Platform Sessions

PS01 Calculating Per Diem Rates for Animal Facilities
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Calculating equitable animal per diem rates is a challenge. Although the Office of Management and Budget circular A-21 provides general rules and guidelines, it does not outline a specific methodology. Most facilities do not have the resources to hire professional consultants or a small army of accountants. At the University of Arizona, we have developed a multi-page spreadsheet format that uses raw data from institutional financial reports and determines per diem rates with concise rate components such as feed and bedding. We begin by identifying the cost centers and cost drivers. We also use time/motion studies as a basis for allocating direct labor and husbandry overhead, rather than effort reports, which proved unreliable. Our spreadsheet format uses the step-down allocation of indirect cost as stipulated in A-21. Because spreadsheets are linked, data changes or "what-if" scenarios are accomplished with minimal effort. Use of this process year after year allows easy comparison of rate components over time and allows analysis of rate changes.

PS02 Engineering a Reduction in Personal Exposure to Laboratory Animal Aeroallergens

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Prevalence of allergy to laboratory animals is associated with the intensity of personal exposure to animal allergens. Studies were conducted to identify factors in the management of laboratory animals that influence exposure to airborne animal allergens. Airborne allergen concentrations were influenced by litter type, cage design, and stacking density. The effect of using positively individually ventilated cage systems, vacuum systems and fume hoods to reduce personal exposure to animal allergens were also objectively assessed. Use of positively individually ventilated (PIV) cages reduced the static mouse urinary aeroallergen (MUA) concentration (n = 24; median = 0.09 µg/m³) to one-seventh of the concentration for open cage systems (n = 12; median = 0.67 µg/m³; P < 0.001). Removing soiled litter with a vacuum (n = 15; median = 25.08 µg/m³) reduced personal exposure to rat urinary aeroallergen (RUA) by one-half, compared with tipping (n = 9; median = 42.99 µg/m³; P < 0.017). Exposure to RUA was 25-fold greater when rats were handled on an open bench (n = 17; median = 54.39 µg/m³), compared with use of fume hoods (n = 8; median = 1.19 µg/m³; P = 0.0001). These results indicated that utilizing existing equipment and modifying laboratory animal management practices can reduce personal exposure to animal aeroallergens.

PS03 The Effects of Routine Animal Husbandry and Experimental Procedures Upon Physiological Parameters of Rats
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Minimizing stress and avoiding distress is a common objective of laboratory animal care. To avoid animal distress, the 1993 AVMA Panel on Euthanasia and the 1996 Guide recommend that other animals should not be present when euthanasia is performed. It was our hypothesis that moving a rat from its home room environment to perform routine procedures will cause a greater variability in physiologic parameters (core temperature, heart rate, blood pressure, and activity) than conducting those procedures in the animal's home room, and procedures, including euthanasia, conducted in the presence of rats in their home room environment would not influence these physiologic parameters. Fourteen, female Sprague-Dawley rats were implanted with a telemetry monitoring system. The system enabled collection of core temperature, heart rate, blood pressure, and activity measurements. Monitoring was adjusted to rotate through the colony, collecting data points at 5-minute intervals, 24 hours per day. Normal circadian rhythm demonstrated a quiet phase during the light cycle (7 a.m. to 7 p.m.) with heart rate around 325 beats per minute (BPM) and an active phase during the dark cycle (7 p.m. to 7 a.m.) with heart rate around 400 BPM. Other measured parameters had similar circadian variation. During a routine cage change, heart rate increased 100 BPM over baseline values and required up to 2 hours to return to normal values. Body temperature increased by up to 2°C and remained elevated for up to 4 hours. Performing a physical examination or weighing an animal in a home room elicited increases in physiologic parameters similar to that of a cage change. When animals were transported to a strange environment, recorded increases were more dramatic. Heart rate increased 50 BPM in transported animals versus those in the home room. Blood collections, minor surgery (osmotic pump implant), anesthesia, and euthanasia (barbiturate overdose, CO₂ exanguination, or decapitation while anesthetized) were performed in the animal's home room. Procedures were done in view of the animals or behind a partition, in rooms with mass airflow and conventional airflow. Procedures performed in the presence of the telemetry-implanted rats caused results similar to baseline data for all measured parameters. Manipulating a rat in its home room en-
environment for routine husbandry and experimental procedures resulted in an increase in the animal’s body temperature, heart rate, blood pressure, and activity. These values were further increased when the animal was transported to a strange environment. Conducting these same procedures in an animal’s home room did not alter the monitored parameters. For rats, our data appeared to support the practice of performing routine procedures, including euthanasia, in the animal’s home room.

**PS04 Suggested Microbial Count Guidelines for Qualifying Sanitization of Animal Caging by Use of Rack Washers**

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Our study offered a practical guideline for microbiologically qualifying the sanitization of animal caging, using rack washers. Procedures were developed for rodent, rabbit, and primate caging, using manifold, rotating-arm, and carousel-type rack washers. Wash cycles were developed on the basis of minimal cycle times, and microbiological monitoring was conducted by using replicate organism detection and counting (RODAC) plates. Acid and detergent were used to clean all caging, except rodent caging, in which only detergent was used. All caging was exposed to a final rinse temperature of 180°F for 3 minutes. Ten to 12 monitoring sites, reflecting the most soiled areas, were chosen, and pre- and postcleaning testing were conducted, using RODAC plates. Colony counts reflecting effective sanitization were established at ≤5 nonsporulating bacteria/monitoring site. Cycle times, detergent, and acid concentrations were adjusted to meet this criterion. Qualification of the procedure required that 3 consecutive runs be conducted to insure reproducibility. This limit of ≤5 nonsporulating bacteria has been used for >15 years at our facility without a report of nosocomial infections that could be traced to inadequate sanitization.

**PS05 Ethical Wildlife Research: The Need for a Team Approach**

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Research that uses laboratory animals is controversial, but is commonplace. However, the traditional spectrum of species is being continually extended to include fish, amphibians, reptiles, birds, and various animals in the wild. Increases in the diversity of species has created challenges for the Animal Care and Ethics Committee (ACEC) at institutions when evaluating the best practices for a wide range of species; for institutional veterinarians when making recommendations on the care, handling, breeding, capture, anesthesia, and analgesia of infrequently encountered wildlife; and for researchers who must convince the ACEC that the welfare of wildlife will not be compromised by an investigation. Even simple procedures such as capture and handling, which are taken for granted in more common species, can be traumatic and life threatening to wildlife. Although it is the responsibility of the ACEC to ensure that wildlife research is humane, it may be dealing with a species about which there may be scant information. This difficulty can be resolved when the ACEC, veterinarians, and wildlife experts work together. Animal welfare regulations in Australia, the ACEC process of review of research protocols involving wildlife, specific areas that the ACEC regards as priorities, and ways of facilitating ACEC approval for wildlife research are described.

**PS06 Management of a Bat Colony**

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The management of a bat colony presents several challenges not encountered when housing more-traditional laboratory animal species. These challenges include: regulatory issues concerning the acquisition and maintenance of these species, employee health issues, design and construction of primary enclosures, and development and implementation of husbandry and veterinary care programs. We will review the national, international, and local regulatory issues that were encountered when housing bats; describe the employee health program that had to be implemented; describe the special housing equipment that was fabricated; discuss recommendations for routine and specialized husbandry practices; and review disease entities that have been encountered in maintaining species of bats, the Mexican freetailed bat (Tadarida brasiliensis mexicana) and the mustached bat (Perisotus narni).

**PS07 Management of American Black Bears Used for Biomedical Research**

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American black bears (Ursus americanus) have a unique metabolic adaptation during denning that has been studied to benefit people with conditions such as osteoporosis. Scant information is available regarding housing, care, and clinical procedures for American black bears used for biomedical research. We used a management system that has successfully maintained bears for several years and allowed for normal metabolic changes associated with seasonal denning. Fourteen bears were housed in indoor-outdoor enclosures in 2 buildings. Twelve 3-year-old bears were housed separately in divided pens (16 x 8 ft. outdoors and 7 x 5 ft. indoors). Two older bears (17 years old) were penned together in a 20 x 12 ft. outdoor pen, which was connected to 2 indoor, cylindrical 3 x 6 ft. dens. Bears were fed ad libitum during spring, summer, and fall and were allowed to den for approximately 4 months during winter. Every 3 to 5 weeks, the bears were anesthetized with tiletamine-zolazepam for body weight determination and collection of body fluids. Blood was collected from the femoral vein, and urine was collected by means of urinary bladder catheterization. The bears remained clinically normal and underwent normal seasonal metabolic changes. Mean body weight decreased by 30% during the most recent denning season and increased by 66% during the subsequent active season. Some seasonal changes in clinical biochemical parameters were associated with denning behavior. For example, mean urea/creatinine ratio decreased 77% during denning. Thus, the husbandry system described was useful for housing and maintaining American black bears for biomedical research.
PS09 The Octodon Degu—A New Experimental Model

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The Octodon degu is a valuable animal research resource, because, unlike other laboratory rodents, the degu is diurnal and long-lived. However, colonies have encountered problems with poor breeding success, low number of pups per litter, maternal death, diabetes, and obesity. To overcome these problems, extensive literature searches, including searches of Internet sites, were used to gather information on degus. A background in guinea pig breeding was very valuable in solving the challenge of poor breeding success. A diet was established that included low-sugar chow supplemented with carrots, seeds, and peanuts. Animal companionship, human interaction, and environmental enrichment devices eliminated abnormal behaviors, including withdrawal, self-mutilation, and aggression. Dams were bred at a younger age than was used in other colonies. Using these guinea pig-related changes, a colony of 10 young degu pairs was monitored, with weight and reproductive status recorded weekly. These pairs have produced 140 live, healthy degu pups in a period of 2.5 years with a mean litter size of 7 pups. In addition, none of the dams have died since diet and breeding-age changes were instituted.

PS10 An Interlaboratory Comparison of Serologic Test Results for Encephalitozoon cuniculi Infection in Rabbits Points to the Need for Accreditation of Laboratory Animal Diagnostic Laboratories

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Several factors should be considered when choosing a diagnostic laboratory to monitor the health of laboratory animals, including accessibility and geographic location, services offered, test methods, costs, and reliability. Confidence in the validity of the test results is perhaps the most important factor in selecting a diagnostic laboratory, but also the most difficult to evaluate. Laboratories that operate under a quality assurance system document elements of this system in a quality manual that contains, where appropriate, reference to proficiency testing, use of reference material, and other information. Laboratory proficiency testing, as defined in the general criteria for the operation of testing laboratories, European Norm 45001, is the determination of laboratory testing performance by means of interlaboratory test comparisons. In an effort to select a diagnostic laboratory, we sent sera from 170 rabbits (Oryctolagus cuniculus) to 4 private laboratories for testing for Encephalitozoon cuniculi infection. The brain, kidney, and liver of 64 of these rabbits were also examined histologically for lesions consistent with E. cuniculi infection. Results indicated that one laboratory failed to diagnose > 40% of the rabbits determined positive by the other 3 laboratories and by histologic examination, and that one laboratory reported a higher number of seropositive rabbits than the other laboratories, including 90 positive rabbits that were all changed to negative when we requested a re-check. Errors, changes in techniques, and differing test methods that included the use of differing antigens, contributed to the discrepancies in serologic diagnoses. Accreditation of laboratories performing diagnostic testing in laboratory animal sciences would standardize and harmonize test methods and evaluate the results through validation schemes that would guarantee that all tests are reliable and yield correct results. The Federation of European Laboratory Animal Science Associations’ Working Group on Accreditation has been charged with interpreting the European Norm 45001 for such diagnostic laboratories to assist them in becoming accredited by the European Cooperation for Accreditation of Laboratories.

PS11 Identification of Widespread Helicobacter hepaticus Infection in Mouse Colonies in Japan

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Helicobacter hepaticus is a recently identified bacterium associated with chronic active hepatitis and hepatic tumors. An antibody test was performed to detect H. hepaticus infection in mouse colonies in Japan from 1994 to 1996. Five commercial breeder (CB) facilities (1,025 mice), 12 pharmaceutical company (PC) facilities (598 mice), and 14 university/institute (U/I) facilities (615 mice) were tested. Blood samples were collected from all mice, and all mice were necropsied. Sera were tested for H. hepaticus antibody by an enzyme-linked immunosorbent assay (ELISA), followed by an indirect immunofluorescent antibody (IFA) test on ELISA-positive sera. H. hepaticus-type strain ATCC 51448 was used as the antigen for serology. In addition, the polymerase chain reaction (PCR) was performed to detect the H. hepaticus 16S rRNA gene, and a histologic examination was undertaken.
Using cecal tissues, 62 H. hepaticus antibody-positive mice were tested by PCR, 59 of which yielded positive results. For the histologic examination, sections of liver were stained with hematoxylin and eosin, Warthin-Starry, and immunohistochemical stains. Antibody was detected in 65 of 354 mice from 3 CB facilities, 94 of 538 mice from 3 PC facilities, and 82 of 604 mice from 11 U/J facilities. Gross lesions of multiple pale to yellow foci were seen in the livers of 69 mice. Focal coagulative necrosis with inflammatory cell infiltration was found in 58 hepatic sections. Numerous H. hepaticus organisms within bile canaliculi were detected in 47 of 58 hepatic sections, and all of these mice were H. hepaticus antibody positive. A strong correlation was obtained for results of the antibody tests, PCR, and histologic examination, suggesting a high prevalence of H. hepaticus infection among mouse colonies in Japan.

PS12 Enzyme-linked Immunosorbent Assay (ELISA) for Fecal IgA to Helicobacter hepaticus: A Rapid, Noninvasive Screening Test for Infection

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Infection with Helicobacter hepaticus can potentially confound research results, because it causes hepatitis and hepatic adenocarcinoma in certain strains of mice. Because H. hepaticus is a fastidious microaerobe, takes 3 to 7 days to grow on selective antibiotic media, and polymerase chain reaction (PCR)-based assays can be expensive and sometimes difficult to interpret, a sensitive and specific noninvasive diagnostic assay for H. hepaticus is needed. Data for A/JCr mice indicated that humoral IgG serum titers increased as male and female mice aged. Therefore, we studied whether a fecal-based enzyme-linked immunosorbent assay (ELISA) would be an earlier diagnostic indicator of H. hepaticus infection as well as being sensitive and specific for detecting infection caused by H. hepaticus. Feces from A/JCr and C57BL/6 mice positive or negative for H. hepaticus by bacterial culture or PCR testing were used to evaluate the sensitivity and specificity of an ELISA that utilized an outer membrane preparation of H. hepaticus antigen. Results indicated that fecal IgA to H. hepaticus can be detected 2 weeks after inoculation in A/JCr mice and 4 weeks after inoculation in C57BL/6 mice. To evaluate cross-reactivity of fecal IgA obtained from H. hepaticus-infected mice with other Helicobacter species, A/JCr and C57BL/6 mice were orally inoculated with H. bilis, H. muridarum, and H. rodentium. Feces were collected every 2 weeks for 2 months, then monthly until necropsy. Fecal IgA cross reactivity was not detected throughout the 20 weeks after inoculation; however, cross reactivity of serum IgG was detectable as early as 4 weeks after inoculation with H. rodentium. These results indicated that the fecal IgA ELISA was a rapid and reliable screening tool for detecting H. hepaticus in mice.

PS13 Detection of Pneumocystis carinii in Immunocompetent Sprague-Dawley Rats, Using a Semiquantitative Polymerase Chain Reaction Assay: Correlation of Detection by Polymerase Chain Reaction Testing With Age in an Enzootically Infected Colony

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Members of our laboratory group have demonstrated that P. carinii could be detected in some naturally infected, unstressed, immunocompetent rats by using a polymerase chain reaction (PCR) assay on DNA extracted from samples of lung tissue. We undertook a study to determine the correlation of age to P. carinii detection by PCR in an enzootically infected breeding colony of immunocompetent Sprague-Dawley rats. A semiquantitative PCR (SQPCR) assay was used to determine the relative number of P. carinii organisms in each lung tissue DNA preparation. The closed commercial barrier production colony was housed without filter boottops, fed a standard sterilized diet formulated for laboratory rodents, and provided hyperchlorinated water ad libitum. On the basis of routine diagnostic surveillance, the colony was known to be free of other common rodent pathogens. DNA was isolated from the lungs of groups of 5 male rats at each of the following ages: 1, 2, 3, 4, 5, 6, 8, 10, and 12 weeks. In addition, DNA was isolated from lung tissue of the dams of the 1-week-old (dam 1) and 2-week-old (dam 2) rats. Two PCR assays were performed on the isolated DNA in the presence of α-32PdGTP. The first PCR amplified a 357-base pair (bp) region of the P. carinii mitochondrion large ribosomal subunit. The second PCR amplified a 400-bp region of the rat globin gene as an internal control. The SQPCR scores were calculated as described previously from densitometric scores of autoradiograms obtained from the PCR assays. Serum and bronchoalveolar lavage (BAL) specimens were obtained at the time of necropsy from the same rats. Humoral recognition of P. carinii-specific antigen was assessed for each rat. Whole, solubilized P. carinii organisms and, separately, a recombinant major surface glycoprotein were used as antigens in enzyme-linked immunosorbent assays (ELISA) for the detection of IgM and IgG in serum and for IgA in bronchoalveolar lavage specimens. The PCR results and SQPCR scores were as follows: week 1 (-3/3), week 2 (-3/3), week 3 (-3/3), week 4 (+1/3, 0.1 ± 0.2), week 5 (-3/3), week 6 (+1/3, 0.5 ± 0.2), week 8 (+2/3, 0.6 ± 0.6, week 10 (+1/3, 0.5 ± 0.3), and week 12 (+3/3, 1.0 ± 0.1). Bronchoalveolar lavage specimens were antibody negative in all samples for anti-P. carinii IgG and IgA. Serum from 1 dam and all 3 offspring were positive for anti-P. carinii IgG, although none were positive for the organism on PCR assays. In addition, the dam's serum was positive for anti-P. carinii IgM. All other sera were negative. We concluded that PCR detection of P. carinii increased between 1 and 12 weeks of age. The SQPCR score was significantly correlated with age (r = 0.72, a = 0.001; Student's t-test, one-tailed, t = 6.04, d.f. = 34). There did not appear to be a correlation between the humoral status of an animal and detection of P. carinii. To the extent that this enzootic P. carinii infection of a rat breeding colony was typical, it appeared that sample groups of 3 Sprague-Dawley males between the ages of 6 and 12 weeks, not enhanced by immunosuppression, were satisfactory for the diagnosis of P. carinii infection by PCR testing.
PS14 Retrospective Analysis of Histologic Materials: Detection of *Pneumocystis carinii* by Polymerase Chain Reaction Testing, Using DNA Isolated From Hematoxylin and Eosin-Stained Sections of Microscopically Normal Rat Lung

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We sought to determine whether DNA sequences of *Pneumocystis carinii* could be detected by polymerase chain reaction (PCR) testing of sections of lung tissue from immune competent rats, even though discernible pulmonary pathologic changes associated with this organism were not apparent during light microscopic examination. Eight archived hematoxylin and eosin (H&E)-stained slides with sections of lungs from 24 rats were selected for comparison of *P. carinii* detection by light microscopy and by PCR testing. The histologic specimens represented tissues of rats from a single commercial Fisher 344 breeding colony known (retrospectively) to harbor an enzootic infection with *P. carinii* during the period when tissues had been collected at necropsy. The slides represented nonstressed health surveillance specimens submitted from the colony every 60 days throughout a 1-year-period. Each H&E slide contained sections of lungs from 3 rats submitted at a single time point. Additional sections from the corresponding paraffin blocks were cut and stained with Gomori methenamine silver (GMS) to enable examination for cyst forms of *P. carinii*. The H&E and GMS slides were given to a pathologist for independent evaluation. The pathologist was specifically asked to evaluate the slides for lesions or forms indicative of *P. carinii*. When the pathologist had completed his evaluation, DNA was isolated from the same H&E-stained tissues for PCR assay. Coverslips were removed, and the tissue was decolored, using a modified protocol originally developed for smears stained with Papanicolaou stain. Tissues were scraped from the slides, and DNA was isolated using standard methods. The 3 lung sections from the rats on each slide were pooled as a single preparation for isolation of DNA. Isolated DNA samples were assayed for *P. carinii*, using a PCR assay specific for the mitochondrial large ribosomal subunit of the organism. The product of the PCR was visualized in an ethidium-stained 2% agarose gel and photographed. Specificity of the amplicon was verified by Southern blot analysis, using a 24-mer oligonucleotide probe 5'-labeled with 32P. The hybridized probe was visualized by autoradiography and compared with a positive-control sample. None of the H&E slides were found to contain lesions suggestive of *P. carinii*, although a single slide had substantial pulmonary perivascular lymphocytic cuffing. Cyst forms of *P. carinii* were not seen in the GMS sections. All 8 PCR assays of the H&E sections had amplicon of the appropriate molecular weight, indicating *P. carinii* DNA isolated from the histologic sections. The identity of the amplicon as being of *P. carinii* origin was confirmed by hybridization analysis. It was concluded that PCR testing was more sensitive than light microscopy of the same histologic sections for detection of subclinical *P. carinii* infection in immune competent rats. This study further underscored the sensitivity of PCR assays to detect latent pathogenic agents (e.g., *P. carinii*) in nonstressed rodents.

