compounds in rats.

P31 The Use of Plastinated Specimens as Teaching Aids

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In the era of the three Rs (reduction, refinement, and replacement), many innovative ideas have evolved in an effort to decrease the number of live animals used in teaching. At the University of California, Davis, veterinary students receive instruction on the anatomy of the oropharynx of common laboratory species. This is done to allow visualization of the structures that are involved in intubation of these species. In the past, these goals were obtained through use of prosections created from animals specifically euthanatized for this purpose. The authors of this abstract have introduced a novel approach to reduce the numbers of animals used by creating plastinated models, which can be used for multiple years. Plastination is a patented technique of tissue preservation that was introduced in Europe in 1979. It has been used in the United States to study anatomic and pathologic details in brains, hearts, lungs, and bones since the 1980s. This process consists of replacement of water and lipids in biological tissues by curable polymers. To create our specimens, representatives of common laboratory animals (including rabbits, guinea pigs, and conventional swine) were anesthetized for perfusion. A catheter was placed in each external carotid artery through which saline, then buffered 10% formalin was infused. The jugular veins were transected allowing outflow of blood and infusates. During this process, care was taken to hyperextend the neck and keep the mouth in the open

position. After decapitation, the head was frozen then transected on a mid-sagittal plane. The tissues were dehydrated in acetone then impregnated with silicone polymer under vacuum in a freeze drier according to the published technique of Gunther von Hagens. After a curing period at room temperature, a permanent, dry, odorless, life-like specimen was then available for use. The plastinated specimens are studied by students prior to attempting intubation of these species. The result has been the use of fewer animals for dissections, and has been met by an overwhelmingly favorable response from the students. These specimens are clearly superior to the only other alternative to fresh tissue, which is tissue preserved in formalin. Formalin-fixed tissues emit a noxious volatile vapor and are becoming an issue for laboratory safety standards. In addition, formalin-fixed wet tissues are delicate and susceptible to damage when handled repeatedly. Clearly, the process of plastination produces stable, durable maintenance-free specimens that are superior to any other alternatives available and allows reduction in the numbers of animals used for teaching.

P32 Dental Radiographic Techniques in the Squirrel Monkey

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In the squirrel monkey, dental abscesses are a frequent health problem that results in facial swelling, pain, and fever. The affected animal will often refuse to eat, and weight loss can be substantial and rapid. Such fulminant dental disease can, in general, be identified by a careful physical examination; however, this late-stage diagnosis is not optimal. The nature of our research use of the squirrel monkey requires application of a close-fitting face mask for inhalation studies, thus even mild or sub-clinical dental disease is undesirable. An aggressive program of dental prophylaxis and radiography over the past two years has greatly reduced the unplanned "lost-time" on studies, the number of emergency dental interventions and the associated discomfort to the animals. The techniques of dental radiography of squirrel monkeys and the interpretation of these radiographs are not well described in literature. However, we have defined techniques that permit early diagnosis of abscess-prone teeth. Because of the small size of the squirrel monkey oral cavity, it is difficult to obtain accurate images of the teeth, using conventional radiographic techniques; tooth roots will often be truncated. Superimposition of surrounding structures will also compromise visibility. The bisecting-angle radiographic technique is widely used in small animal practice to overcome these difficulties. We describe this technique in the squirrel monkey as well as other radiographic techniques useful in permitting diagnosis and prognosis of dental problems in the squirrel monkey.

P33 Comparison of Several Materials to Cool Metabolism Cage Collection Tubes During Use