PS15 Evaluation of Ranitidine-Bismuth Citrate and Clarithromycin Combination Treatment for Eradication of *Helicobacter mustelae* From Ferrets

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*Helicobacter mustelae*-infected ferrets are used as a model of *Helicobacter pylori* gastritis. Ranitidine-bismuth citrate (RBC) and clarithromycin, alone in combination, were used to evaluate eradication potential, gastritis severity, and development of clarithromycin resistance. Serum IgG antibody titers to *H. mustelae* were determined by using an enzyme-linked immunosorbsent assay (ELISA) and were monitored throughout the course of treatment. Twenty-four *H. mustelae*-infected spayed female ferrets were randomly assigned to 4 groups: group 1 received 48 mg of RBC/kg of body weight, group 2 received 24 mg of RBC/kg, group 3 received 24 mg of RBC/kg and 12.5 mg of clarithromycin/kg, and group 4 received 12.5 mg of clarithromycin/kg. Ferrets received each agent orally 3 times daily for 14 days. For all groups, gastric endoscopic biopsy was performed prior to treatment, 1 week after initiation of treatment, and at 24 hours, 1 week, and 4 weeks after termination of treatment. Group 1 ferrets were euthanized at 4 weeks after termination of treatment. Additional biopsy specimens were obtained from group-2 ferrets 16 weeks after termination of treatment, and from group-3 and -4 ferrets at 16, 32, and 48 weeks after termination of treatment. Quantitative bacterial cultures were performed on endoscopic biopsy specimens obtained from the body and antrum; samples were evaluated histologically, using sections stained with hematoxylin and eosin and Warthin-Starry stains. Serum IgG titers for *H. mustelae* were evaluated at each time point. Minimum inhibitory concentration (MIC) of clarithromycin was determined for isolates before and after clarithromycin treatment. All ferrets in groups 1 and 2 were colonized within 7 days of termination of treatment. Eradication was achieved in all 6 ferrets in group 3 and in 4 of 6 ferrets in group 4. Clarithromycin resistance was recorded in *H. mustelae* isolates after clarithromycin treatment in 1 ferret. Mean baseline ELISA titers were positive for all groups and remained positive throughout the study for groups 1 and 2. Antibody titers decreased with time for groups 3 and 4. With some exception, biopsy specimens obtained throughout the posttreatment period for ferrets in groups 3 and 4 indicated a trend towards reduction in gastritis scores. This study revealed that RBC and clarithromycin combination treatment was 100% efficacious in eradicating *H. mustelae* and that decreasing *H. mustelae* IgG titers can be used to monitor eradication. These findings paralleled results for human beings and validated the use of ferrets in evaluation of antimicrobials for the treatment of *H. pylori* infections.

PS16 Idiopathic Lung Lesions in Rats: Search for an Etiologic Agent

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Granulomatous lung lesions of unknown cause were identified in laboratory rats from multiple specific-pathogen free breeding colonies. Our objective was to characterize the natural
biologic course of the disease and to examine tissues for an etiologic agent. Groups of 5 Sprague-Dawley rats (ages 3, 6, 8, 10, 12, and 18 weeks) from an affected colony were necropsied. Blood and respiratory tract tissues were collected for serologic and histologic examination, bacterial and fungal culturing, and polymerase chain reaction (PCR) testing. Serum antibodies to adventitious agents, including known respiratory tract pathogens of rats, were not found. Multiple small gray nodules were evident on the lungs of four 10- to 18-week-old rats. Histologic evaluations revealed mild to moderate multifocal granulomatous alveolitis and perivascular lymphoid cuffing in nineteen of twenty 8- to 18-week-old rats. Lesions were most severe in 10- to 12-week-old rats. Bacterial or fungal organisms were not identified in affected lungs by culture, histochemical staining, or PCR testing. The PCR assays included those for Mycoplasma sp., cilia-associated respiratory (CAR) bacillus, and a generic bacterial PCR with primers that amplified a region of the 16S rRNA gene from all known bacterial species. Inoculation of mammalian cell cultures with tissue homogenates from affected rats produced cytopathic effects, whereas tissues from unaffected control rats did not. Sera from affected rats yielded positive results when tested by immunofluorescence assay with the injected mammalian cell cultures, whereas sera from unaffected colonies were negative. Taken together, these results suggested a novel virus in lung tissues of diseased rats. Further studies are underway to characterize this agent and to determine whether it is involved in respiratory tract disease of rats.

PS17 Fatal Infection With Pneumocystis carinii and Pasteurella pneumotropica in B-cell Deficient Mice

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Fifty percent mortality was reported among adult barrier-reared CB17 and MRL/lpr mice homozygous for a targeted mutation of the Jb region of the Ig heavy-chain locus that rendered them B-cell deficient. Three to 4 days prior to death they became inactive and dyspneic and developed hunching and ruffled coats. Necropsy findings on affected mice included consolidated lungs with multifocal cream-colored spots. Microscopic changes included diffuse, nonsuppurative, interstitial pneumonia with a superimposed pyogranulomatous lobar pneumonia. Aerobic bacteriologic culturing of pleural necropsy and lung yielded pure cultures of Pasteurella pneumotropica. Necropsy of aged-matched, clinically normal mice of both genotypes revealed interstitial histiocytic pneumonia without lobular pneumonia. Histochemical stains of lung tissues revealed scattered cysts of Pneumocystis carinii, primarily in the interstitium. Indirect immunofluorescence staining of frozen lung sections, using serum from immunocompetent cage mates, also illuminated interstitial cysts consistent with Pneumocystis carinii. Microscopically, the characteristic trophozoite-driven proteinaceous alveolar exudate of Pneumocystis carinii infection in T-cell deficient mice (nu, rag) or combined T- and B-cell deficient mice (scid) is unlike the prevailing cyst form and interstitial pneumonia in these strict B-cell deficient mice. Pneumonia caused by dual infection with Pasteurella pneumotropica and Pneumocystis carinii was associated with clinical signs of disease and high mortality in B-cell deficient mice, whereas Pneumocystis carinii alone caused subclinical pneumonia in age-matched mice of the same strain. This result indicated the potential pathogenicity of Pasteurella pneumotropica in immunosuppressed mice. All B-cell deficient mice were medicated with enrofloxacin and sulamethoxazole administered in the water. These mice are currently being rederived by coordinating enrofloxacin treatment, cesarean section, and isolator rearing.

PS18 Effect of Long- and Short-Term Anesthesia Induced by Various Methods on Acid-Base and Blood-Gas Parameters of Neonatal Rats

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There is little published information on physiologic parameters of anesthetized neonatal rodents. We examined the effects of short- and long-term anesthesia induced by various methods on acid-base and blood-gas variables of neonatal rats. Fifty-eight 1- to 4-day-old Sprague-Dawley pups from 7 litters were subjected to short-term anesthesia (just to the point of surgical anesthesia) induced by chilling (n = 9) or methoxyflurane (MOF; n = 10); or to 30 minutes of anesthesia induced by chilling (n = 10), MOF (n = 10), pentobarbital (20 mg/kg of body weight, intraperitoneally) plus chilling (n = 9), or pentobarbital plus MOF (n = 10). Rectal temperatures were measured immediately before anesthesia induction, at the onset of surgical anesthesia (all groups), and following 30 minutes of anesthesia (30-minute groups only). Mixed venous blood was obtained from anesthetized pups by cardiac puncture after the final temperature measurement. Parameters measured included partial pressure of oxygen (PO2), partial pressure of carbon dioxide (PCO2), pH, bicarbonate, base excess, and total carbon dioxide (TCO2). Mean blood pH was normal in pups anesthetized short- or long-term by chilling (7.48 and 7.45, respectively), but was low in pups anesthetized short- or long-term with MOF (7.3 and 7.2, respectively). The pH was lowered by the addition of pentobarbital to chilling (7.33) or MOF (7.17). The PCO2 increased progressively with duration of anesthesia, from 21.87 mm of Hg after short-term chilling to 25.58 mm of Hg after 30 minutes of chilling, and from 47.28 mm of Hg after short-term use of MOF to 55.2 mm of Hg after 30 minutes of MOF. The PCO2 was increased by the addition of pentobarbital to chilling (30.52 mm of Hg) or MOF (62.72 mm of Hg). A generally similar trend was seen with PO2, which was lowest in pups anesthetized for 30 minutes by chilling (4.52 mm of Hg) and highest in pups anesthetized by pentobarbital plus MOF (47.81 mm of Hg). All groups tested had similar mean values for TCO2 (range, 22.7 to 27.6 mmol/L), bicarbonate (range, 20.6 to 24.9 mmol/L), and base excess (range, -2 to -10 mmol/L). Mean rectal temperature remained at baseline values for pups maintained on a heating pad during long-term anesthesia with MOF (36.1°C) or pentobarbital plus MOF (35.8°C), but was progressively lower in pups anesthetized short-term with MOF (34.1°C) or by chilling (13.7°C), or long-term with pentobarbital plus chilling (10.5°C) or by chilling (9.6°C). Of the anesthetic methods studied, chilling was previously found to be associated with the shortest induction and recovery times. Results of our study indicated that it is also the least likely to cause disturbances in acid-base balance.

PS19 Contrast Radiographic Evaluation of a Gall Bladder-Cannulated Dog Model

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Intravenous cholangiography and cholecystography have been used to evaluate the integrity of a gall bladder-cannulated (GBC) dog model and to document pathophysiologic biliary shunts. Although the formation of portosystemic shunts is well chara-
terized, biliary shunts are not well documented in the literature. In our laboratory, 8 GBC dogs underwent periodic clinical assessment, including contrast radiographic evaluation. For the radiographic procedure, dogs were anesthetized with propofol, and survey radiographs were taken initially to optimize the radiographic technique. A radiopaque contrast agent, 52% iodipamide meglumine solution, was administered i.v. at a dosage of 0.6 ml/kg of body weight throughout a 10-minute period. Subsequent serial radiographic exposures were made at approximately 30-minute intervals until optimal visualization of the biliary ducts, gall bladder, and cannula was achieved. In Beagles, maximal filling of the gall bladder and cannula was observed at approximately 2.5 hours after administration. Radiographic evaluation allowed the diagnosis of biliary shunt formation in 2 chronically instrumented GBC dogs. In these 2 affected dogs, a moderate decrease in bile flow rate was detected 2 to 3 months after surgery. Although the decrease in bile flow indicated a potential problem with respect to the integrity of the animal model, the dogs remained clinically normal. The primary differential diagnoses for decreases in bile flow include a normal physiologic decrease in bile production, hepatic injury, biliary obstruction, catheter leakage, or the formation of a biliary shunt. Because the development of a biliary shunt presents a diagnostic challenge and compromises the integrity of the animal model, the use of radiographic evaluation was critical to identification of the problem. It is our belief that the use of contrast radiography is an essential component for the validation and periodic assessment of any chronic biliary cannulation model.

PS21 Development and Characterization of the hHGF/SCID Mouse as a Model to Evaluate Hepatocyte and Hematopoietic Cell Transplantation

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Human hepatocyte growth factor (hHGF) is a pleiotropic non-species-specific cytokine that regulates cell growth, motility, and morphogenesis of various types of cells via specific binding to its receptor c-met. The HGF is synthesized primarily in the liver and in lesser amounts in other tissues. The HGF-transgenic mice have increased hepatocyte growth and liver DNA content and have been primarily used to study the in vivo effects of HGF on mouse liver and regulatory genes including c-myc and c-jun. Evidence indicates that hematopoietic stem cells (HSC) express c-met and have a marked response to in vitro treatment with HGF. The SCID-hu chimeric mice have been used to evaluate the engraftment capacity of reconstituted human HSC. The HGF/SCID mice are immunodeficient and have marked hepatocyte growth evidenced by bromodeoxyuridine labeling and an increased prevalence of tumors. The transgenic mice produce hHGF in the range of 20 to 200 pg/ml and, after hepatectomy, have about eightfold higher bromodeoxyuridine labeling than controls. We are currently using the HGF/SCID-hu (human) and HGF/SCID-mac (macaque) models to evaluate human fetal hepatocytes for growth and differentiation in vivo (subcapsular kidney and subcutaneous routes), reconstitution with HSC derived from fetal hepatocytes, human and macaque bone marrow, and cord blood.

PS22 The Nonobese Diabetic (NOD) Mouse: A Model for the Study of Fatty Acid Oxidation in the Pathogenesis of Insulin-Dependent Diabetes Mellitus

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Insulin-dependent diabetes mellitus (IDDM) is a devastating disease affecting millions of people worldwide. In addition to altered glucose metabolism, insulin deficiency affects fatty acid metabolism leading to a potentially fatal ketoacidosis. Long-term sequelae include retinopathy, neuropathy, and vascular disease, which are believed to be a direct result of long-term aberrant metabolism. The nonobese diabetic mouse (NOD/Ltj) may develop insulinitis as early as 5 weeks of age and insulin deficiency by 16 to 24 weeks of age. Affected adults have serum glucose concentrations of 400 to > 1,000 mg/dl (normal range, < 500 mg/dl). They also develop hyperlipidemia, fatty liver, and fatal ketoacidosis, closely mimicking the human disorder. Immuneologic defects of their disease have been studied in detail, but little information is available on the metabolic aspects. We used the NOD/Ltj mouse to study the role of mitochondrial fatty acid oxidation in the pathogenesis of IDDM. Mitochondrial fatty acid oxidation is a cyclic, four-step pathway that oxidizes straight chain fatty acids and produces acetyl-CoA for either the tricarboxylic acid (TCA) cycle or ketogenesis. The acetyl-CoA dehydrogenases are a family of chain-length specific enzymes that catalyze the initial dehydrogenation of the fatty acid substrate. This family includes very-long-chain, long-chain, medium-chain, and short-chain acyl-CoA dehydrogenases (VLCAD, LCAD, MCAD, and SCAD, respectively). We measured steady-state mRNA concentrations of these enzymes as well as those of phosphoenolpyruvate carboxykinase (PEPCK) and carnitine palmitoyl transferase I (CPT-I). We found a significant increase (P < 0.05) in mRNA expression of hepatic MCAD, LCAD, VLCAD, PEPCK, and CPT-I in the diabetic NOD/Ltj, compared with age-matched, nondiabetic controls. These results suggested that insulin deficiency creates a drive on fatty acid oxidation that leads to pathogenic consequences. To evaluate transcriptional regulation in IDDM, we have developed a series of NOD/Ltj transgenic mouse lines with variable lengths of the MCAD promoter driving a chloramphenicol acetyltransferase reporter gene. These transgenic models will allow us to identify important regulatory elements involved in acetyl-CoA dehydrogenase gene expression in IDDM.

PS23 Characterization of the Rhino Rat: A Model of Dermal Hyperkeratosis

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The purpose of the study was to characterize a heritable condition referred to as rhinoism that developed in a colony of Sprague-Dawley rats. This condition was first observed as naturally developing hair loss on the muzzle of certain 6-month-old outbred Sprague-Dawley rats in a breeding colony. Environmental and infectious causes were ruled out, and an increasing program was begun to investigate a genetic basis. Offspring of these crosses exhibited a condition inherited in Mendelian fashion, referred to as rhinoism. Rhino rats develop hair loss and thickening of the skin beginning at 4 weeks of age. The condition appears on the muzzle and progressively affects the entire
haired body (the tail is unaffected). As the skin thickens, a characteristic folding pattern develops. Skin sections reveal follicular cysts varying in size up to 6 mm and characterized by keratinized epithelium. In progressively larger cysts, intervening dermal collagen appears, sebaceous glands atrophy, and hair shafts disappear. Mucous membranes can be affected, leading to lacrimation, ptosis, poor lactation, respiratory distress, and other secondary disorders. This condition has not been reported in rats; the findings of this condition resemble those of mice reported by Kligman in 1989. This animal model may be useful in the study of related human skin disorders.

PS24 Characterization of Large-Cell Lymphomas in Ferrets

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Ferrets (Mustela putorius furo) develop a large-cell polymorphic lymphoma resembling adult T-cell lymphoma in human beings. The purpose of the study was to examine the cellular origin of large-cell lymphomas in 4 ferrets to characterize the disease and to evaluate ferrets as a potential model for the syndrome in human beings. All of the ferrets were adults between 3 and 6 years old. Two ferrets had lived with other ferrets that developed lymphoma, and 1 had received cell-free lymphoma culture supernatants in a virus-inoculation experiment. Grossly, the lymphomas in all 4 ferrets were multicentric; 1 ferret had cutaneous lymphoma. The microscopic appearance of the lymphomas was variable among ferrets, but all had large anaplastic cells admixed with medium-sized and small lymphoid cells and a few Reed-Sternberg-like cells. Immunohistochemically, the lymphomas comprised predominantly of CD3+ CD79- cells and were interpreted to be T-cell in origin. In human beings, large-cell lymphomas of T-cell origin have been linked with human T-cell lymphotropic virus infection, although the etiology is poorly understood. A retrovirus has yet not been clearly linked with lymphoma in ferrets. Our findings suggested that large-cell polymorphic lymphoma in adult ferrets may provide a model to further understand adult T-cell lymphoma in human beings.