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Cooling of metabolism cage collection tubes is often required to protect the integrity of urine and fecal samples for subsequent chemical and biochemical analyses. Ice is generally used to cool urine and feces to prevent evaporation and sample degradation, but it has several disadvantages. Ice lasts approximately 16 h; however, a typical collection period may last up to 24 h, leading to frequent ice changes. Additionally, ice cannot be reused. Five cooling materials were evalu-

ated over a 28-h period: ice, dry ice, ice substitute frozen at -20°C, ice substitute frozen at -70°C, and ice substitute frozen on dry ice. For each material, a polycarbonate metabolism cage was placed in a styrofoam cooler with the feces and urine collection tubes extending into the cooling material. A rat urination pattern was simulated by adding 1 ml of saline (38°C) to the urine collection tube at 4-h intervals. Temperature was measured immediately prior to saline addition and 15 min after saline addition. Data were analyzed by use of a single-factor ANOVA, and a Student's *t*-test. Ice and dry ice required replacement after the 16-h measurements. Results indicated that ice maintained the saline temperature at about 0°C, whereas all ice substitute groups had an increase in temperature over a 24-h period from about -2°C to about 8°C. Dry ice kept the temperature lower than all other cooling materials, making it the best option for volatile substance collection. Ice substitute, regardless of storage conditions, maintained the samples within acceptable facility temperature ranges for standard collections.

P34 Techniques for Serial Bone Marrow Aspirations in Growing Yucatan Minipigs

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Serial bone marrow aspirations of 1 ml were required in the development of the Yucatan minipig as an animal model for hematopoiesis. Sample collection began two weeks after birth and progressed at two- to four-week intervals for 4 months in 13 minipigs. Three adult minipigs also had samples taken. Light anesthesia was achieved in all minipigs with isoflurane and nitrous oxide before the procedure. We discovered that the optimal location and size of the needle used varied depending on the weight of the minipig. From 2 to 9 kg, the cranial dorsal iliac spine was easily palpable. An 18-gauge Jamshidi bone marrow needle was introduced medially 2 cm ventral from this landmark for successful bone marrow aspiration of up to 3 ml. At the same sampling site, for minipigs weighing 10 to 20 kg, either a 16- or 13-gauge bone marrow needle was needed. The size required was dependent on the development of the gluteal muscles and the strength of the cortical bone. An 18-gauge needle could also be used on the second sternebra for a 1-ml draw. A high degree of precision was required for this approach, however, taking care not to puncture through the sternebra and enter the thoracic cavity. Approaches attempted on adult pigs (80 to 90 kg) included varying locations on the mandible, sternum, tibia, and ilium. The mandible, although easily palpable, could not be pierced with the lowest gauge needle available (8-gauge). The ilium was difficult to palpate and unreachable with the longest available needle (4 in) due to gluteal muscle development. Bone marrow was accessible in the second sternebra, using a 13-gauge needle. Marrow was also accessible by introducing an 11-gauge needle perpendicularly into the flat proximomedial aspect of the tibia halfway between the tibial tuberosity and the distal end of the tibial crest. The techniques described here allowed successful serial marrow aspiration with minimal trauma in minipigs.

P35 Chronic Intestinal Cannulation for Site-Specific Dosing in Conscious Microswine

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Similarities in gastrointestinal tract physiology with humans support the use of swine in pharmacokinetic studies determining oral bioavailability of new drugs. Subcutaneous ports with 5-F silastic catheters were used to develop an intestinal cannulation model in Yucatan microswine for site-specific dosing, allowing determination of absorption kinetics within different segments of the gastrointestinal tract. A ventral midline approach allowed optimal exposure of the small and large intestines, though the voluminous spiral colon situated along the abdominal floor complicated access to the small intestine. Familiarity with gross intestinal anatomy, including length of mesenteric attachments, lymphatic structures, and the diameter, shape, and salient external features of various intestinal segments, expedited accurate identification of specific cannulation sites while minimizing surgical exposure. Subsequent use of contrast radiography alleviated concerns regarding intra-abdominal catheter kinking and verified continued intraluminal placement and patency of cannulas within the intestinal tract of all seven instrumented animals for the 3-month duration of animal model maintenance. Long-term cannulation of the intestines in Yucatan microswine using subcutaneous access ports provided a manageable, appropriate model to study gastrointestinal tract absorption of test compounds in a conscious large animal species.

P36 Basic FGF Protects Myocardium at Risk in Dogs With Temporary Coronary Artery Occlusion Without Affecting Regional Blood Flow

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It has previously been reported that reduction in infarct size after treatment with basic FGF (bFGF) is mediated by angiogenesis. We studied the effects of bFGF on infarct size, regional myocardial flow, and shortening fraction in 11 anesthetized dogs undergoing 180-min LAD coronary artery occlusion and 5-min reperfusion. Fifteen min after occlusion, 6 dogs received an intracoronary bolus of bFGF (20 µg) and 5 received placebo. Heart rate and aortic and left atrial pressures were similar in both groups throughout. Infarct size was measured by computer-assisted planimetry of myocardial sections after ex vivo staining of the circumflex (Evan's blue) and LAD (tetrazolium chloride) territories. Regional flow was measured 1, 30, and 180 min after occlusion, using radio-labeled microspheres. The infarct zone shortening fraction was measured, using mid-myocardial sonomicrometer crystals. Flow in the distal LAD territory was similar in both groups and did not increase during occlusion; however, in dogs receiving bFGF, infarct size was reduced and shortening fraction improved (data are expressed as mean ± SD).

	Placebo	bFGF	PValue
Infarct size (% area at risk)	25.8 ± 8.2	14.2 ± 5.2	0.016
LAD endocardial flow at 1 min (ml/min/g)	0.05 ± 0.08	0.14 ± 0.11	NS
LAD endocardial flow at 180 min (ml/min/g)	0.03 ± 0.03	0.09 ± 0.08	NS
Shortening fraction at 1 min (%)	-4.2 ± 4.5	-2.5 ± 3.5	NS
Shortening fraction at 180 min (%)	-3.1 ± 4.7	2.7 ± 4.1	0.049
Shortening fraction 5 min after reperfusion (%)	0.4 ± 0.9	4.6 ± 3.4	0.23

In a period too short for neovascularization to occur, bFGF given after the onset of ischemia protects myocardium at risk without affecting regional blood flow.

P37 The Hanford Miniature Pig as a Model for Cardiac Electrophysiologic Testing

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Techniques for cardiac pacemaker implantation and noninvasive electrophysiologic (EP) testing have been developed in Hanford miniature swine to address various clinical issues in human cardiology. Recently, 14 Hanford swine (45 to 55 kg) have been studied to develop a novel pacemaker lead that provides single-lead dual-chamber pacing. Sixteen Hanford swine (19 to 23 kg) implanted with pacemakers have also been used to determine whether cardiac hypertrophy renders the heart arrhythmogenic. Hanford swine were chosen as the model for these studies because of the anatomic and electrophysiologic similarities of the heart to that of the adult human. Both studies require surgical implantation of pacemakers, dual-chamber pacing, and EP testing. For pacemaker and lead implantation, anesthesia was induced with ketamine (33 mg/kg of body weight, IM) and acepromazine (1.1 mg/kg, IM), then animals were intubated, and anesthesia was maintained with 2% isoflurane delivered in oxygen:nitrous oxide (1:1). The left external jugular vein was surgically isolated for insertion of atrial and ventricular pacing leads, and a pocket was created to accommodate the pulse generator and excess length of leads. Pacing leads were advanced and positioned in the cardiac chambers under fluoroscopic guidance. Capture and pacing of each chamber was confirmed, and stimulation thresholds and intracardiac electrograms were recorded for both chambers prior to wound closure. Noninvasive EP testing of conscious animals in both groups was performed for up to 12 weeks, using a programmable telemetry system. Successful completion of EP studies in 30 Hanford swine has generated a data base for cardiac EP parameters that can be compared with those parameters in humans.