PS25 Choanal Atresia in Llamas—A Model for Human Beings?

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A portion of a long-term project to study choanal atresia in llamas (Lama glama) was to determine whether llamas can be used as a model of the human condition. Choanal atresia develops in human beings in approximately 1 of every 5,000 to 8,000 live births. The syndrome in people varies from unilateral to bilateral and results in a membranous-to-bony blockage of the choanae or caudal nares. In human neonates, complete choanal atresia creates a respiratory emergency, because newborns are obligate nasal breathers. Current theories to explain choanal atresia include persistence of the buccopharyngeal membrane, persistence of the nasobucal membrane of Hochstetter, abnormal persistence or location of mesodermal adhesions in the choanae, and misdirection of neural crest cell flow secondary to local genetic factors. Other developmental defects associated with choanal atresia in human beings include coloboma, congenital heart disease, retarded growth and development, genital anomalies in males, ear anomalies, and deafness (CHARGE syndrome). The only species with an incidence of choanal atresia similar to or exceeding that of human beings is the llama. Though the true incidence in llamas is not known, choanal atresia appears to be more common in North American llamas than in human beings, with a probable prevalence of approximately 1 of every 200 to 2,000 births. Llamas in the study included females and 1 male with surgically corrected choanal atresia, females carriers of choanal atresia genetics, and crias and fetuses with choanal atresia. The crias and fetuses were necropsied and evaluated for choanal atresia and other developmental defects. Results from 20 necropsies were compared with published descriptions of human choanal atresia. Choanal atresia in llamas can be partial or complete, unilateral or bilateral, and bony or membranous. Associated defects observed in llamas included congenital heart defects, retarded growth and development, urogenital anomalies, wry face, lack of olfactory tracts, small olfactory tracts, and lack of a rhinencephalon. Though there appeared to be some differences, similarities in the anatomy of choanal atresia and the defects associated with choanal atresia in llamas may provide valuable information as a model of the human condition. It will be especially useful to study the embryologic development of the condition in llamas to help determine the pathogenesis.

PS26 Animal Models of Androgen-Independent Prostate Cancer

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Prostate adenocarcinomas develop when prostate epithelial cells, which are normally androgen dependent, acquire malignant characteristics and progress to androgen-independent growth. These cancerous cells resist androgen-withdrawal therapy. Prostate cancers involve a variety of mechanisms. Apoptosis has been shown to be mediated by a family of proteinases called interleukin converting enzyme (ICE) proteases. A viral protein, CRMα, blocks apoptosis by inhibiting ICE protease activation. We examined the role of ICE protease activity in androgen withdrawal apoptosis in androgen-sensitive prostate cancer cells. We generated cell lines that overexpress CRMα and mutated functional CRMα and examined the effect of various apoptosis-inducing agents in these cell lines. Overexpression of CRMα, an potent inhibitor of ICE protease, blocks androgen withdrawal mediated apoptosis in LNCaP prostate cells. CRMα also blocks tumor necrosis factor (TNF)α, Fas, UV, and chemotherapeutic drug-induced apoptosis in prostate cancer cells in vivo. Mutated nonfunctional CRMα did not have a significant effect on blocking apoptosis. These results suggested that abrogation in signal-mediated activation of ICE proteases may play a role in androgen-independent growth of prostate adenocarcinomas. We also examined androgen-withdrawal apoptosis in vivo by transplanting prostate cancer cells expressing CRMα and mutated CRMα into castrated athymic nude mice. We developed transgenic mice that overexpress CRMα specifically in the ventral aspect of the prostate by using the probasin promoter, which is prostate specific. Transgenic mice may serve as useful models for androgen-independent prostate adenocarcinomas.
PS27 Inbred Mouse Strain With an Anomaly of External Genitalia: Animal Model for Hypospadias in Human Beings

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We identified mice with an anomaly of the external genitalia. Mice were of the AQ strain (tentative name) derived from a cross of BALB/cA and an inbred strain originated from ICR outbreeding. Results of a morphologic study, growth and reproduction, and genetic crosses to reveal the cause and inheritance of the anomaly are described. In the morphologic study, external genital areas of normal and abnormal mice in both sexes were observed in detail. Abnormal mice with the anomaly were characterized as having a substantially short distance between penis and clitoris and anus in both sexes, a severe opening of scrotal and vaginal areas along the midline, and a substantially small glans penis and glans clitoris. The anomaly in both sexes was developmentally caused by hypoplasia in the genital area, because these characteristics were observed in mice at birth. In the growth and reproduction study, male mice with the anomaly were completely sterile, and females naturally mated with normal mice had poor reproduction. Spermatozoa activity and number were normal. Development of testis and ovary was morphologically normal. For the genetic cross study, mating experiments were carried out by using in vitro fertilization, because we believed this anomaly was governed by recessive gene(s) on the basis of family records. Increases of abnormal AQ males and AQ females produced abnormal mice (homozygotes), and crosses of abnormal AQ males and normal C57BL/6 females produced normal mice (F1 hybrids; heterozygotes). In F2 offspring obtained by matings between F1 females and F1 males, normal and abnormal mice were produced at a ratio of about 6:1. These results revealed that the anomaly was governed by autosomal recessive gene(s). It was strongly suggested that the anomaly of external genitalia observed in the AQ strain could be the same as hypospadias reported in human beings and might be a novel model for comparative medicine in human beings and other animals.

PS28 Enterohpatic Lesions in SCID Mice Infected With Helicobacter bilis

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Helicobacter bilis is a recently identified organism that colonizes the intestinal tract and liver of mice. In immunocompetent mice, infections have been associated with mild hepatitis, but intestinal lesions associated with Helicobacter infection have not been described. In our study, female C.B-17- scid (SCID) mice were co-housed with B6D2F1 mice naturally infected with Helicobacter bilis. After 8 and 17 weeks of cohabitation, SCID mice were positive for Helicobacter bilis on the basis of bacterial culture of feces. After 11, 18, 34, and 45 weeks of cohabitation, groups of B6D2F1 and SCID mice were euthanized, and livers and intestinal tracts were examined for histologic lesions. In SCID mice, moderate-to-severe proliferative typhlocolitis was seen in the majority of mice from all groups. Moderate necrotizing hepatitis was evident sporadically in mice from the 34- and 45-week cohabitation groups. To further assess whether Helicobacter bilis caused these lesions, 6-week-old SCID mice were orally gavaged 3 times with approximately 1 x 10^7 Helicobacter bilis organisms. Groups of mice were euthanized and necropsied at 12 and 24 weeks after inoculation. Mild-to-moderate proliferative typhlocolitis was evident in all mice in the 12-week postinoculation group, and milder typhlocolitis was evident in the majority of mice in the 24-week group. Cecal lesions were similar between genders and, in general, less severe than those of the normally exposed SCID mice. Mild-to-severe necrotizing hepatitis was evident in all male mice from both groups and 1 of 3 female mice from the 24-week group. These results suggested that Helicobacter bilis can cause proliferative bowel lesions and necrotizing hepatitis in immunodeficient mice, although lesion severity and character may be dependent on several host or bacterial factors.

PS29 Experimentally Induced Helicobacter bilis Infection Causes Inflammatory Bowel Disease in Defined-Flora scid Mice

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Helicobacter bilis has been isolated from aged inbred mice in association with multifocal chronic hepatitis. We also have clinical evidence linking Helicobacter infection with diarrhea, proliferative typhlitis, and colitis in scid mice with conventional flora. To determine the pathogenic potential of Helicobacter bilis, we inoculated defined-flora scid mice with ATCC 51630, the type strain of Helicobacter bilis. Fifteen 4-week-old female Taci:CreHa(ICR)-scid DF mice were inoculated by intraperitoneal injection of 0.5 ml of phosphate-buffered saline solution (OD_600 ~ 0.3) containing Helicobacter bilis organisms. Ten control mice were sham-dosed with phosphate-buffered saline solution alone. Infection with Helicobacter bilis was confirmed by bacterial culture and polymerase chain reaction (PCR) assay of feces. Mice were housed throughout the study in autoclaved microisolator caging and fed sterilized food and water to maintain their defined-flora status, which was confirmed by aerobic and microaerobic bacterial culture of feces. Mice were euthanized and necropsied at 7 weeks after inoculation. All inoculated mice were confirmed to be Helicobacter bilis-colonized on the basis of bacterial culture of cecal contents. All sham-inoculated mice were negative for Helicobacter bilis and did not have important gross or histopathologic lesions. In contrast, all 15 of the Helicobacter bilis-infected mice had varying degrees of inflammatory bowel disease. Mild-to-moderate proliferative typhlitis was the most prominent feature. Cecal proliferation was characterized by focal areas of increased crypt length (maximum crypt length [mean ± SD], 284 ± 104 μm; n = 15), compared with 170 ± 52 μm (n = 10) in control mice (P = 0.001), with increased mucosal epithelial cell hyperchromicity and increased crypt cell density. Bromodeoxyuridine staining revealed an increased labeling index in the ceca of 15 Helicobacter bilis-infected mice, compared with 8 control mice (21.7 ± 7.1 vs. 8.5 ± 4.2%, respectively; P = 0.0044). The cecal inflammatory infiltrate consisted predominantly of mononuclear cells with numerous eosinophils. Colonic proliferation and colitis were also observed. Focal-to-segmental areas of mild-to-moderate colonic hyperplasia were observed in the distal part of the colons of Helicobacter bilis-infected mice (maximum crypt lengths of 170 ± 44 μm; n = 15) versus 140 ± 25 μm (n = 10) in control mice (P = 0.037). Bromodeoxyuridine staining revealed an increased labeling index in the distal part of the colons of Helicobacter bilis-infected mice, compared with control mice (10.8 ± 6.7%, n = 15 vs. 4.3 ± 1.6%, n = 9; P = 0.002). Colitis was observed in 6/15 mice; the colonic inflammatory infiltrate consisted predominantly of mononuclear cells. Substantial hepatic lesions...
were not seen. This is the first report that experimentally induced infection with *H. bilis* causes inflammatory bowel disease in specific-pathogen-free mice. These results confirmed a pathogenic role for *H. bilis* in mice and provided a new model relating a specific microbial agent with inflammatory bowel disease.

**PS30 An Outbreak of Diarrhea Associated With Dual Helicobacter bilis/Helicobacter rodentium Infection in a Colony of scid Mice**

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An outbreak of diarrhea was reported in a breeding colony of *scid/p53* knockout mice. Clinical signs were evident in approximately a third of the 150 mice in the colony and ranged from mucoid-to-watery diarrhea to severe hemorrhagic diarrhea and death. Aerobic and anaerobic bacterial culture of feces did not yield known murine pathogens, and endoparasites were not seen. Microaerobic bacterial culture of feces or cecal contents revealed infection with a urease-positive and a urease-negative *Helicobacter* organism. The urease-positive organism was *Helicobacter bilis*, and the urease-negative organism was *H. rodentium*. Mixed infections were confirmed by bacterial culturing and polymerase chain reaction (PCR) assays in several mice, although some mice tested positive for only *H. bilis* or *H. rodentium*. Sentinel animals exposed to bedding from cages of affected mice were rapidly colonized with both species of helicobacter. Affected mice had histologic lesions of inflammatory bowel disease consisting of multifocal-to-segmental proliferative typhilitis, colitis, and proctitis. The most severely affected mice had necrocerative lesions throughout the cecum, colon, and rectum. Affected mice were treated with food washes containing antimicrobials (1.5 mg of amoxicillin, 0.69 mg of metronidazole, and 0.185 mg of bismuth/mouse/d) that have reportedly been effective in eradicating *H. hepaticus* in immunocompetent mice. This treatment resulted in a resolution of chronic illness, but did not eradicate the infection; mice continued to be positive on PCR assays after a 2-week treatment regimen, and clinical signs returned in some mice when treatment was suspended. This outbreak suggested that *H. bilis, H. rodentium,* or both can be important pathogens for *scid* mice. Experimentally induced infection of defined-flora *scid* mice with *H. bilis* caused diarrhea and proliferative typhilitis, although signs were not as severe. *Helicobacter bilis* has been isolated from the bile, liver, and intestines of aged inbred mice in association with multifocal chronic hepatitis; this is the first report of *H. bilis* or *H. rodentium* causing substantial acute diarrheal disease.

**PS31 Role of Helicobacter hepaticus in Confounding Results of a Triethanolamine Carcinogenesis Bioassay in B6C3F1 Mice**

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*Helicobacter hepaticus* has been associated with naturally developing hepatitis in certain inbred and random-bred strains of mice and has been linked in A/JCr mice to the development of hepatocellular tumors. Recently, the carcinogenicity of triethanolamine (TEA) was tested in a long-term rodent bioassay, because it is used in a wide variety of industrial and consumer products. In the initial evaluation of the TEA dermal studies, there was equivocal evidence of carcinogenic activity in male B6C3F1 mice. This was based on a marginal increase in hepatocellular adenomas and hepatoblastomas and detection of *H. hepaticus* on selected silver-stained sections of liver plus the detection of karyomegaly and oval cell hyperplasia (previously used in A/JCr mice as biomarkers of *H. hepaticus* infection), which complicated interpretation. Some evidence of carcinogenic activity was also evident in female mice, because of an increase in hepatic tumors. However, *H. hepaticus* was not considered a complicating factor, because the livers of female mice did not have histologic features compatible with *H. hepaticus* infection. A retrospective analysis, using polymerase chain reaction (PCR) assays of frozen hepatic tumor samples from 44 mice (25 female and 19 male) without characteristic hepatic lesions, indicated that 12/25 (48%) and 10/19 (53%) of the livers amplified *H. hepaticus*-specific DNA. Fourteen of these mice (7 male and 7 female) also yielded *H. hepaticus* on bacterial culture of their frozen hepatic tumors. A positive correlation (i.e., concurrence on both positive and negative results) of results of culture of *H. hepaticus* and PCR assay of *H. hepaticus*-specific DNA amplification was recorded for 20/25 (80%) and 16/19 (84%) of female and male mice, respectively. An additional 5 male mice (from the high TEA dosage group) with hepatic tumors had *H. hepaticus* evident on Warthin-Starry silver-stained sections of liver, had hepatic karyomegaly and oval cell hyperplasia and also yielded *H. hepaticus* on bacterial culture of their frozen livers. Because of the confirmed intercurrent *H. hepaticus* persistent infection in both male and female B6C3F1 mice in this study and the fact that *H. hepaticus* has been documented to have a causal role in hepatic tumor development in mice, a diagnosis of primary carcinogenic activity in the liver of mice to which TEA was dermally applied could not be determined. It is recommended that all B6C3F1 mice involved in the NTP bioassay program and other *in vivo* mouse studies involving hepatic tumorigenesis be screened for *H. hepaticus* prior to initiation of experiments.

**PS32 In vivo Administration of Helicobacter hepaticus Cytoxin Is Associated With Hepatic Inflammation and Necrosis**

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*Helicobacter hepaticus* is associated with chronic hepatitis and hepatocellular neoplasia, particularly in male A/JCr mice. The type strain of *H. hepaticus* possesses a cytoxin associated gene (*cagA*) and expresses a homologous *cagA* protein. In *H. pylori*, the *cagA* gene is associated with peptic ulcer disease, gastric carcinoma, and production of a cytoxin. Additionally, the type strain of *H. hepaticus* as well as several other *H. hepaticus* isolates secrete a toxin that has a cytotoxic effect *in vitro* on a mouse liver cell line (ATCC No. CCL 9.1). To investigate whether this cytoxin has an *in vivo* role in the pathogenesis of hepatic disease, 0.5 ml of a cell-free saline extract (CFSE) of *H. hepaticus* containing 5 x 10⁴ or 5 x 10⁵ units of cytoxin activity was injected intraperitoneally into 16 male A/JCr mice. Four groups of mice were injected every other day for 8, 16, or 20 days, Mice in group 4 were rested for 1 week and then received daily injections for an additional 4 days. Control mice received 0.5 ml of saline solution intraperitoneally at the same frequency as group 4. Necropsy was performed 1 day after mice received the final injection. Serum alanine aminotransferase (ALT) levels were measured prior to initial injection, and at necropsy (24–48 h after final injection). ALT levels were significantly increased above baseline in all mice receiving the CFSE (64%, P < 0.005); however, these values were not significantly different from the controls (40%). The primary histopathologic change was a segmental mononuclear phlebitis affecting primarily hepatic sublobular veins and,
less frequently, hepatic portal and central veins. It has been observed that multifocal intrahepatic phlebitis and perivenous inflammation are primary components of hepatitis observed in A/JCr mice naturally infected with *H. hepaticus*. Multifocal necrosis of individual hepatocytes was also frequently observed, accompanied by an inflammatory infiltrate composed of neutrophils, macrophages, and eosinophils as well as a few multifocal areas of hepatic capsular inflammation. In addition to our *in vivo* model, splenic mononuclear cells were harvested from naïve and CFSE-injected mice and cultured *in vitro* with the CFSE. Cells were assayed for proliferation by means of tritiated thymidine incorporation. Cells from both naïve and CFSE-exposed mice proliferated in response to the CFSE, indicating a mitogenic effect. The stimulation index of proliferation was significantly (P < 0.009) higher in CFSE-exposed mice, suggestive of possible immune recognition of specific antigen(s). This *in vivo* system provided a standardized model for studying the effects of *Helicobacter* infection and its role in the development of hepatic disease and neoplasia.

**PS33 Chronic Active Hepatitis Induced by Helicobacter hepaticus in A/JCr Mice is Associated With a Th1 Cell-Mediated Immune Response**

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Chronic infection of the hepatic biliary system with *Helicobacter hepaticus* in A/JCr mice results in a high incidence of chronic active hepatitis and an increased risk for hepatic tumors. Infected A/JCr mice develop inflammatory lesions in the liver characterized by numerous foci of mononuclear and plasmacytic infiltrates surrounding the bile ducts, suggesting that substantial humoral and cell-mediated immune responses develop to *H. hepaticus* antigens localized in the liver. The propensity for A/JCr mice to develop hepatic lesions secondary to *H. hepaticus* infection despite a high serum IgG response is additional evidence to support that the immune response of A/JCr mice to *H. hepaticus* is nonprotective. Because a T-helper type 1 (Th1)- or Th2-like immune response can be associated with susceptibility or resistance to bacterial disease on the basis of host genetics, our study profiled the Th-helper immune response of A/JCr mice experimentally infected with *H. hepaticus*. Humoral immune responses, measured as development of serum IgG and mucosal IgA released in bile and feces after infection, and cell-mediated immune responses, measured as mononuclear cell cytokine release and proliferative responses to *H. hepaticus* antigens *in vitro*, were ineffective in preventing chronic infection or development of hepatitis. Subclass isotyping of the serum IgG response indicated that the infected A/JCr mice produced predominantly IgG2a serum antibodies to *H. hepaticus* (P < 0.05), which is consistent with a Th1 immune response similar to that reported for human beings infected with *H. pylori* and mice infected with *H. felis*. Splenocyte mononuclear cells isolated from infected A/JCr mice proliferated *in vitro* to *H. hepaticus* antigens and produced more γ-interferon than interleukin-4 or interleukin-5 (P < 0.05), which also is characteristic of a Th1 immune response. Thus, the results indicated that A/JCr mice develop a nonproductive Th1 immune response to *H. hepaticus* infection. This model will be valuable for testing preventative and therapeutic approaches that have the potential to manipulate the mucosal and systemic immune response to *Helicobacter* organisms and whether such manipulations will contribute to or inhibit lesion development.