P38 A New Catheter for the Collection of Bile in Conscious Dogs

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A novel, totally implantable catheter system to allow bile collection in conscious, freely moving dogs is described. This set up allows either the complete collection of bile or the delivery of bile to the duodenum. Patency durations for functional bile collection in eight of eleven implanted animals averaged 181 (range, 16 to 553) days. In selected animals, parameters indicative of liver function (serum alanine transaminase, alkaline phosphatase, γ-glutamyltransferase, and total bilirubin) were within normal ranges six months after surgery. Four animals have been used in a total of 18 drug studies where bile was successfully collected for 24 h. Bile has been collected, using either a tethering system or a protected pouch arrangement. Two animals have been used to validate the model's complete collection of bile via biliary recovery of an intravenously administered dose of radiolabeled glycocholic acid. Compared with exteriorized catheter models, this system requires less maintenance and is better tolerated by the animals. The potential for a longer functional lifespan for individual animals and more normal liver enzyme activities are other advantages of this model.

P39 Effect of AIN and NIH-07 Rodent Diets on the Cecum of Gnotobiotic Rats Inoculated With the CRAS Flora

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Metabolism of rodents is profoundly affected by their intestinal anaerobic bacterial flora, their diet, and interactions between the two. We are attempting to determine optimal diets and intestinal flora to be used to standardize these variables. We compared the effects of the rodent diets recommended by the American Institute of Nutrition (AIN) on the cecum of rats with a standardized enteric flora. We used 16 male and 16 female, axenic rats maintained in a sterile gnotobiotic isolator and fed autoclaved food since weaning. Five weeks before beginning the study, when the rats were 9 weeks old, they were inoculated with the CRAS flora (8 species of anaerobes). One week before the start of the study, the animals were transferred to two isolators, one for males and one for females, and were maintained using gnotobiotic techniques. Groups of 4 male and 4 female rats were formed by use of a table of random numbers, and each was fed either NIH-07, AIN-76a, AIN-93, or AIN-93 with 2% pectin (substituted for 2% starch) for four weeks. All diets were sterilized, using 4 megarads of gamma irradiation. Food was removed the night before the animals were euthanized by use of CO₂, and body weight, brain weight, cecum weight, and volume of cecum contents were determined. Analysis of variance and the Kruskal-Wallis test for ranks (in the case of unequal variances and small sample sizes) were used to compare treatment groups at $\alpha = 0.05$. Tukey's test was then used for post-hoc multiple comparisons of pairwise treatment differences among means, while maintaining study-wide $\alpha = 0.05$. Males weighed 398.81 ± 14.25 g, and females weighed 270.73 ± 12.53 g at study termination. Male brains weighed 1.917 ± 0.074 g and female brains weighed 1.815 ± 0.046 g. Neither of these variables differed significantly among the diet treatment groups even when the data were stratified by sex. Cecum weight ($F = 11.16, P = 0.0001$), cecum contents volume ($F = 6.05, P = 0.003$), and brain weight/cecum weight ($F = 6.1, P = 0.0025$) differed significantly among treatment groups. These differences were attributed to rats in the NIH-07 group. All these differences except cecum contents volume were significant when the data were stratified by sex. In conclusion, the weight and volume of the contents of the cecum are significantly different in rats of either sex that have the same intestinal flora and fed NIH-07, compared with the AIN rodent diets. When rodent diets are developed for or used in studies, the effect on the cecum should be considered.

P40 Multichannel Telemetric Monitoring of Urodynamics in Rhesus Monkeys

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Telemetric monitoring of research animals has been in use for many years. Recent advances in computer, electronics, and battery technologies now allow more practical monitoring systems. This system was designed with the goal of monitoring bladder pressure, abdominal pressure, electromyography (EMG) of the external urethral sphincter, volume of urine voided, and urine flow rates in conscious rhesus monkeys for extended periods without the stress to the animal of being physically tethered to any piece of equipment. Bladder and abdominal pressures are measured via solid-state transducers.

The bladder pressure transducer is implanted in the bladder wall, and the abdominal pressure transducer is left free in the abdominal cavity. The active EMG lead is buried in the pelvic floor musculature near the external urethral sphincter. The transducers and EMG leads terminate in a radio transmitter implanted subcutaneously in the animal's back. Signals from the implants are picked up by antennas mounted on a specially modified metabolic cage. Urine volume voided data are measured by a uroflowmeter load cell transducer placed under the cage. All data are acquired by use of a PC-based data acquisition system located in a remote laboratory. This system allows real-time monitoring of data for periods of up to 23 h per day. The animals are generally monitored for one or two weeks, then returned to their home cage. Initial studies focused on feasibility of this system. Current studies are investigating differences in measuring internal pressures in the body by implanted solid-state transducers versus measurements via water-filled catheters. We have found that this system is reliable, accurate, and able to successfully monitor for extended periods without any stress to the animals.