**PS34 Decreased Inflammatory, Proliferative, and Immune Responses to Helicobacter felis Infection in Mice Carrying a Truncated Apc Gene**

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*Helicobacter pylori* infection and adenomatous polyposis coli (*Apc*) gene mutations have been linked to gastric cancer in human beings, but a possible synergistic interaction between these risk factors has not been examined. Fourteen C57BL/6 wild type and 14 *Apc* 1638 mice were inoculated with *H. felis* at 6 weeks of age and compared at various time points with a similar number of uninfected control mice of the same genotype. Both infected and uninfected *Apc* 1638 mice had a limited number of atypical proliferative foci in the mucosa of the antrum and pyloric junction at 4.5 and 6 months of age, whereas gastric foci of the antrum and pylorus were evident in all mice, regardless of infection status, at 7.5 months of age. In contrast, altered gastric mucosal foci were not observed in control or infected C57BL/6 mice at any time. Interestingly, the *Apc* 1638 mice had less epithelial proliferation and inflammation in the body of the stomach, lower anti-*H. felis* serum IgG antibody responses (although both the wildtype and *Apc*-mutant mice had a Th1-like immune response, determined on the basis of a predominantly IgG2a immunoglobulin response), and higher bacteria and urease scores, compared with wild type C57BL/6 mice. The *Apc* 1638 truncating mutation leads to gastric dysplasia and polyposis of the antrum and pyloric junction, but in 7.5-month-old *H. felis*-infected *Apc*-mutant mice did not predispose strongly to gastric cancer. In addition, our data suggested this *Apc* mutation may lead to decreased immune, inflammatory, and proliferative responses to *Helicobacter* infection, suggesting the possibility of a novel role for this tumor-suppressor gene in the immune response to gastric bacterial infection.

**PS35 Intestinal Helicobacter spp. Associated With Inflammatory Bowel Disease and Colon Cancer in a Colony of Cotton-top Tamarins (Saguinus oedipus)**

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A disease similar to ulcerative colitis in human beings has been identified in cotton-top tamarins (CTT; *Saguinus oedipus*) in captivity. Clinical signs include weight loss, diarrhea, and rectal bleeding with the pathologic features and biochemical abnormalities of ulcerative colitis. Approximately 25 to 40% of these tamarins develop colon cancer after 2 to 5 years of captivity. An infectious agent has been proposed; however, a microbial agent has not yet been identified. *Helicobacter* spp. have been associated with enterocolitis and inflammatory bowel disease (IBD) in human beings and other animals. Infection with *H. pylori*, *H. hepaticus*, or *H. mustelae* is also associated with an increased risk of gastric adenocarcinoma, hepatic carcinoma, and lymphoma of the mucosa-associated lymphoid tissue, respectively. The aim of this study was to assess a colony of CTT with a high prevalence of IBD and colon cancer to detect colonic *Helicobacter* spp. Colonic biopsy specimens were obtained from 24 CTT in a colony at the New England Regional Primate Research Center, which
had been described previously as having a high incidence of colonic diseases. The colitis evident in members of this colony was characterized in the acute phase by crypt abscesses, loss of goblet cells, and infiltration of the lamina propria and epithelium by neutrophils. Chronic mucosal changes include loss of crypts, atrophy of the mucosa, and infiltration of the lamina propria by chronic inflammatory cells. A polymerase chain reaction (PCR) assay was performed on DNA extracted from colonic specimens, using primers for a conserved region of the 16S rRNA gene that amplifies a 1.2-kb region. These primers reliably detect members of the genus Helicobacter. Twenty-four samples (63.4%) were positive for Helicobacter spp. by PCR testing. The DNA sequencing of a 1.2-kb PCR product of the 16S rRNA gene identified homology to Helicobacter spp. Over the length of 1,140 base pairs, the sequence had 93.8% identity and 96.4% similarity to H. canis (NCTC 12739). Thus, we have identified a colonic Helicobacter spp. in a colony of C57 mice that have a high prevalence of IBD and colon cancer. Further studies to characterize this organism and its role in the pathogenesis of ulcerative colitis and colonic adenocarcinoma in C57 are underway.

**PS36 Innate Immune Mechanisms in Antimycoplasmal Defense**

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Innate immune mechanisms involving alveolar macrophages (AM) are believed to play an important role in antimycoplasmal host defense. We compared the effects of AM depletion on intrapulmonary killing of Mycoplasma pulmonis during the early phase of infection in mycoplasma-resistant C57BL/6Ncr (C57BL) and susceptible C3H/HeNcr (C3H) mice. More than 80% of AM were depleted in both strains of mice by intratracheal instillation of liposome-encapsulated dichloromethylene bisphosphonate, compared with a non-significant AM depletion in either strain following instillation of liposome-encapsulated phosphate-buffered saline (PBS) solution, PBS solution alone, or no treatment. The AM depletion exacerbated the infection in C57BL mice by reducing killing of the organism to a level comparable to that of undepleted C3H mice. In contrast, AM depletion did not alter killing in C3H mice. These results suggested that differences in mycoplasmal killing by AM may explain the resistance of C57BL mice vs. susceptibility of C3H mice to mycoplasmal infection. On the basis of these findings, we studied the potential role of surfactant protein A (SP-A) in early antimycoplasmal defenses, using AM from C57BL/6 mice. Dose-dependent binding of SP-A to AM was revealed by immunofluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA) testing. Dose and calcium-dependent SP-A binding to mycoplasmas was detected by ELISA. To study the role of SP-A-mediated mycoplasmal killing by AM, activated AM were incubated with SP-A, washed to remove unassociated protein, and infected with mycoplasmas. The SP-A enhanced mycoplasmal killing by 79% (P < 0.005) at 6 h after infection. The addition of 1 mM N^2-monethyl-L-arginine to AM cultures prior to addition of SP-A and mycoplasmas abolished the SP-A-mediated mycoplasmalcidal activity and suppressed nitrite concentrations in the media. Thus, SP-A mediated mycoplasmal killing with the AM as the main effector cell, possibly through a nitric oxide-dependent mechanism.

**PS37 Prevalence of Growth Factor-dependent Pasteurellaceae (Haemophilus) in Rodents and Their Phenotypic and Genetic Classification**

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Few reports exist in the literature about growth factor-dependent Pasteurellaceae in rodents and their taxonomic relationship to other species of the family. On the basis of classical criteria for differentiation, these organisms are usually classified as Haemophilus spp. We repeatedly isolated such organisms during routine health monitoring and, therefore, started a systematic study. These bacteria were isolated from 574 of 1,900 (30%) rats and from 107 of 4,500 (2%) mice that were monitored during the past years. Animals harboring Haemophilus organisms originated from various conventional or barrier-maintained experimental colonies and from several commercial breeders. The microorganisms were most frequently isolated from the lungs and the trachea; some were cultured from the nasal cavity or from mucous membranes of the genital or digestive tract. Forty biochemical criteria were determined for 900 isolates. From analysis of these data, groups of bacteria with similar biochemical properties were formed, using exploratory statistical methods. The majority of isolates formed 5 homogeneous groups. These groups have biochemical properties of H. parainfluenzae biotypes. The remaining 200 isolates formed 3 major and a few smaller phenotypic groups. Ten isolates representing 8 major phenotypic groups were submitted to 16S rRNA sequencing. These data confirmed the close relationship of some phenotypic groups to type strains of the H. parainfluenzae complex that is believed to be human-specific. Four isolates clustered together with members of the Pasteurella pneumotropica complex. These data revealed that some growth factor-dependent Pasteurellaceae found in rats and mice are members of the genus Haemophilus and might be transmitted between human beings and rodents. Other organisms are members of the P. pneumotropica complex and seem to be rodent-specific.

**PS38 Pathogenesis of Adenovirus Infection in Guinea Pigs**

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The existence of guinea pig adenovirus (GPAV) has long been suspected on the basis of histologic and serologic results, but has not yet been confirmed by isolation of the virus. In susceptible animals it may cause severe bronchopneumonia and death. Inclusion bodies similar to those associated with adenovirus infections in other animals have been observed in the lungs of animals with clinical disease, and adenovirus-like particles have been detected by electron microscopy. The prevalence of the infection is unknown, because reliable detection methods do not exist. Recently a polymerase chain reaction (PCR) assay was described, which was able to selectively detect GPAV. The sequence of the 280-base pair PCR product located in the hexon gene had a number of differences, compared with adenoviruses of mouse or human origin, which further supported that GPAV is indeed an adenovirus distinct from mouse isolates. We attempted to determine the pathogenesis of GPAV and experimentally inoculated guinea pigs with lung homogenate from an ill animal. A PCR assay was used to trace the infection,
because virus isolation has not yet been successful. None of the infected guinea pigs developed clinical disease, and histologic changes were not found in the lungs. The target sequence of the GPAV genome was detected in nasal swabs on day 4 through 14 after inoculation. Nasal mucosa collected during this time contained infectious virus, as evidenced by re-infection of naive guinea pigs. Virus was not detected in the guinea pigs after day 14. Virus was not found in the lungs of the guinea pigs at any time. Small intestine and adrenal glands of 1 guinea pig tested positive by PCR assay on day 7 after inoculation. These findings suggested that GPAV caused a transient subclinical infection of the upper respiratory tract and may only be found in the lungs during clinical disease.

**PS39 FVB/N Pseudopregnant Mice Receiving DNA-Injected Embryos Have Predictable Pregnancy Rates**

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The FVB/N mice have been a popular inbred strain for generating transgenic lines because of ease of microinjection and efficient breeding. However, it is used less frequently as a pseudopregnant recipient of cDNA-injected embryos. Because our Transgenic Resource Program maintained an in-house breeding colony of FVB/N mice, we evaluated the use of FVB/N females as embryo transfer recipients. We compared the ampulla size of FVB/N pseudopregnant females to that of ICR pseudopregnant females and determined pregnancy rates by using (C57BL/6 X C3H) F1 embryos injected with nonlethal cDNA constructs. During a 3-month period, one-cell embryos were collected, and 15 respective cDNA constructs were injected directly into the pronucleus. Embryos were incubated overnight for embryo transfer. Pseudopregnancy was induced in 7- to 8-week-old FVB/N and ICR mice by using vagectomized FVB/N males. On the day of embryo transfer, these mice were surgically prepared, and 14 two-cell embryos were transferred into their oviduct through a nick approximately 10 mm proximal to the left ampulla of each mouse. Ampulla grading was classified as follows: 1 = normal size; 2 = 2x normal size; 3 = 3x normal size; and 4 = 4x normal size. A normal-sized ampulla was determined as a typical ampulla seen in anestrous. Of 115 FVB/N plugged females, 59 became pregnant, whereas 56 did not (51% pregnancy rate). Females with grade 4 ampulla had a pregnancy rate of 80% (36 of 45), whereas those with grades 3, 2, and 1 had pregnancy rates of 44% (14 of 32), 27% (8 of 30), and 13% (1 of 8), respectively. Of 126 ICR plugged mice, 89 became pregnant, and 37 did not (71% pregnancy rate). The ICR females with a grade 4 ampulla had a 74% pregnancy rate (40 of 54), whereas mice with grades 3, 2, and 1 had pregnancy rates of 72% (51 of 43), 62% (13 of 21), and 63% (5 of 8), respectively. The results indicated that ampulla size was a good predictor of pregnancy in the FVB/N mouse, but not necessarily in the ICR strain. Because more FVB/N females were required to obtain pregnancy than ICR females when using the same cDNA constructs, we concluded that ICR mice are a more reliable embryo transfer recipient under our transgenic breeding conditions.

**PS40 An Easy Approach for Rat Intubation, Using Readily Available Equipment**

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Intubation of rats for chronic experiments permits the control of the status of the arterial blood gases during experimental procedures and the recovery to spontaneous breathing thereafter. However, rat intubation is technically difficult. Here we describe an approach that uses simple equipment to facilitate the procedure. For positioning of rats, we used a readily available plexiglass box (35 x 20 x 20 cm) with openings on one short (top) and one long (front) side. A wire was fixed across the top of the box (i.e., the open short side). After induction of deep anesthesia with halothane, rats (330 to 370 g) were suspended by their teeth on the wire. The mouth was kept open with a small retractor placed against the inside of the cheeks, and the tongue was pulled to the side to give an unobstructed view of the pharynx. We observed the pharynx through a surgical microscope with a magnification of 2x and a fiber-optic light source attached close to its lens. After the pharynx was dried and the epiglottis lifted ventrally by briefly inserting a cotton-tipped swab, the larynx with both vocal cords could be seen easily. We used a guide wire (outside diameter, 0.6 mm) with its tip rounded to avoid injury. This guide wire was placed, using direct observation, into the trachea. The intubation tube, a 14-gauge cannula taken from a set for intravenous cannulation, was inserted 2 cm beyond the larynx. The guide wire was removed, and the tracheal cannula was carefully sutured to the cheek to avoid displacement. By using the described approach, each intubation could be completed successfully in less than a minute (n = 30 rats). When rats were placed on their backs for several hours during experiments, we observed gastric regurgitation in some cases, which complicated respiratory recovery after extubation. We developed an approach for intubation of rats, using a readily available plexiglass box, a guide wire, and a 14-gauge intravenous cannula.

**PS41 A Simple Magnetic Resonance Imaging-Compatible Device for Administration of Gas Anesthesia to Neonatal Rodents**

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Magnetic resonance imaging (MRI) of laboratory animals is becoming an increasingly important tool in academic research and in the pharmacologic industry to noninvasively monitor the progress of test drugs in various animal models. To prevent motion, which causes severe image degradation, animals must be paralyzed or anesthetized. In many studies, delivery of gas anesthetics such as fluorothane and halothane is required, and this presents substantial practical problems for small animals such as neonatal rodents. Commercially available devices often contain metallic components or are too bulky to fit into the MRI probe assembly. We constructed a simple all-plastic device designed to deliver gas anesthetics in the enclosed space of an animal MRI scanner. Principal features of this device include a stage for supporting the animal’s body, a close-fitting holder/immobilizer for the head over which the MRI probe assembly can be fitted, and a mask connected to the anesthetic hoses. We have used this device during diffusion MRI examinations of the brain of neonatal rats and rabbits in studies of hypoxia/ischemia. Each animal’s head was placed into the opening of the holder
to induce anesthesia prior to surgery. A low maintenance dose of halothane was used throughout the imaging procedure (2 to 3 hours) without detectable leakage (i.e., gas odor) from around the mask/head-holder device. During the imaging procedure, the head-holder successfully prevented respiration-induced head motion in the extremely motion-sensitive diffusion MRI scan, and high-quality images were produced.

**PS42 Two Methods for Collection of Rat and Rabbit Fetal Blood, Using Isoflurane Anesthesia**

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In rabbit-development toxicity studies, dams were treated from gestation day (GD) 7 through 20; laparotomies and fetal examinations were performed on GD 28. In separate toxicokinetic studies, maternal and fetal blood samples were collected for analysis of drug concentrations on GD 20. Previously, we developed methods for fetal blood collection via umbilical vessels on GD 28 after dams were euthanized with sodium pentobarbital or CO₂ asphyxiation. These methods, however, are not effective for fetal blood collections on GD 20 because of the smaller diameter of the umbilical vessels and the rapid constriction of vessels after the small cut is made that is necessary for blood collection. As an alternative, we developed a method by which the dam is anesthetized with isoflurane and fetuses remain in situ until immediately prior to blood collection. Fetuses are removed sequentially from the uterus, and blood is collected into capillary tubes after thoracotomy and making a small incision in the great vessels. Advantages of this method are that maternal/fetal blood circulation remains intact, which increases the volume of fetal blood that can be obtained, and that fetuses are anesthetized via the maternal/fetal blood supply so that additional agents need not be administered prior to blood collection. In rat-development toxicity studies, fetal examinations were performed on GD 21. In separate toxicokinetic studies, fetal blood samples were collected on GD 20. In contrast to the method used for rabbits, fetal blood from rats can be successfully collected from the umbilical vessels.

**PS43 Hypnotic Sedation: An Alternative to Chemical Sedation During Minor Diagnostic Procedures for Rabbits and Guinea Pigs**

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In laboratory animal medicine, veterinary technicians are called upon to perform diagnostic procedures on various anesthetia-sensitive animals, particularly rabbits and guinea pigs. These animals, especially in a debilitated state, are frequently poor anesthetic risks. Diagnostic procedures for these animals would normally require a sedated or manually restrained subject; however, the use of a chemical neutralizing agent or stressful physical restraint may not be prudent. Technicians need a method that is quick, easy to master, and does not adversely affect an animal's physical condition. One such method, the technique of hypnosis, is a widely acknowledged form of sedation in rabbits, but is not routinely used on guinea pigs or for minor diagnostic procedures. Hypnosis is a noninvasive and nonpharmacologic method that may be used to induce short-term sedation and immobility in rabbits and guinea pigs. The hypnotic state can be identified by the loss of spontaneous movement, failure to respond to mild stimuli, and absence of a righting reflex. Hypnotic sedation can be used to restrain rabbits and guinea pigs for many diagnostic procedures. An animal that is to be hypnotized is placed on its back on a flat surface or, alternatively, in a padded V-shaped trough. The cheeks are massaged in a circular motion with one hand, while the abdomen is gently stroked with the other. As the animal relaxes, the forelimbs and hind limbs are carefully stretched while maintaining the animal in dorsal recumbency. This technique is especially useful for obtaining radiographs of rabbits and guinea pigs. After the hypnotic state is produced, electrocardiograms can also be obtained, and venipuncture, cystocentesis, or urinary catheterization can be performed in minutes with minimal stress to the animal and the technician. This technique is useful for obtaining samples from animals that are poor anesthetic risks due to age, preexisting illness, or pregnancy. Because there are no drugs to be metabolized, animals recover in seconds and can be immediately returned to their environment. Technicians do not lose valuable time monitoring a lengthy recovery, and there is less chance of animals becoming excited and injured while recovering. Hypnotic sedation is a valuable alternative to more-traditional methods commonly used to induce short-term sedation and immobility in these animals and should be considered for these and other nonpainful procedures. By eliminating stress in awake animals or lengthy anesthetic recovery times, hypnosis can help refine the process of sample collection in rabbits and guinea pigs.