P41 A Procedure for Improved Safety When Injecting Infective Agents (Including HIV) Into Mice and Obtaining Multiple Retro-Orbital Blood Samples From Infected Mice

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Loading of syringes, delivery of the infective agent and blood withdrawal present a risk of personal exposure from sharps. Within a biological safety cabinet, the infective agent is drawn up into a self-locking 3-ml syringe with a flexible intravenous catheter unit (18 gauge x 1 and 1/4 in). The catheter needle is removed and disposed of prior to drawing up HIV or other infective agents, providing the syringe with only the flexible catheter attached. The substance is carefully drawn into the syringe until the desired dosage is achieved, and the flexible catheter is removed from the syringe, using a pair of hemostatic forceps. Using the same forceps, a 27-gauge 1/2-in needle is secured on the syringe and the sheath is removed (with the forceps). Excess air in the syringe can safely be expelled into a sterile bottle of saline. The loaded syringe is carefully placed into a holder (we use a 6-ml syringe cartridge) to prevent accidental contact. To reduce the risk of a needle stick, only intraperitoneal injections (for HIV) are performed in anesthetized mice. Intravenous injections are prohibited. Blood samples can effectively be obtained retro-orbitally from anesthetized mice by use of a hand-held micropipette (capacity $200 \pm \mu\text{l}$) and appropriate disposable plastic tips. Depending on the size of the mouse, blood samples between 25 and $500 \pm \mu\text{l}$ can routinely be obtained. Blood can easily be dispensed into an appropriate container, and the plastic tip can be injected into a sharp container. On completion of the procedure, the eye is treated with sterile ocular lubricant to reduce irritation and minimize the possibility of injury. In summary, intraperitoneal injections in anesthetized mice provide a safer technique than do intravenous tail injections or cardiac puncture. Use of a flexible catheter to draw up infective agents reduces risk of a needle stick during the process. Using a micropipette for performing retro-orbital blood withdrawal is as effective as the traditional glass Pasteur pipet method, but substantially safer, as an individual's hand is always at a safe distance from infected blood.

P42 A Conceptual and Methodological Model for Evaluating Toxicant and Drug-Induced Perturbations in Reproductive Function in the Rat (*Rattus norvegicus*).

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The female rat has been used as a standard model in studies of reproductive function and dysfunction. Using this model, it has been found that environmental toxicants and certain drugs adversely influence pregnancy and fetal outcome by interfering with one or more aspects of the reproductive process: estrus cyclicity; mating behavior; conception; before and after implantation of the conceptus in the uterus; and fetal development. Thus, thorough assessment of toxicant- or drug-induced abnormalities in pregnancy or fetal outcome should address each of these steps. This has rarely been done in most studies, and consequently, the mechanisms underlying the adverse effects of drugs or environmental toxicants on reproduction are poorly understood. Three factors seem to contribute to lack of a systematic and comprehensive analysis of the impact of toxic agents on the reproductive process. In some instances, investigators are simply interested in whether a drug or toxicant influences pregnancy outcome, regardless of what the mechanisms might be. Also there is a surprising degree of inconsistency in the methods and nomenclature used to characterize the normal female reproductive system of the rat, the changes that occur during pregnancy and fetal outcome. Finally, the methods to carry out these analyses have either been incompletely described or scattered over many reports, in some instances, dating back to 1922. The purpose of the study reported here was to develop a comprehensive conceptual and methodologic model that could serve as a framework to assess the effects of drugs or environmental toxicants on the multiple steps involved in reproduction, pregnancy, and fetal outcome, which has recently emerged as an important and rapidly expanding area of research. The model incorporates methods to examine the effects of exogenous agents on the estrous cycle, copulatory behavior, conception, implantation of the conceptus into the uterine wall, postimplantation loss, and fetal viability.