**PS44 A Surgical Method for Inducing Cryptorchidism in Rabbit Pups**

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Surgical methods for induction of cryptorchidism in rabbits have been described in adult animals only. A reproductive toxicity study required a control group of rabbits whose testes had never descended through the inguinal canal. Testes descend to a scrotal position approximately 5 to 6 weeks after birth in rabbits; therefore, a study was conducted that used six 3-week-old Dutch-Belted specific-pathogen free rabbits to develop a protocol that would prevent testicular descent. Rabbits were anesthetized and maintained on isoflurane via mask during the procedures. The caudal ventral part of the abdomen was prepared for aseptic surgery. A 1-cm incision was made just cranial to the pubis. Undescended testes were located cranial to the inguinal canal, and the gubernaculum was identified. Three surgical techniques were performed for comparison: transection of the gubernaculum and closure of the inguinal ring, excision of a segment of the gubernaculum without closure of the inguinal ring, and excision of a segment of the gubernaculum and closure of the inguinal ring. Each procedure was performed in 2 rabbits. Special magnification was not required to observe structures or perform the procedures. Subcutis was closed with 4-0 dexon; skin was closed with wound clips. Rabbits were given fluids (s.c.) and butorphanol postoperatively and were returned to the dam. All rabbits recovered uneventfully. Rabbits were palpated weekly until 3 months of age to monitor testicular descent, but all remained cryptorchid. At 3 months of age, 2 rabbits (1 each from procedures 2 and 3) were euthanized and pelvic organs examined to rule out unintended lesions. At 5 months old (age of puberty), the remaining 4 rabbits were trained to ejaculate into an artificial vagina, and 6 seminal ejaculates were collected dur-
ing a period of 3 weeks. At 6 months of age, all remaining rabbits were euthanized. All rabbits evaluated had azoospermia (typical of cryptorchidism), but sexual behavior and sexual capacity were not compromised. On necropsy, testes were found in the caudal part of the abdomen. Except for mild fibrinoid adhesions, conspicuous deformity was not observed in any rabbits, including those euthanized at 5 months of age. The 3 techniques were equally effective for induction of cryptorchidism in rabbit pups.

PS45 Hypothermia Reduces Neurologic Deficits Associated With Placement of Vascular Prosthesis in the Abdominal Aorta of Rabbits

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Rabbits have been preferentially used in experimental vascular grafting procedures, because they have many advantages over other laboratory animals. Arteries of rabbits are similar to those of human beings in their thromboplastic and fibrinolytic properties. Furthermore, rabbits are easier to obtain and handle than dogs or nonhuman primates. The rabbits described here were implanted with polytetrafluoroethylene (PTFE) vascular grafts, which were sutured intraluminally with autologous endothelial cells prior to grafting in an attempt to prevent thrombus formation by accelerating the establishment of an endothelial lining within the lumen of the PTFE graft. Eight rabbits undergoing implantation of PTFE vascular grafts in the infrarenal part of the abdominal aorta developed hind limb neurologic deficits. The neurologic complications resulted from focal ischemic damage to the spinal cord attributable to temporary vascular clamping of the abdominal aorta during placement of the vascular grafts. In subsequent studies, induction of mild systemic hypothermia decreased the rate of neurologic deficits from 80 to 9% without perioperative or postoperative complications related to decreases in body temperature. A model for prediction of neurologic outcome dependent on body temperature and aortic-occlusion time was developed. We determined that relatively minor decreases of body temperature were sufficient to afford protection from ischemic injury to the spinal cord in rabbits.

PS 46 Techniques for Effective Administration of Heparin to Rabbits After Vascular Grafting

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Postoperative heparin treatment after vascular grafting has been an accepted means of preventing vascular thrombosis in human beings. Vascular grafting research protocols that use animals require concurrent thrombosis prevention. Maintaining an intravenous indwelling catheter in a peripheral vein in a rabbit is typically difficult due to their dexterity and aggressive grooming behaviors. Central venous lines are also difficult to maintain due to the behavior of rabbits. We developed a catheter-based system for the effective, long-term administration of heparin to rabbits after bilateral venous autographs to the carotid arteries. During the carotid grafting procedure, a 6.0-French indwelling silicone catheter was inserted into the external jugular vein for vascular access. The indwelling catheter was exteriorized at the dorsal aspect between the scapulae (at the base of the neck). Rabbits were fitted with a tubular elastic dressing retainer around the thorax to serve as a netted jacket. The forelimbs were placed through openings in the jacket on the ventral surface. A disposable coiled intravenous extension line on a rotating tether system was connected to the catheter. The rotating tether line was then secured to the jacket near the junction of the indwelling catheter. The jacket relieved tension on the indwelling catheter, thereby preventing inadvertent removal of the catheter. Accurate amounts of heparin were administered via a programmable infusion pump that connected to the tether system. Rabbits remained on the heparin infusion for 1 to 7 days, and the indwelling catheter was successfully maintained for up to 6 weeks after discontinuation of the infusion. Postinfusion catheter maintenance consisted of periodic blood collections and daily flushing with normal physiologic saline solution followed by a heparin lock of 0.5 to 1.0 ml (20 to 25 U of heparin/ml). Anti-coagulation efficacy was measured by the activated clotting time (ACT) method. Typical postoperative ACT times are 150 to 200 seconds. Results of complete blood counts and ACT were evaluated on a daily schedule for several days postoperatively. This delivery system has been used in more than 100 procedures with rabbits and allows for accurate and effective administration of heparin postoperatively in rabbits.

PS47 Surgical Implantation of Telemetry Instrumentation for Cardiovascular Monitoring in Beagle Dogs

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Although extensive cardiovascular research has been conducted with cocaine, the studies frequendly have used anesthetized or restrained animals. We developed a program for monitoring cardiovascular parameters in freely moving dogs by infrared-based telemetry. Telemetry allows for evaluation of acute and chronic effects without the interference that restraint of animals causes. The goal of our research was to better understand how drugs of abuse, such as cocaine, alter cardiovascular parameters, alone and in interaction with compounds that will be used to treat cocaine abuse. Beagle dogs were surgically implanted with a left ventricular pressure transducer, an aortic flow probe, and a descending aortic pressure transducer. Following anesthetic induction with thiopental and maintenance with 1.5% isoflurane, a thoracotomy through the left fifth intercostal space was performed. The first set of leads consisted of a left ventricular pressure transducer and an aortic flow probe. A stainless steel trocar was used to produce a small defect in the apex of the left ventricle. A pressure transducer was then inserted into the defect and secured in place with stay sutures. Adipose tissue surrounding the ascending aorta was bluntly dissected free, and a flow probe was placed around the ascending aorta and secured. The second set of leads consisted of a descending aorta pressure transducer along with an echocardiography spurt that was placed outside the thoracic cavity in the subcutaneous tissues in the area of the sixth intercostal space. The proximal part of the descending aorta was dissected free of adipose tissue, the side was clamped, an arteriotomy incision was made, and a pressure transducer was inserted. The pressure transducer was secured with suture, and the arteriotomy was repaired. Skin sutures for both sets of probes were exteriorized in the dorsal interscapular area and secured. The thoracotomy incision was closed in a routine manner. Analgesia was provided by preoperative administration of an epidural of morphine, intraoperatively by use of a costal nerve block with lidocaine, and postoperatively by s. c. adminis-
tration of morphine and oral administration of carprofen. After a minimum 14-day postoperative recovery period, baseline cardiovascular monitoring was obtained by placing backpacks on the dogs with sending units and batteries for remote monitoring of dogs in runs for 48 hours. Vascular parameters obtained from the aortic pressure transducer included systolic, diastolic and mean arterial pressures. Cardiac parameters obtained from aortic flow or left ventricular pressure probes included cardiac output, total peripheral resistance, stroke volume, left ventricular end-diastolic pressure, maximal increase in ventricular pressure, and increase in ventricular pressure at 50 mm of Hg. Electrocardiographic parameters were also obtained from the electrocardiography leads.

PS48 Portal Vein Vascular Access Ports and Intestinal Access Ports in Cynomolgus Monkeys (Macaca fascicularis)

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Pharmacokinetic characterization of novel compounds often requires determining bioavailability, intestinal absorption, and hepatic clearance. These studies require access to the intestinal lumen and the portal venous system. We evaluated a model of portal vein vascular access ports (PVVAP) and duodenal access ports (DAP) in cynomolgus macaques (Macaca fascicularis). From October 1993 through March 1997, 16 male cynomolgus macaques were implanted with a PVVAP (SLA-3.5H-14 or TA2000-3.5H-14) and a duodenal access port (modified GPV-7S). At the time of analysis, 5 macaques had fully functional ports (infusion-and withdrawal). These PVVAP and DAP had been implanted for a range of 302 to 579 days, with a mean functional duration of 465 ± 136 days. Eleven macaques had nonfunctional ports or had been euthanized. Seven macaques developed complications associated with the PVVAP. The PVVAP lost patency in 5 macaques (4 due to portal vein thrombosis at 839, 805, 578, and 293 days after implantation and 1 due to a kink in the catheter within the portal vein at day 440). One PVVAP was confirmed to be causing an infection at day 219 and was removed. One PVVAP became nonfunctional at day 450 due to ulceration of the skin over the dome. Mean functional duration of the 7 macaques with nonfunctional PVVAP due to complications was 517 ± 238 days. The DAP were functional in all 7 of these macaques. Three macaques developed complications associated with the DAP. Two macaques were euthanized due to complications with the DAP at day 28 and 58. One DAP caused infection at day 515 after it had migrated too far into the intestinal lumen. The PVVAP were functional in these 3 macaques. One animal was lost to a drug-related death at day 210, but it had functional PVVAP and DAP. These macaques were used in between 2 and 21 pharmacokinetic studies, a mean of 10.5 studies/macaque. This suggested that PVVAP and DAP can serve as long-term devices for the study of novel compounds.

Poster Sessions

P01 Assessment of Pulmonary Responses to Ascaris suum Antigen in Anesthetized Squirrel Monkeys, Using Forced-Oscillation Mechanics

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Conscious, trained ascaris-sensitive squirrel monkeys (Saimiri sciureus) have proven to be an appropriate model for preclinical screening of leukotriene D4 (CysLT1) receptor antagonists. However, this model (dual plethysmograph) has limitations as a high-capacity screen due to a shortage of trained ascaris-reactive monkeys. We developed an alternative method to rapidly identify ascaris-reactive squirrel monkeys. This technique involves measuring lung resistance in propofol-anesthetized monkeys, using a forced-oscillation mechanics apparatus from Buxco Electronics, Inc. Male or female squirrel monkeys (0.8 to 1.2 kg) had food withheld overnight, were sedated with ketamine hydrochloride (10 to 15 mg/kg of body weight, i.m.), and then were injected with a bolus dose of propofol (5 to 12 mg/kg, i.v. or followed by continuous i.v. infusion of propofol (10 to 30 mg/kg/h) via the saphenous vein. Monkeys were intubated (2 to 2.5 mm i.d. endotracheal tube), and after stabilization, were attached via the endotracheal tube to the forced-oscillation apparatus. Baseline measurements of lung resistance, respiratory rate, heart rate, blood pressure, blood temperature, and arterial oxygen saturation (SaO2) were recorded continuously throughout the experiment. Only 12 of 56 squirrel monkeys screened had reproducible airway responses to aerosolized ascaris antigen (1:25 dilution; 3 min; particle size, 1 to 5 μm) delivered by a DeVilbiss ultrasonic nebulizer (# 25). All monkeys, however, had some degree of airway responsiveness to an aerosol challenge with methacholine (1 to 10 mg/ml; 30 s). These findings indicated the utility of propofol anesthesia and the forced-oscillation system for identifying squirrel monkeys that possess lung reactivity to ascaris antigen. Advantages of this technique include rapid and safe recovery from propofol anesthesia as well as the ability to use untrained squirrel monkeys for identifying and studying allergic lung responders.

P02 Determination of a Reference Range for Bleeding Time in the Common Marmoset

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The bleeding time (BT) test is the most frequently used test of primary hemostatic competence. It consists of measuring the duration of blood flow from a skin incision to test the functional integrity of the platelet-microvessels interaction. There currently is a lack of BT data available on the common marmoset (Callithrix jaccus; CJ). Methods for performing the BT test on CJ were developed, and the test was administered on both forelimbs of each of 50 clinically normal CJ that were under ketamine anesthesia (26 mg/kg of body weight, i.m.). A disposable pediatric BT device with a spring-loaded blade was used to provide a uniform incision 5 mm long and 0.5 mm deep. A BT reference range for CJ was determined (n = 50; mean ± 2 SD = 61 to 105
sec). An aspirin-sensitivity test (12 mg/kg, p.o.) resulted in a prolongation of the BT by 34.1% in healthy CJ. Factors that may have affected the BT such as age, weight, sex, and right or left forelimb were evaluated, and only increases in age significantly increased BT (Student's t-test, P < 0.01). Mean BT of the older age group (49 to 96 months; n = 39) was 21.5% greater than that of the younger age group (0 to 48 months; n = 11). These data were subsequently used to document increased BT in 2 CJ with clinical bleeding abnormalities, suggesting a defect in primary hemostasis. The described BT test methods and establishment of a reference range provide a simple diagnostic test for use in evaluation of hemostasis in the common marmoset.

P03 Evaluation of Hypothermia-Associated Analgesia in Leopard Frogs (Rana pipiens)

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Hypothermia induces diminished voluntary muscle activity and is frequently used as a means of providing deep anesthesia to amphibians, although it is unclear whether hypothermia produces pain insensation. The purpose of the study described here was to determine whether hypothermia produces analgesia in amphibians. A needle-probe thermometer was used to determine that local hypothermia (10.0°C) could be induced in leopard frogs (Rana pipiens) by placement of a leg with a tourniquet into ice water (6°C) for 10 minutes, contrasted with the contralateral leg without a tourniquet that was not in ice water (21.8°C). Analgesia was tested by placement of 30 μl of acetic acid diluted to 10 strengths equally spaced on a logarithmic scale from 0.26 to 15 M and numbered from 1 to 10 with increasing concentration (glacial acetic acid = 11). Results for an initial group with a restrained, uncooled, leg with a tourniquet revealed that the contralateral leg would respond by attempting to wipe the acetic acid from the restrained leg. Further tests that used groups of 10 frogs revealed that frogs with local hypothermia tolerated significantly (P < 0.001) greater concentrations of acetic acid than morphine-treated (10 mg/kg of body weight) or non-treated frogs (acid concentrations of 11.0, 9.6, and 5.8, respectively). Additional studies revealed that naloxone reversed morphine-induced analgesia at dosages as low as 0.01 mg/kg and hypothermia-induced analgesia at 10 mg/kg. Naltrexone reversed morphine-induced analgesia at dosages as low as 0.01 mg/kg and hypothermia-induced analgesia at 0.10 mg/kg. Results of our study indicated that hypothermia induced significant pain insensation in amphibians and that this analgesia was reversed by naloxone and naltrexone, suggesting that the effect is mediated at least partially by opioid receptors.

P04 Rectal Temperature After Recovery From Anesthesia in Laboratory Rats (Rattus norvegicus)

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Recovery from anesthesia is often regarded as being complete once the righting reflex has returned. However, a study conducted by the authors revealed profound behavioral effects in the early recovery period with a number of anesthetic regimens.

The study reported here was performed to determine whether postanesthetic hypothermia may have been responsible for these changes. Six female Wistar rats were anesthetized with halothane (5%, followed by 1.5%), ketamine-xylazine (50 and 10 mg/kg of body weight, respectively, i.p.), pentobarbitone (50 mg/kg, i.p.), and fentanyl-alfaxone-midazolam (0.14, 6.75, 2.38 mg/kg) in a randomized order, with a 7-day recovery period between each anesthetic episode. Anesthesia was maintained at a surgical plane for 30 minutes, then after recovery of righting reflex, supplemental heat was withdrawn, and the rats were returned to their cages (22 to 24°C). Rectal temperature was measured at 15-minute intervals until normothermia was maintained for 3 successive measurements. Pentobarbitone anesthesia produced a decrease of 2.2°C (SD, 0.7°C) which lasted between 120 and 240 minutes. Ketamine-xylazine produced a similar, but less pronounced reduction. These results indicated that heat supplementation must be maintained until an animal is fully recovered from the effects of an anesthetic for several hours after return of the righting reflex after use of these common injectable anesthetic regimens.

P05 Benefits of Noninvasive Versus Direct Blood Pressure Measurements in Spontaneous Hypertensive Rats/Stroke Prone

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Spontaneous Hypertensive Rats/Stroke Prone (SHR/SP) were used to study pathophysiologic mechanisms and potential interventions for the prevention and control of hypertension, brain ischemia, and other associated complications in human beings. These unique rats tend to develop cerebral hemorrhage or infarcts that progress to a stroke in addition to developing well-documented cardiovascular malfunctions secondary to chronic hypertension. Current research protocols frequently require multiple determinations of blood pressure in SHR/SP for extended periods. These rats are considered high anesthetic risks, making surgical placement of indwelling catheters and continued maintenance of such devices difficult. An accurate noninvasive method for monitoring blood pressure is critical for investigators to identify hypertensive rats and monitor disease progression over time. We report here that noninvasive monitoring via a tail cuff is safe, efficient, and accurate in evaluating blood pressure in SHR/SP with progressive hypertension. A commercially available system enabled us to evaluate the blood pressure on a group of SHR/SP rats (n = 25) over several weeks without adverse effects attributed to testing. Invasive procedures typically required more time to accomplish and required use of anesthetics. Additionally, peripheral arteries of rats are relatively small and difficult to cannulate for direct pressure measurements. Noninvasive measurements were analogous to direct pressure measurements (± 10%). We concluded that although direct pressure measurements may be preferred for accuracy, long-term evaluation can be successfully performed via noninvasive methods.
P06 A New Feeder for Diet Optimization in Rats

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In The Guide for the Care and Use of Laboratory Animals, it is stated that “Moderate restriction of calorie and protein intakes for clinical or husbandry reasons has been shown to increase longevity and decrease obesity, reproduction and cancer rates in a number of species” and that “calorie restriction is an accepted practice for long-term housing of some species, such as rodents and rabbits.” Diet optimization is a procedure in which an animal is given unlimited access to a daily allotment of nutritionally adequate food that exceeds the minimum daily nutritional requirements and is sufficient to allow for normal growth and development, but will prevent severe obesity. Studies supporting the benefits of diet optimization have been published in peer-reviewed journals for > 40 years; however, a practical means to feed a specific amount of diet to a large number of rats has not been available. Faced with the trend of increasing body weights and decreased survival in our rat studies that are of 2- years’ duration, we developed a new feeder that presented a specific amount of ground diet formulated for rodents to singly housed rats. This feeder was used with an automatic filling station. Food consumption, body weight, and body-weight gain data from rats fed a control optimized diet by using the new feeders were compared to data from rats fed a control ad libitum diet.

P07 A Novel Ventilated Transport Container for Movement of Laboratory Animals

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A novel ventilated transport container was developed to allow safe movement of laboratory animals among biosafety level (BL) 4, BL-3, and conventional animal holding areas or laboratories. This specialized device allowed the simultaneous transport and concurrent isolation of 2 potentially infected, unanesthetized nonhuman primates by using standard transport boxes as the primary enclosure. Its use substantially reduced the risk to the primates by eliminating the need for anesthesia during movement between containment areas and, in addition, improved the margin of safety during transport. The device was easily decontaminated and was also appropriate for transport of other small laboratory animals among biocontainment areas.

P08 A Model for Determining Simultaneous Vascular and Lymphatic Clearance Model in Beagle Dogs

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Pharmaceuticals may be cleared from extracellular fluid via the vasculature, the lymphatic system, or both. Targeting specific therapeutic and diagnostic agents for selective lymphatic clearance after s.c. or i.m. injection could augment the detection, diagnosis, and treatment of lymphatic disease and staging of malignant tumors. However, the physical and chemical characteristics required for selective lymphatic clearance of pharmaceuticals must be evaluated. A simultaneous vascular and lymphatic clearance model was developed using an isoflurane-anesthetized beagle dogs. The thoracic duct was approached via an incision along the left external jugular vein. Careful dissection permitted identification of several tracheal lymph vessels and the junction (bulb) at which the tracheal lymph ducts and thoracic duct coalesce to drain lymph into the cardiovascular system. A microcatheter was placed in a tracheal lymph duct and fed distally into the lymph bulb. All other lymph ducts entering the bulb, except the thoracic duct, were ligated, and lymph drainage from the thorax was collected into preweighed tubes at 5- to 10-minute intervals. After collection of control samples of venous blood and lymph, Evens' blue (EB) or indocyanine green (ICG) dye was injected s.c. in a hind limb. Dye first appeared in lymph samples within 5 minutes; peak dye concentrations were detected within the first 2 hours. After 3 hours, approximately 1% of the injected dose of EB was collected in lymph samples and 0.01% in plasma samples, whereas ICG was not detectable in lymph samples, and only approximately 0.2% of the injected dose was detected in plasma samples. At the end of the experiment, much of the dye remained at the injection site bound to local tissues. Results of these preliminary studies indicated the utility of determining simultaneous vascular and lymphatic clearances to explore physical and chemical variables (size, shape, charge) and enhance selective lymphatic clearance of therapeutic and diagnostic agents from extracellular fluid.

P09 A Magnetic Resonance Imaging-Compatible Respiration Monitor for Use in Small Animals

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Physiologic monitoring of laboratory animals during magnetic resonance imaging (MRI) scans presents major technical problems. For these scans, animals are placed inside the MRI probe assembly, which, in turn, is positioned inside the bore tube of the MRI system’s magnet. Bore tubes on small animal scanners are typically 6 to 10 inches in diameter, and investigators do not have physical access to animals during the scans. It is also often impossible to visually monitor the animals; therefore, all monitoring must be performed remotely by using various nonmagnetic/nonmetallic sensors. In large animals, respiration can be monitored by using an air-filled balloon placed next to the abdomen. In some studies, however, vibrations caused by the MRI system during imaging can disrupt pressure-based respiration measurements. Finally, for small animals such as neonatal rodents, placing a balloon on the abdomen may restrict breathing and may not reveal detectable pressure variations. For these reasons, we developed an optical-monitoring device that can be used to measure displacement of the abdomen due to breathing without physical contact. The device consisted of a diode that emits infra-red light and photo-transistor pair connected to a simple battery-powered bridge circuit. The emitter/detector assembly is about 8 mm in size, was placed 5 to 10 mm from an animal’s body, and was pointed at the part of the abdomen that seemed to be moving the most prior to positioning of the animal/probe assembly into the magnet. It was connected to the circuit (outside the magnet) by 1 m of cable. Respiration-induced variations in light reflectance from an animal’s body were converted to a voltage waveform and recorded on a computerized chart recorder. For scans in which an animal’s abdomen was inside the MRI probe, a 50-cm optical fiber pair was connected to the emitter and photo-transistor, because placing the metallic components of the emitter/detector inside the
probe resulted in noise and other problems with the MRI scans. We have used the device to monitor breathing during MRI scans of the brains of neonatal rat pups.

**P10 Comparison of Results of Helicobacter Tests Performed by Commercial Laboratories**

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Our objective was to assess the variability among commercial laboratories for results of *Helicobacter* testing. Fecal and serum samples were submitted to commercial laboratories for polymerase chain reaction (PCR) analysis (3 laboratories) and for serologic analysis (2 laboratories). Replicate samples were submitted from 20 transgenic K14a mice (C57BL/6 background) suspected of harboring *Helicobacter* organisms and 5 heterozygous nude mice (CD-1 background) believed to be negative for *Helicobacter* spp. Live mice were submitted to a laboratory for bacterial culture and PCR analysis of fresh fecal specimens. The PCR results of replicate samples for one laboratory indicated that none of the mice were positive for *H. hepaticus*, but another laboratory reported that all 20 mice were positive for *H. hepaticus*, and a third laboratory reported that 12 mice were positive by PCR analysis for a *Helicobacter* organism. The number of mice reported to be positive by serologic analysis was 1 for a *Helicobacter* sp. by one laboratory and 7 for *H. hepaticus* by another laboratory. The PCR and serologic results for the replicate samples submitted from heterozygous nude mice were negative for all laboratories, except for 1 equivocal and 2 positive results by PCR assay and 1 positive result by serologic analysis. Bacterial culture and PCR analysis of fresh fecal samples revealed that 16 of the 20 suspect positive mice were positive on culturing, yielding a *Helicobacter* sp. (not *H. hepaticus* or *H. bilis*), and all 20 were positive for a *Helicobacter* sp. (not *H. hepaticus* or *H. bilis*) by PCR assay. Bacterial culture and PCR analysis of fresh fecal specimens yielded uniformly negative results for the heterozygous nude mice. Therefore, due to the discrepancy of results between commercial laboratories, researchers should use caution when interpreting results from commercial laboratories of *Helicobacter* tests.

**P11 Effect of Controlling Tongue Temperature on Pulse Oximetry Monitoring**

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Pulse oximetry is an integral part of advanced intraoperative clinical monitoring. The effect of factors such as probe-site temperature on measurements is not known. We investigated the effect of controlling tongue microenvironmental temperature on pulse oximetry measurements obtained during noninvasive and invasive procedures performed on dogs. Thirty intubated, anesthetized dogs were instrumented to enable measurement of tongue and body temperature, expired CO$_2$, SpO$_2$, blood gases, and electrocardiography. Tongue microenvironment was controlled by using a plastic bag around the lower jaw. Noninvasive groups had weak correlations between tongue temperature and SpO$_2$ ($r^2 = 0.65$), with and without the bag. For invasive groups, correlations were even weaker, but there were strong correlations between tongue and body temperature ($r^2 \geq 0.85$). Invasive procedures performed on septic dogs had strong correlations between body temperature and SpO$_2$, SaO$_2$, pCO$_2$, and pH, and between SaO$_2$ and SpO$_2$ ($r^2 \geq 0.78, 0.96, 0.81, 0.88$, and 0.84, respectively). Tongue and body temperatures were significantly different with and without the bag. Controlling tongue microenvironmental temperature did not appear to significantly affect or improve SpO$_2$, although body temperature was significantly affected. Isolation and containment of the air around the tongue appeared to increase temperature and humidity immediately adjacent to the tongue, resulting in decreased radiant and evaporative cooling. Controlling tongue temperature did appear to decrease loss of body heat during surgical procedures and may therefore indirectly improve the outcome of those surgical procedures.

**P12 Establishment of a Uniform Monitoring System for Breeding of Transgenic Mice**

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Transgenic facilities monitor a multiplicity of projects for a number of investigators. However, cage-cards, the most accessible daily checklist of animal status, are often maintained in formats specific for each investigator or project, reflect incomplete or inaccurate information, and are difficult to comprehend. We instituted a standardized cage-card format to accurately record all genetic information, develop complete histories on each animal, and allow technicians to easily monitor all facility projects. In a consistent and comprehensible fashion, these cards provide detailed pedigree, mating, weaning, and embryo transfer data and could be used to accurately trace complete transgenic lines. Technicians developed a better understanding of each project, because causative factors for producing transgenic models were clearly reflected in standard scientific nomenclature. They more readily identified phenotypes, recognized outliers, tracked reproductive physiologic events with fewer errors, fully documented relevant statistics, and moved between projects with ease. Therefore, a uniform, comprehensive cage-card system decreased training time and increased technical expertise as laboratory science was incorporated into daily animal care and resulted in greater compliance with internal protocols and external guidelines. Most important, the reproductive health of the colony improved as tracking errors diminished; phenotypes, overage breeders, and viable new breeders were more readily discerned; and unexpected outcomes were clearly recognized.

**P13 Gavage Dosing of New Zealand White Rabbits With Fumonisins B1, Using a Computer-Based Automated System**

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A computer-based automated system was used for gavage dosing of New Zealand White rabbits with fumonisin B1, a mycotoxin. Minimal biohazardous risk exposure was evident for technicians, while there was increased accuracy and reproducibility of dose delivery when compared with manual techniques. The automated system allowed technicians to weigh and dose each rabbit daily without having to perform manual calculations. The computer program used the body weight of each rabbit and multiplied it by the dose amount, and then divided that number by the concentration of the test article to obtain the required volume of dosing solution. The computer delivered the data to the automated dosing machine, which used it to regulate the amount of
solution to be administered to each rabbit. The dosing machine dispensed through 1 or 2 lines, depending on study requirements with respect to concentration or volume delivered. Once a rabbit received a dose, the system prompted by asking if dosing was successful, and the technician responds by typing “yes” or “no.” At the completion of dosing, computer output reports are generated to indicate mixture usage, mixture usage summary, and actual dosing transactions. These reports can be monitored by quality assurance inspectors for accuracy of dosing and for maintenance of an accountability record of test article usage.

**P14 An Efficient Tape Technique for Detecting Pinworms**

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Tape testing rodents for pinworm eggs is an important part of rodent disease surveillance programs in research facilities. The technique described here was developed so that a single technician could collect a fingerprint-free test sample. A small piece of tape was attached to the flossing fork of a disposable dental floss device. A slide was labeled with each rodent’s identification number. Rodents were grasped by the base of the tail and allowed to grasp the side of the cage. The applicator was held in the free hand, and a finger was used to press the tape against each rodent’s perianal region. Rodents were returned to their cages, and the tape was removed from the applicator and applied to the slide. This cost-effective procedure reduced the amount of tape needed per test. Three small pieces of tape easily fit on 1 slide, reducing the number of slides used. The technicians are inexpensive, can be sanitized, and are reusable, and only 1 technician is required to accomplish the task in an efficient and effective manner.

**P15 Management of a Canine Donor Colony and Blood Bank**

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A blood bank can provide blood components for a wide range of therapeutic and research uses. In our facility, dogs are used as blood donors to support a Factor-VIII deficient hemophiliac colony. Blood-donor dogs are housed separately or in pairs, depending on size and temperament. Routine health care, including physical examination, blood biochemical analysis, hematologic analysis, blood typing, and fecal examination are performed quarterly. A unit of whole blood can be collected in approximately 10 minutes by 2 technicians. The whole blood may then be processed into plasma, packed red blood cells, cryoprecipitate, or cryoprecipitates. Cryoprecipitate is a concentrated source of Factor VIII and other essential clotting factors. To allow for frequent blood donation, processed red blood cells are reconstituted with saline solution and infused into the donor dog via an indwelling catheter. This provided an efficient, safe, and easy way to maintain a supply of blood products from a limited number of donors.

**P16 Novel Techniques for Testing Esophageal Irritancy of Liquids and Tablets in Dogs**

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Esophageal irritation can be an adverse effect of medications in human beings, but this potential complication is rarely evaluated in animal studies, which usually deliver test compounds directly into the stomach. Our toxicology unit required development of a model to test esophageal irritancy of compounds in liquid and tablet form. A novel technique was devised to allow delivery of liquids into the esophagus of anesthetized dogs. After dogs were anesthetized, a 5.0-mm (i.d.) endotracheal tube was passed into the esophagus, and the cuff was inflated directly caudal to the larynx. An 8-French red rubber catheter was then passed through the tube until it extended 1 inch past the end of the tube. An infusion pump delivered the test liquid through the catheter throughout a 30-minute period. The cranial portion of each dog was elevated so that the liquid would flow aborally. Infusions generally were administered for 5 consecutive days, with dogs being euthanized on the fifth day to enable gross and histologic examination of the esophagus. This technique was successfully performed without complication on > 80 dogs. For delivery of compounds in tablet form, a clear tube was passed into the cranial third of the esophagus. A tablet, tied to a premeasured length of suture, was introduced into the esophagus through the tube by using an endoscopic retrieval forceps. Tablets were left in place for 1 hour, and dogs were then euthanized and examined. These techniques allowed for accurate and reliable testing of the esophageal irritancy potential of compounds in liquid or tablet form in dogs.

**P17 Reduction in Animal Use Through Simultaneous Dosing of Multiple Compounds of Rats Used for Pharmacokinetic Evaluations**

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Approximately 30% of tumors in human beings harbor an activated ras gene, and its product (p21) is believed to be essential to maintain the tumorigenic phenotype. The p21 protein is unable to transform cells if it is not farnesylated; therefore, inhibitors of ras farnesyltransferase (ras FT) could be potent anti-tumor agents. As part of our preclinical pharmacology efforts, we needed to rapidly identify potent compounds with favorable pharmacokinetic (PK) characteristics that were potential development candidates. In traditional PK studies, single compounds are administered to each animal; however, with the advent of detection systems such as liquid chromatography tandem mass spectrometry (LC/MS/MS), it is possible to administer mixtures of compounds to each animal to screen several compounds simultaneously for specific PK traits. In addition to increasing the throughput of compounds being evaluated, the number of animals is reduced. To validate this approach for ras FT inhibitors, we administered 3 compounds intravenously as single agents (10 mg/kg of body weight) or as a mixture (3.3 mg of each/kg) to male Sprague-Dawley rats (~ 300 g). The 3 compounds differed by a single substitution on a benzodiazepine nucleus. Plasma samples were obtained during a 24-hour period and analyzed by LC/MS/MS. The following PK parameters were estimated for
obtaining the core body temperature of pigs has been use of thermometers inserted in the rectum and held for 2 to 3 minutes, which requires that the pig be captured and manually restrained. A new method was developed for obtaining core body temperature in < 5 seconds. The Michelangelo® scanner (BioMedic Data Systems, Maywood, New Jersey) was designed for use in small laboratory animals. This commercially available system consists of a small implantable transmitting device and a portable reader with digital display. When the implant was placed in an appropriate area of the body, it constantly emitted a signal containing information on core body temperature and animal identification number. Using a 12-gauge implanting needle, implants were injected 1 cm deep in pigs, immediately lateral to and slightly dorsal to the anus. The reader wand was touched to the implant site, and the temperature was displayed within 5 seconds. To test the accuracy of this device in swine, body temperature of 10 pigs weighing approximately 20 kg were obtained daily for 10 days, using a thermometer inserted in the rectum and the scanner. Pigs received a bacterial inoculum, causing an increase in body temperature. After comparing the values from each device, we found that the scanner closely matched the thermometer with an error margin of less than 0.5°F; therefore, the scanner was acceptable for use in our studies.

P19 Use of an Implantable Device to Monitor Body Temperature in Swine

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Data collection with animal models often requires handling test subjects multiple times in a day. This is especially troublesome when working with swine, a species particularly prone to stress from handling. Pigs that are stressed have reduced immunologic function due to increased concentrations of serum cortisol. Many test variables including blood counts, serum biochemical analysis, and body temperature may also be affected. Minimizing the duration of handling and restraint of test animals benefits the pigs physiologically, aids in protecting integrity of the data, and is a human safety measure, because swine are not the most tractable of species. The historical method in our laboratory for obtaining the core body temperature of pigs has been use of thermometers inserted in the rectum and held for 2 to 3 minutes, which requires that the pig be captured and manually restrained. A new method was developed for obtaining core body temperature in < 5 seconds. The Michelangelo® scanner (BioMedic Data Systems, Maywood, New Jersey) was designed for use in small laboratory animals. This commercially available system consists of a small implantable transmitting device and a portable reader with digital display. When the implant was placed in an appropriate area of the body, it constantly emitted a signal containing information on core body temperature and animal identification number. Using a 12-gauge implanting needle, implants were injected 1 cm deep in pigs, immediately lateral to and slightly dorsal to the anus. The reader wand was touched to the implant site, and the temperature was displayed within 5 seconds. To test the accuracy of this device in swine, body temperature of 10 pigs weighing approximately 20 kg were obtained daily for 10 days, using a thermometer inserted in the rectum and the scanner. Pigs received a bacterial inoculum, causing an increase in body temperature. After comparing the values from each device, we found that the scanner closely matched the thermometer with an error margin of less than 0.5°F; therefore, the scanner was acceptable for use in our studies.

P20 Use of Intravascular Ultrasonography for Measurement of Arterial Luminal Diameter in Laboratory Animals

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Pigs, sheep, and calves are routinely used in preclinical testing of biomedical devices intended for cardiovascular and other vascular applications. One critical component in the selection of the appropriate animal model and the test procedure itself is accurate knowledge of expected and actual diameter of the lumen of the vessels in which the device will be tested. Intravascular ultrasonography has become the clinical standard for measuring the internal diameter of blood vessels. Data from our surgical laboratory, including the internal diameters of the most commonly used blood vessels such as abdominal aorta, carotid artery, renal artery, iliac artery, femoral artery, and coronary arteries of miniature swine, sheep, and calves were determined. This data can help researchers select the most appropriate species and vessel in which to test candidate biomedical vascular devices as directed by the U.S. Food and Drug Administration, International Organization for Standards (ISO 10993-1), and European Union Medical Devices Directive.

P21 Effect of Time and Temperature on Results of Complete Blood Counts

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It is generally accepted that most diagnostic specimens should be refrigerated when they cannot be immediately processed, particularly blood samples for hematologic analysis. When a large number of samples is obtained at a time, there is often a delay of one to several hours before samples can be analyzed. We collected blood samples from mice, rats, rabbits, and monkeys in EDTA-coated evacuated tubes for analysis by complete blood counts (CBC). Several samples were collected from each animal, and tubes were subjected to varying time and temperature...
conditions prior to analysis. Samples were immediately refrigerated (or put in an ice bath), held at room temperature for 2 to 6 hours and then refrigerated, or held at room temperature until analyzed. The CBC were conducted at 2, 6, 12, and 24 hours after samples were obtained, and these results were compared with counts conducted immediately after sample collection. All samples suitable for analysis, whether immediately refrigerated, held at room temperature prior to refrigeration, or held at room temperature for up to 24 hours maintained constant values for leucocyte counts, erythrocyte counts, hemoglobin content, hematocrit, and differential leucocyte ratios. Standard Deviation from the mean of counts obtained at the various times (t = 2, 6, 12, and 24 hours) was not significantly different (P < 0.05) from the variation among the samples (n ≥ 3) at the initial reading (t = 0 hours). The only variable with a noticeable change was platelet counts, and these values did not vary significantly until 24 hours after sample collection. These results indicated that immediate refrigeration was not necessary when performing cell counts on EDTA-fixed blood samples and that properly collected blood samples held at room temperature can provide adequate data for ≥ 24 hours after collection.