P43 Random-Source Swine as an Alternative Model in a Cardiovascular/Physiology Laboratory for Second-Year Medical Students at Ohio University College of Osteopathic Medicine

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At Ohio University College of Osteopathic Medicine, second-year medical students participate in a cardiovascular laboratory. The model of choice had previously been random-source dogs; however, there were a number of drawbacks associated with this model. Because the dogs were random source, the students often found themselves comparing the model with similar animals with which they had been familiar or had owned or still owned as companions. This placed them in an extremely uncomfortable position with regard to this terminal teaching laboratory. Over the course of the 4-h laboratory session, a few students found themselves becoming ill. Some found they had a moral dilemma regarding the use of dogs. Others refused to participate in the session, placing their grade in jeopardy. After much discussion and research toward finding a suitable alternative, random-source swine were selected as an appropriate model. During the first session using the new model, the change in attitude displayed by the students was pronounced. They were more accepting of the

swine model, which has led to an improved atmosphere more conducive to learning. In subsequent sessions conducted using the swine model, we have had no incidence of the illness or nausea, which had been relatively common when dogs were used. There have been only isolated refusals to participate in the sessions, and the students do not seem to have the difficulty with the finality of their procedures that had been observed. Swine are anesthetized prior to the beginning of the 4-h terminal laboratory session, then continually maintained at a surgical plane with a ketamine/pentobarbital combination administered intravenously. During this class, a number of surgical procedures are undertaken by the students, including tracheal cannulation, catheterization of blood vessels, vagal nerve stimulation, thoracotomy, and external heart massage. Electrocardiograms and arterial pressure traces are displayed on a 2-channel oscilloscope coupled with a Windows-based data acquisition package. The software (AcqKnowledge Ver 3.0) and hardware (Gateway 2000 computer P4D66) allows real-time display of acquired data with subsequent analyses of hemodynamic and electrocardiographic variables. In a hands-on laboratory environment, the medical students obtain immediate feedback on various physiologic characteristics of the circulatory system. Physiologic manipulations (vagal stimulation, carotid occlusion, atrial and ventricular fibrillation with conversion) are complemented by a wide range of pharmacologic manipulations that examine the autonomic control of the circulation. Pharmacologic experimentations include studies on dose-dependency as it pertains to the administration of epinephrine and norepinephrine, differentiation of cholinergic muscarinic and nicotinic responses, and desensitization, as well as an introductory study on autonomic pharmacology. The knowledge and experience gained in this laboratory is an important part of the overall education of students about to enter the field of medicine.

P44 Telemetry: A New Method to Measure Cardiotoxicity in Freely Moving Mice

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Measurements of the electrocardiogram (ECG) and heart rate by telemetry has been described for many animals, but not mice. By using telemetry, measurements in freely moving animals are more efficient, reliable, and less labor intensive. The effect of surgery for implanting the transmitter, handling, and anesthesia on the changes in ECG and heart rate were examined. Clinical use of the antitumor agent doxorubicin is limited by doxorubicin-induced cardiotoxicosis. Therefore, the effect of chronic doxorubicin administration on the ECG also was determined. Balb/c mice ($n = 8$) were equipped intraperitoneally with a telemeter, then were given 6 weekly doses of 4 mg of doxorubicin/kg of body weight intravenously. A saline-treated group ($n = 4$) served as control. The ECG was measured in the freely moving animal 2 times per week. To assess the effect of doxorubicin on the ECG, the following complexes were analyzed in detail: QRS-complex and QT- and ST-intervals. At the end of the study, the QT- and ST-intervals widened to 17.7 ± 2.9 and 16.7 ± 2.7 milliseconds (mean \pm SEM, $P < 0.01$, compared with controls). The ECG in the control animals did not change during the entire study. It is concluded that this new method is sensitive, uses small numbers of mice, and enables us to monitor the development of cardiotoxicosis over time.