P22 A Novel Technique for Noninvasive Intrapulmonary Administration of Bacterial Inoculum in Rats

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Experimentally induced models of bacterial pneumonia in rats are used commonly in efficacy studies of antibiotic compounds. Accurate intrapulmonic administration of inoculum is critical for successful development of the models and proper interpretation of results. Noninvasive methods typically have the disadvantage of blind intubation or reusable needles, which must be disinfected before use in subsequent animals. Other methods employ tracheal cut-down procedures that use injectable anesthetic agents and require several minutes for completion. The following technique was noninvasive and could be performed easily in rats anesthetized with an inhalant anesthetic agent. This procedure used a Plexiglass stand to suspend and support the body of an anesthetized rat. Inoculum was loaded into a 1-ml syringe affixed to a sterile, disposable closed-ended 3.5-French 5.5-inch tomcat catheter, and the catheter tip was dressed with a viscous lidocaine gel. The rat was positioned on the rack vertically; the rack was slanted slightly to provide adequate body support while the animal was suspended by its incisors. After the tongue was withdrawn, a 4-mm otoscope ear cone was placed such that the tapered end was positioned in the pharyngeal cavity, exposing the epiglottis. Using an external light source, the tracheal lumen was observed during inspiration. The catheter was then threaded into the lumen of the cone, and intubation was possible during inspiration. Accurate endotracheal placement may be verified visually and by extension to a natural stopping point at the bronchial bifurcation. This noninvasive technique has proven to be 100% accurate and is easily performed within 30 seconds after induction of anesthesia.

P23 A Method for Intravenous Infusion of a Test Compound, Using an Indwelling Catheter in Adult New Zealand White Rabbits

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Daily intravenous administration of solutions of known vascular irritancy can pose technical problems related to venous obstruction secondary to marked inflammation or thrombosis at the injection site. Similar problems can develop after repetitive administration of solutions that require a large volume to be given at a slow rate. We developed a nonsurgical procedure, using commercially available indwelling catheters inserted in the lateral auricular vein of adult nonpregnant and pregnant New Zealand White Rabbits, for daily intravenous infusions of up to 2-hours' duration. This method has been used for range-finding, developmental-toxicity, and toxicokinetic studies in rabbits in which the duration of drug administration was 2 weeks; however, because the catheters were well-tolerated by the rabbits, longer treatment intervals can be used for other study designs. Because rabbits must remain calm and relatively immobile during infusions, commercially available plastic restraint boxes were customized by the addition of eye covers to limit head movement and minimize distractions. Rabbits were acclimated to the restraint boxes during a 4-day period. One day prior to the start of infusions, a catheter was inserted into the lateral auricular vein and secured to the ear by use of a combination of sutures and surgical glue. An injection port was attached, and nonheparinized, sterile saline solution was flushed through the catheter to determine patency. Catheter placement and patency were verified daily. Solution delivery rate was controlled by a Harvard 22 programmable infusion pump, and dosing solutions were infused into the vein from the syringe via a microbore extension set. Each rabbit was returned to its own cage after completion of dosing. Because the catheters were well-tolerated, Elizabethan collars were not required. The cornerstone for success of this method, which minimized daily trauma to the rabbits, was in the selection and maintenance of the indwelling catheters.

P24 A Catheter Modification for Drug Infusion and Repeated Blood Sample Collection Via the Lateral Auricular Vein in Rabbits

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Procedures including infusions and timed, serial blood collections often require multiple venipunctures or restraint, both of which are very stressful to rabbits. However, the simple modification of a venous catheter eliminated multiple venipunctures and minimized restraint and stress. A 19-cm in length, 24-gauge pediatric venous catheter was threaded into the jugular vein via the lateral auricular vein of an anesthetized rabbit. The Luer hub of the catheter was removed with hemostats, and a 20-cm section of appropriately sized polyethylene (PE) tubing was inserted in the catheter lumen. Although the PE tubing fit snugly in the catheter, a drop of tissue adhesive often was used to secure it. The PE tubing was trimmed to a length of 8 cm, and this combination of PE tubing and catheter was referred to as the modified catheter. Although serial blood collection could be per-
formed by inserting a 23-gauge Luer adapter directly into the PE tubing and attaching a syringe, a coupler unit was necessary to attach the modified catheter to an infusion apparatus. The coupler unit consisted of a 1-cm section of silicone tubing with a 20-cm length of PE tubing attached to a 23-gauge Luer adapter, which could be attached to the infusion apparatus. The modified catheter was flushed with isotonic saline solution, filled with undiluted heparin (heparin sodium, 1,000 units/ml) of an appropriate volume for the length of the modified catheter; and the end of the PE tubing was heat-sealed with a cautery unit. The modified catheter was anchored to the skin with sutures or tissue adhesive. During infusion or sample collection, the tip of the sealed catheter was cut, and the Luer adapter or coupler unit was attached for blood collection or infusion. The tip was sealed again after each procedure. Aseptic conditions were maintained throughout all of the aforementioned procedures. Advantages of this modification were that it was relatively noninvasive, it allowed the rabbits to remain unrestrained between infusions or sample collections, it permitted consistent access to the vein, it remained patent for $\geq$ 48 hours, and it minimized stress to the rabbits.

**P25 A Simple Technique for Bone Marrow Aspiration in Macaques**

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Bone marrow serves as an important reservoir for hemopoietic stem cells. Investigating the structure and function of these populations is increasingly important in toxicologic studies. Techniques for bone marrow aspiration have been described extensively for dogs, cats, cattle, horses, and human beings, but reports are rare concerning nonhuman primates. Most published methods for these species involve collection sites on the ribs, sternum, proximal portion of the femur, or iliac crest. These sites may be difficult to use in primates, and techniques require small skin incisions and specialized bone marrow biopsy needles. We developed a simple alternative procedure for macaques, using regular laboratory supplies to enable us to aspirate up to 1.0 ml of marrow from the iliac tuberosity. The technique was simple, quickly performed,atraumatic, and yielded samples of high-quality bone marrow suitable for cytologic examination and other applications. This procedure has been successfully performed hundreds of times without complication.

**P26 Apparatus and Technique Used to Condition Goats for Repeated Blood Collection**

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A discarded metabolic crate was adapted for use as a safe and efficient restraint apparatus during collection of volumes of blood from a small herd of donor goats. Modifications were designed to minimize labor requirements. Unique features were designed to allow collection from various-sized goats and was dependant on voluntary entry by each goat. To develop this behavior, a sensitization/reward-based conditioning program was implemented. The conditioning program was begun with aged, experienced donors and continued with their kids. On the basis of subjective evaluation, the conditioning method was a total success in all goats, except for 1 adult and 1 kid. The herd was conditioned during 11 randomly scheduled sessions spanning a 47-day period. Interval between conditioning sessions was 1 to 21 days. The conditioning has been retained by the goats for $\geq$ 2 years. Sugar cubes were used as the reward. The bleeding crate was also used as an environmental enrichment device.

**P27 Blood Collection via the Jugular Vein in Rats**

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Blood collection via the jugular vein in rats is warranted for sample collection at multiple intervals or when a large volume of uncontaminated blood is desired. The equipment we used included a custom-made restraining board, plastic head cover, 3-cc syringe, and 23-gauge 0.75-inch needle. Each rat was restrained on the board so that its forelimbs formed a straight line perpendicular to its midline. A cover was placed over the rat’s head to control movement and was rotated until the skin was taut. A needle was inserted under the ventral aspect of the clavicle, 1-cm lateral to the midline and to a depth of 1 cm, by a second technician. Negative pressure was applied during needle insertion. The syringe was held stationary while obtaining blood. Digital pressure was applied immediately after the needle was removed and prior to release of the rat. Jugular venipuncture in rats is used for withdrawal of substantial quantities of blood from unanesthetized rats. Skilled teams can obtain 1 ml of blood from each of 60 rats in 1 hour.

**P28 Influence of Blood Collection Sites and Use of Anesthesia on Plasma Glucose Concentration in Mice**

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Blood collection from various sites in animals with or without use of anesthesia is commonly reported. To investigate the potential influence of these variables on plasma glucose concentration, a study was conducted to compare relative plasma glucose concentrations in blood samples collected with or without use of anesthesia from the tail vein (TV) versus the retroorbital sinus (RO) in 8-week-old C57BL/Ks male mice. Anesthetic agents evaluated included sodium pentobarbital (SP), methoxyflurane (MF), and propofol. Blood was collected through a 25-gauge needle with a 1-inch needle holder. Samples were screened prior to the study to determine plasma glucose concentrations and grouped such that the mean and distribution of plasma glucose concentrations were equivalent in each group. In conscious mice, plasma glucose concentrations were higher when blood samples were collected from the RO than the TV (189.0 ± 3.1 vs. 158.1 ± 6.1 mg/dL, P < 0.0001 ANOVA). However, significant differences were not observed between the RO and TV, respectively, for mice anesthetized by use of SP (216.0 ± 2.6 vs. 224.9 ± 5.0 mg/dL), MF (179.0 ± 3.9 vs. 171.5 ± 6.8 mg/dL), and PH (206.3 ± 8.8 vs. 196.5 ± 4.0 mg/dL). The use of anesthesia resulted in higher plasma glucose concentrations, compared with conscious mice (TV = 158.1 ± 6.1 mg/dL, RO = 189.0 ± 3.1 mg/dL). When samples were collected from the TV, the increases in plasma glucose concentrations were: SP (224.9 ± 5.0 mg/dL, P < 0.0001), PH (196.5 ± 4.0 mg/dL, P < 0.0001), and MF (171.5 ± 6.8 mg/dL, P = 0.0736). When samples were collected from the RO, concentrations were higher in mice anesthetized with SP (216.0 ± 2.6 mg/dL, P = 0.005) and PH (206.3 ± 8.8 mg/dL, P = 0.0220), whereas values in mice anesthetized with MF were not significantly higher.
A vascular cannula for use in rats was sought that would maximize ease of preparation and duration of patency with a minimum of maintenance. Jugular cannulas made of polyethylene (PE) tubing are easy to prepare and surgically place, but do not provide long-term patency due to clot formation at the cannula tip. Silicon rubber as part of a cannula material has improved anti-thrombogenic characteristics, but its permeability allows for aqueous diffusion through externalized material. A hybrid cannula consisting of silicon rubber tubing intravascularly and PE tubing extravascularly was designed and evaluated. Parallel groups of jugular and carotid vessels in male Sprague-Dawley rats were cannulated, using PE and hybrid cannulas. Cannulas were identical in length and method of surgical placement and were flushed once per week. Groups varied only in construction of the tip (PE or silicon rubber) and solution used to fill each cannula (heparinized saline or viscous heparin solution). The hybrid cannula group filled with viscous heparin outperformed all other groups, 10/10 remaining patent for ≥2 weeks. This modified cannula appeared to be a simple and highly effective device for use in vascular cannulation in rats. It provided extended duration of patency with minimal use or maintenance flushing.

A new surgical technique for placing a cardiovascular monitoring catheter designed for long-term use in the femoral artery of juvenile pigs was developed. This technique did not cause problems of ischemia in the hind limbs that have been associated with other methods. The femoral artery was isolated via a medial approach and cleared of connective tissue. A triangular- or trapezoidal-shaped pursestring was sewn into the tunica media of the artery, using 4-0 silk suture. Silk sutures were placed on both sides of the pursestring to temporarily occlude the artery, and then a small incision was made inside the boundaries of the pursestring. A surgical-grade catheter (0.05 i.d., 0.09 o.d.) was threaded through the incision and advanced past the proximal occluding suture into the abdominal aorta. The pursestring was then tightened and tied around the catheter at its base, after which the distal occluding suture was released. The catheter was manipulated through the subcutaneous tissues to the dorsum and exited on the midline, just caudal to the scapulas. The catheters were maintained twice daily by withdrawing a small amount of blood followed by flushing with a saline solution and injecting a heparinized solution as a lock. This method of catheterization has been successful in nineteen 20-kg Yorkshire pigs, maintaining a patient catheter for a minimum of 5 days without physical evidence of lameness, paraparesis, cyanosis, or necrosis. This procedure provided a safe method for catheterization of the femoral artery in juvenile pigs.

A new procedure for the diversion, exteriorization, and collection of bile in Beagle dogs was developed. This novel gall bladder-cannulated (GBC) model provided an alternative to bile duct cannulation for the long-term exteriorization of bile flow required in some drug metabolism studies. The surgical procedure involved the placement of one end of a unique, flexible plastic cannula in the gall bladder; the other end was secured within the lumen of the duodenum, distal to the major and minor duodenal papillae. Ligation of the common bile duct was necessary for the complete diversion of bile flow into the gall bladder and cannula. A segment of the cannula was passed through a 1-cm incision in the right side of the body wall, caudal to the last rib. After passing the cannula segment subcutaneously to a point between the scapulas, the segment was exteriorized and secured within the pocket of a nylon vest worn by the dog. This procedure has been used in 8 dogs with minimal postoperative or long-term complications. A complete blood count and serum biochemical analysis are obtained for each dog prior to surgery and then biweekly postoperatively to help monitor animal health. Intensive postoperative clinical care and assessment provided assurance of cannula patency and have helped maximize the longevity of each dog as a functional model. Our results suggested there were several advantages to this biliary cannulation technique. The new surgical approach was minimally affected by variations in hepatobiliary anatomy and provided enhanced exposure for cannula manipulation during the surgical procedure. The ability to use a large diameter cannula minimized intraluminal biliary pressure, bile stasis, and the potential for hepatic injury, which are common complications of bile duct-cannulation models. The GBC-dog model permitted an easily achieved diversion and collection of bile in unrestrained, conscious dogs that have normal enterohepatic circulation during non-collection periods. The GBC-dog model has proven useful in several drug metabolism studies.

Hardwood bedding can be a source of pathogenic agents and is sterilized prior to use in many animal research facilities. In our facility, 40-lb bags of hardwood bedding are sterilized in nonperforated 2-ply paper bags (2.2 ft²), in a bulk steam sterilizer containing 2 racks (4 shelves/rack). The sterilization cycle consisted of 2 prevacuum pulses, a 30-minute exposure time at 270°F (28 to 30 psi), and a 20-minute drying time. Recently, 50 to 60% of the bags burst during some cycles. Visual observations indicated that the supply steam pressure (SSP) decreased from...
50 to 60 psi to 30 to 35 psi and that the bags were excessively wet after autoclaving. Autoclave cycles were modified by reducing the exposure time and temperature; however, 50 to 60% of the bags still burst during some cycles. The autoclave was subsequently refurbished, and all cycles were computer programmed. An initial study was conducted to evaluate factors that may have caused the bags to burst, such as SSP, total autoclave time, and use of perforated (pinpoint holes) versus nonperforated bags containing 37 to 41 lb of bedding. In a second study, we evaluated a new wet-strength 2-ply paper bag (2.2 R²) containing 34 to 36 lb of bedding. All bags and bedding were purchased from the same vendor, and weight of each bag was recorded. The perforated and nonperforated bags were alternately placed 6/ shelf, lengthwise on edge, with the filling spout turned down. The top shelves contained four bags placed horizontally. The autoclave cycle selected included a 3-minute purge, 2 vacuum pulses, a 15-minute exposure time at 250°F (23 to 26 psi), and a 20-minute drying time. The SSP was observed and recorded. Effectiveness of the sterilization procedure was determined by using biological indicators placed in the center of a bag located on a lower shelf and microbial culturing procedures. In the initial study, when SSP decreased from 50 to 60 to 30 to 55 psi, total cycle time increased by 20 minutes, resulting in prolonged exposure to steam and a subsequent wetness problem that caused about half of the bags to come unglued at the seams. When SSP was low, 34 of 56 (60%) nonperforated and 6 of 18 (33%) perforated bags containing 37 to 41 lb of bedding burst. In contrast, when SSP remained > 50 psi, total cycle time was not increased, and 13 of 80 (16%) nonperforated and 2 of 37 (5%) perforated bags containing 37 to 41 lb of bedding burst. Most bags burst at the filling spout, suggesting that these bags were overfilled. In the second study, when SSP remained > 50 psi, none of the new improved wet-strength paper bags burst. Biological indicators and microbial culture results confirmed that bedding was properly sterilized. Also, the pinpoint holes were offset to reduce the risk of re-contamination after autoclaving. The sterilized bedding was maintained in a sanitized area and used within 48-hours. It was concluded that low SSP increased total cycle time, resulting in a wetness problem that caused the bags to burst; perforated bags were less likely to burst than nonperforated bags; bedding should be autoclaved only when SSP is maintained at > 50 psi; and < 36 lb of bedding should be placed in 2.24 R² paper bags to allow adequate room for expansion during autoclaving.

P33 A Novel Approach to Staffing a Long-Term Multi-Shift Project

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To support a 4-month research project that required 24-hour shift coverage, it was necessary to use people in the research environment who were not laboratory animal personnel. We hired third-year students attending a local nursing school to serve as temporary staff. Each student was interviewed to determine their technical qualifications and views on animal research. All students agreed with the necessity of animal research and appeared to be comfortable with the use of dogs as the animal model for this project. All students attended a 3-day course in which they received training on the basis of animal research, good laboratory practices, and operation of equipment in the intensive care unit (ICU). After their orientation, all were assigned to work with laboratory animal technicians who had experience working in the animal ICU. Animal technicians served as mentors to the nursing students and were assigned as shift leaders. All students had some initial problems becoming accustomed to the logistics associated with the animal ICU but learned quickly and adjusted to the ICU routine. After completion of 2 weeks, all students were able to function without close, direct supervision and were comfortable with the animal ICU. This process will assure success in future research projects of this magnitude.

P34 A System for Transporting Clean Microisolator Cages in a Specific-Pathogen Free Container

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A common approach to achieve sterility of microisolator cages and accessories after sanitation is to steam sterilize these components (and entire racks) immediately prior to housing animals in them. This procedure requires purchase and operation of high-capacity autoclaves and results in increased labor costs because of sterilization procedures and supply costs because of the fastened deterioration of plastic cages and filter tops from repeated high-temperature exposure. We created an equipment transportation system that protected cages and accessories after washing and allowed the transfer of these components from washers to animal rooms without the use of special packaging and steam sterilization. Soiled cages and components were sanitized in a pass-through tunnel washer, which reached a minimum rinse-water temperature of 182°F. The clean wash room was treated as if it were a specific-pathogen free (SPF) rodent room (i.e., personnel were required to wear protective clothing and shoe covers and restrictive procedures for entry to the room were enforced). In this room, sanitized cages and bottles were filled with corncob bedding and filtered tap water, respectively, and stacked on mobile shelving units (MSU). The MSU were nylon-fabric covered and Velcro-sealed stainless-steel wire shelves (53 x 49 x 24 in.) on wheels. Prior to use in the SPF clean wash room, MSU and covers were sanitized in a pass-through rack washer. The MSU were loaded with cages and accessories in accord with requisitions for specific animal rooms. The MSU were then closed and transported to specific SPF animal housing rooms; wheels of the MSU were disinfected with a bleach solution prior to room entry. Once inside a housing room, the MSU was opened briefly to enable the clean cages and accessories to be moved to a laminar flow hood. All animal transfers took place within this HEPA-filtered hood. Soiled cages and accessories were then loaded into the empty MSU for transportation to the soiled wash room for sanitation. These procedures were implemented for > 50 SPF-rodent rooms in 1992. In 1993, there were only 3 viral disease outbreaks in these rooms. Each outbreak was confined to the rodents of a single investigator, and the husbandry procedures were not incriminated. There have not been any viral outbreaks since 1993. This transportation system has enabled our institution to use microisolator cages effectively without requiring use of steam sterilization of the cages and accessories. We calculated that we saved $130,000 to 240,000 for every 10,000 cages purchased by using regular-washed cages instead of autoclavable plastic cages. This savings in replacement supply costs coupled with reduced labor and equipment costs enabled us to lower SPF-rodent per diem rates by 35% in 1995.
P35 Biodigestion—An Alternative to Incineration?

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One issue that an animal facility administrator faces early in their career is to ensure that their facility is properly disposing of animal waste. Geographic locations vary with regard to regulations for incineration of pathologic wastes, without exception, each state and many local municipalities have specific regulations. There are additional federal regulations administered by the Environmental Protection Agency (EPA). A newly developed technologic advancement installed at one of our facilities provided an efficient and effective alternative to the incineration process. The process, reductive cremation, hydrolyzed vertebrate, invertebrate, and microbial tissues in 12 to 14 hours through the application of heat, pressure, and a dilute aqueous alkali solution. The final product was reduced in volume by 97% and was free of infectious agents such as fungi, bacteria, and viruses. The principle of this process is that tissues are essentially water and protein. Using concentrated sodium hydrochloride, peptide bonds are destroyed, resulting in single amino acids. Because the process also destroyed aldehydes on an equal-weight basis, it was useful for reducing formaldehyde-saturated (i.e., formalin-fixed) tissues. Likewise, it could be used to dispose of radioactive biological waste. However, the process did not reduce or destroy non-biological products, such as rubber gloves, bedding, and suture. When compared to incinerators, this equipment required less space, was less expensive, and was monitored under different regulations. It did not release particulates into the atmosphere, and the concentrated basic solution used in the process was easily diluted with standard effluent waste streams that are generated at most research sites.

P36 Design and Application of a Unique System for Weighing and Transferring Unanesthetized Nonhuman Primates

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A unique new system was designed to allow accurate weighing and safe transfer of conscious nonhuman primates between cages. The system comprised two separate chambers (each chamber was 16 7/8 x 11 3/8 x 24 in.) mounted in a vertical orientation (i.e., one-over-one) on a portable frame. The chambers, frame, and components were constructed of 304-grade, glass-bead polished stainless-steel. The top, back, and sides of each chamber were solid. The front of the chamber incorporated a 2-direction sliding door made from horizontally oriented welded 0.25-inch stainless-steel wire on 0.75-inch centers. The solid floor of each chamber was removable and could be easily and quickly replaced with the weighing platform of a portable electronic scale. The weighing/transfer system was designed to be used with a quad (two-over-two) housing rack with 6 ft³ cages equipped with squeeze-back mechanisms. When the system was placed face-to-face with the housing rack, the doors of each chamber were aligned with the cage doors. Metal clips were used to attach the weighing/transfer frame to the cage rack. Adjustable metal guards provided a seal between the chamber and cage doors to prevent animals from escaping. After conscious animals were moved from their cages into the chambers, the frame/chamber assembly was disconnected from the housing rack and moved as needed. When chambers were fitted with electronic platform scales, animals could be weighed and returned to their home cage or transported as needed. Chambers also could be removed from the frame to facilitate transport of one animal. This new system provided an efficient method for weighing and transferring conscious nonhuman primates, minimizing the safety risks associated with handling nonanesthetized animals, and helping to prevent injury and stress to animals.

P37 Efficacy of a Separator-Tube Caging System in a Breeding Colony of Short-Tailed Opossums (Monodelphis Domestica)

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The gray, short-tailed opossum (Monodelphis Domestica) has become a standard laboratory animal since its introduction approximately 15 years ago. Its small size, ability to breed well in captivity, and marsupial characteristics facilitate its use in many diverse applications. Although a viable breeding colony can be maintained by using standard cages for rats, we found the tendency for conflicts between females and males on initial contact decreased the number of successful matings. We designed a separator-tube caging system that facilitated familiarization of the pair before they were allowed to breed. A male and female were placed in separate cages joined by threaded polyvinyl chloride collars and a tube in which a slotted polyvinyl chloride screen was placed. The screen allowed minimum physical and maximum sensory contact. After 1 week in this caging system, a new tube without the separator screen was placed between the cages, allowing the pair to freely associate. Each cage also contained a stainless-steel nesting box filled with shredded paper towels, which provided an added level of security and physical isolation. We found such an arrangement also decreased the amount of fighting between breeding pairs. During the last 3 years, there have been 120 pairings attempted by using the separator-tube caging system. Approximately 77 (64%) have produced neonates. Although caretaking procedures were slightly more complicated with the separator-tube caging system, use of the system resulted in maintaining a healthy stable breeding colony.

P38 Cross Training Animal Caretakers for Success

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Our University Animal Care group devised a training method that ensures all technical personnel are trained in the same manner, using the same standard operating procedures, and that periodic updates are performed for all personnel on each animal species. All incoming employees adhere to an outline with strict guidelines during the first 6 months of employment. Each new employee is accompanied by a trainer during the first 12 weeks of employment until each task is learned. A new task is not attempted until old tasks are assimilated. At the end of the first 3 months of employment, an appraisal is given to the employee by the trainer and by the supervisor. During the next 12 weeks, each technician is evaluated on how well they perform their assigned duties without constant supervision. Before the end of the 6-month period, which coincides with the university's probationary period, each new employee will be notified of their continued employment status. Evaluations are made daily and
filed in each technician's personnel folder. These records indicate those tasks for which the employee has already been trained and also those tasks on which they have not been trained. New employees start work in the cage wash area, move to the weekend shift, and then, if they so choose, work a Monday-through-Friday schedule. Work assignments in each of these areas stay the same until a new employee is hired, another technician wishes to change job assignments, or the management team feels a change is needed to improve the quality of animal care. Having permanent trainers on staff has greatly improved morale, as all employees, old and new, have been trained or retrained to conform to the standard operating procedures. Employees who are on leave know that their animal rooms will be cared for in an appropriate manner and that they will not come back to a disaster. All employees know the performance standards and use peer pressure to enforce these standards.

P39 An Organizational Tool for Managing Information and Reference Materials for Officers of a Branch of the American Association for Laboratory Animal Science (AALAS)

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Each year, new officers and board members are elected to conduct activities of a Branch of the American Association for Laboratory Animal Science (AALAS). In most cases, at least one newly elected candidate is unfamiliar with the responsibilities of their position. To aid in the orientation of new officers and board members, an organizational tool (notebook) was created to provide information on position responsibilities. In addition, the notebook also provided information on previous activities (meeting minutes), by-laws, responsibilities, and a phone roster for that Branch. The notebook could be customized to the needs of committees and other organizations. The first edition of this notebook was created for use by the California Veterinary Medical Association Registered Veterinary Technician Committee, and it was enthusiastically approved and adopted. The notebook has also been adopted by the Palms-to-Pines AALAS Branch. By providing a historical perspective, reference information, and position duties to newly elected officers and board members, these new members can become oriented more quickly and function as active participants more effectively in Branch activities.

P40 Workshop on Surgery of Rodents: Tips, Tricks, and Practices

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The 1996 Guide for the Care and Use of Laboratory Animals indicates that people performing or assisting with surgical procedures often have widely varying backgrounds and experience, which may require specific training programs to address surgical procedures. Responsibility for ensuring personnel are appropriately qualified and trained in the procedures to be performed is assigned to an Institutional Animal Care and Use Committee. At the National Institutes of Health (NIH), 3 Institutes joined in a collaborative effort to provide training in all aspects of surgery of rodents, because those surgeries are difficult to monitor on a daily basis within each Institute. Our objective was to develop a course in surgery of rodents that would teach humane methods of restraint, aseptic surgical preparation and technique (including donning of gloves), choice of suture, and basic incision/closure. We created a 2-part syllabus: didactic and hands-on. Initially, we planned a limited enrollment, but we had to modify our workshop due to demand across NIH. We delivered the seminar (didactic) portion of the workshop approximately 1 month prior to the hands-on segment of the workshop. The seminar was open to anyone who wanted to attend. However, to preserve an effective student/teacher ratio for the hands-on portion, enrollment was limited to those researchers who attended the seminar and who were involved in surgery procedures of rodents. When openings were available, technicians involved in assisting surgical procedures of rodents could attend. We modified our hands-on session considerably from the initial workshop. We found that researchers had limited experience in proper aseptic technique, donning of gloves, maintaining sterile fields, suture selection, and proper suturing technique. To allow additional time for practicing these basic skills, we eliminated the use of live animals and developed a simple "practice mouse" for participants to use when learning how to drape and suture while maintaining a sterile field. The workshop has been so popular that we now offer it every 6 to 8 weeks to all NIH researchers. We are currently discussing criteria necessary for enrollment in a second workshop that would require participants to correctly restrain, anesthetize, and prepare a mouse for surgery.

P41 Task Evaluation and Solution Development for Ergonomic-Related Injuries at a Research Animal Facility

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Ergonomic-related injuries at a research animal facility prompted scrutiny of procedures. Physical therapists were contracted to assess functional facility operations. The assessment allowed broad evaluation of ergonomic issues, but lacked the data to allow us to evaluate specific tasks and to develop a mechanism for solution development. In follow-up to the evaluation, a departmental ergonomic team was formed. The team received training in basic ergonomic principles and prioritizing tasks for solution development. Tasks with the highest potential for ergonomic injury were identified and evaluated according to the frequency and severity of the motions used. A Task Evaluation/Prioritization Form enabled risk identification classified according to section/body part and risk rating as low, moderate, or high. Solution development proved problematic for the team due to time constraints, lack of ergonomic information specific to the field, and difficulty in identifying vendors of specialized equipment. An ergonomic consultant was contracted to assist the team with solution development. Solutions for filling and transporting racks of water bottles and disposing of dirty bedding have been developed. Current projects include developing standardized rodent workstations and improving procedures for manual handling of material. Constant evaluation and improvement of procedures is critical to improving ergonomic-related conditions.
P42 Survey of the Membership of a Representative Branch of the American Association for Laboratory Animal Science (AALAS)

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Membership in a Branch of the American Association for Laboratory Animal Science (AALAS) is essential for the continued growth of the national organization. It is also an important source of laboratory-animal related information for technicians, veterinarians, and allied tradespeople who attend. The amount of participation of Branch members depends on each individual's commitment to their local meetings. It also has the potential to impact on each member's contribution to animal well being at the personal, job-related, and national level. The Upstate New York Branch of AALAS used a survey to obtain opinions and comments from each member. It was divided into 4 areas. Questions on the frequency of general meetings and their content was designed to gain insight into what a member would consider a worthwhile meeting. Input regarding site selection for the annual meeting and meeting content could greatly enhance efforts of those coordinating a potentially sizeable event. Employer involvement and dedication to AALAS can have an impact on an employee's desire to belong to AALAS at the local (Branch) or national level. The personal survey provided a sketch of who attended meetings and their level of involvement. Those who seek change should have contributed some personal experiences with their Branch. However, every member was a contributor with valued input. The reasons that attendance increased or decreased was not always evident. Attendance records alone did not provide evidence of the degree of satisfaction members were receiving from attending meetings. The level of active or passive participation of the membership may determine the growth potential of each Branch. Each Branch member has some perception of what and who makes up their particular Branch. Results of the survey of one representative Branch of AALAS could shed some light on those perceptions.

P44 Environmental Conditions in a Chamber When Using Dry Ice for Euthanasia

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Dry ice is used in research laboratories as a source of CO₂ for euthanasia of rodents. This study was performed to evaluate environmental conditions created in a standard (9-L) bell jar. Chamber filling rate at room temperature with a known quantity of dry ice cut into cubes (3 x 5 x 5 cm), maintenance of a 70% level of CO₂ with that amount of dry ice, and concentration of CO₂ maintained in the chamber when the top was removed and replaced was measured at a point 7.5 cm above the platform, using a portable gas analyzer. Water was not added to the dry ice to assist sublimation. Ambient temperature in the chamber and temperature of the platform were measured, using a digital resistance temperature detector panel and a D-TG thermocouple module. Results indicated that 500 g of dry ice was required to maintain the filling rate and CO₂ concentration at levels recommended by the American Veterinary Medical Association Panel on Euthanasia; that after removal of an animal, the lid should be replaced for 1 minute before subsequently placing another animal in the chamber; and that ambient temperature in the chamber never was <14°C during an 8-hour period, but that the platform temperature decreased to 0°C within 80 minutes. On the basis of these studies, it was found that the use of ≥ 500 g of dry ice to produce CO₂ could meet recommendations for filling rate and concentration, that time must be allowed between placement of subsequent animals in the chamber for the CO₂ concentration to return to 70%, and that platform temperatures can reach a level considered painful after 80 minutes of use. Consideration should be given to changing the platform at an interval of < 80 minutes.

P43 A Systematic Approach to Implementation of an Enrichment Program for Primates

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Despite much debate regarding interpretations of enrichment and physiologic well being, the reality remains that institutions must abide by mandates of the 1985 amendments to the Animal Welfare Act. The behavioral needs of various nonhuman primate groups can be complex. Design of a psychologic enrichment protocol to meet these needs can be an exercise in futility unless the designers have a clear concept of the available resources, such as personnel, time, and budget capabilities. Organization is essential to the design and execution of an enrichment program that provides variety and novelty as well as a practical structure that can be applied easily. We developed an adaptable model that other facilities could use to accommodate their needs for enrichment programs. The enhancement protocol was twofold, including guidelines for general enrichment as well as a set of procedures to address the needs of clinically stressed primates. Our interpretation of a clinically stressed primate was based on identification of clinical signs of psychologic distress, such as hair pulling, stereotypic movements, self-mutilation, psychosomatic indications, and atypical species behaviors. Our model included a wide variety of enrichment options, such as vertically oriented cages, various food treats, unique combinations of toys, videotapes and auditory stimuli, and specialized enrichment techniques. This allowed for more complexity and flexibility in the program while not substantially affecting labor requirements. During a year, 55 primates with various clinical indications of stress have been rehabilitated by means of the augmented program. Within the first 2 months, 78% recovered and were removed from the program. Within 3 months, an additional 9% were removed due to substantial improvements. Only 13% of the clinically stressed primates have remained on the program for more than 4 months due to marginal evidence of recovery. We believe our model fulfilled the goal of complying effectively with the regulations by using an efficient, cost-effective enrichment paradigm that provides a better quality of life for laboratory-maintained nonhuman primates.

P45 Modified Restraint Chairs for Nonhuman Primates

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Some studies require restraint of nonhuman primates for extended periods. To improve the quality of animal comfort during these studies, our staff designed a jacket and chair for use in the restraint of primates. A major design goal included the ability of
the chair to fit a wide range of animals, including large male baboons and small macaques. To meet this goal, all vital parts of the chair were designed with adjustability in mind. The chair was composed of a metal frame consisting of a number of adjustable plates and dividers. Chair dividers separated an animal's forelimbs and hind limbs and also separated the hind limbs from each other. Jackets used in conjunction with the chair provided the means to separate an animal's forelimbs from each other. In addition, the skirt on the jacket aided in separating an animal's forelimbs from its hind limbs. By using the chair and jacket dividers, an animal could be restrained without tying its limbs. Animals restrained by using the chair and jacket have a reduced incidence of stress, bruising, and trauma.

P46 The Baboon Suite: Novel Method to Increase the Size of a Baboon Cage to Meet Requirements for the Care and Use of Laboratory Animals

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The 1985 revisions to the Guide for the Care and Use of Laboratory Animals divided nonhuman primates into 6 groups on the basis of approximate size (body weight) of the species commonly used in biomedical research. Baboons and nonbrachiating species that weigh > 15 kg were assigned to Group 5 with a recommended minimum cage floor space of 8.0 ft². On the basis of this recommendation, we purchased commercially manufactured cages that encompassed approximately 10 ft² of space to accommodate all our baboons. In the 1996 revision, the number of primate groups was expanded and categorized on the basis of body weight rather than species type. As a result, adult male baboons now would be included in Groups 5, 6, and 7, requiring a minimum of 8.0, 10.0, and 15.0 ft² of space, respectively. Approximately half of 60 animals at our institution were in Group 7. A 15.0 ft² cage could be purchased for approximately $7,800/cage, resulting in a total expenditure for our 60 animals of approximately $250,000. These cages would be difficult to move to the cage washing facilities because of their size and weight, and they would not fit through standard 42-inch wide door frames. For these reasons, we designed a cage addition (baboon suite) that provided the additional square footage needed to meet requirements, was appreciably cheaper than the cost of a new larger cage, could be attached to the front of the existing cages with minimal cage adaptation, used the existing cage-squeeze mechanism, permitted relatively easy removal to allow sanitation of the original cage and the addition, and increased the complexity of the cage environment for the benefit of animals. A prototype cage addition was constructed and tested. Baboons fully used the additional space and seemed to spend more time in the addition than in the cage. Preliminary evaluation revealed minor changes that should be made to allow easier mounting, removal, and transport to the cage washer. Additional items, such as a resting perch and an environmental enrichment panel, will increase the utility of the addition. Initial cost estimate for the baboon suite was approximately $2,500, a considerable savings over the purchase price of a new cage.

P47 A Way Into the Classroom

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An important element of improving public awareness about the use of animals in medical research is informing young people by discussing with students the issues involved. Under the guidance of the Animals in Medicine Research Information Center (AMRIC) and the Biomedical Research Education Trust (BRET), which also provides speakers' training, and in keeping with staff wishes we developed a program for staff volunteers to visit schools to talk about these issues. These speakers were consistently rewarded by positive feedback from their audiences who, when given the opportunity, were usually open to considering mainstream scientific and medical opinions realistically. Our earliest approaches to schools resulted in few invitations to visit. During a 4-year period, we refined our approach, taking into account the views of teachers and organizers of similar programs. For example, by adjusting the time of year when we first make contact, the content of the introductory letter, and by providing a simple means for teachers to respond we have increased the number of invitations fourfold. These organizational aspects have also helped us to plan our speakers' involvement more effectively.

P48 A Tissue Harvesting Program as a Method for Implementing the Three Rs of Biomedical Research

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A tissue harvesting program is a vehicle through which investigators share animal tissues whenever possible, providing a valuable method by which to reduce the number of animals used in biomedical research. Motivation for such a program includes conservation of animal resources, reduction in bureaucratic burden, and fulfillment of the Three Rs. Provision and purchase of tissues through the tissue harvesting program is described in an Institutional Animal Care and Use Committee protocol that includes all databases, methods of euthanasia, and specific procedures for collection; only tissues harvested from animals after death or anesthetized animals undergoing nonsurvival surgical procedures are approved. The tissue harvesting program was computerized and maintained in the surgical resource facility. Information on investigators' protocols and type of species used was maintained so that necropsies and animal donations could be scheduled when needed. A list of vendors from which tissues could be purchased also was available, along with a brochure that included the standard operating procedure and all necessary forms. Investigators accessed these databases via electronic mail when needed. Participation in the program began when an investigator completed a background information form, which outlined tissue needs or donation capabilities. A surgery technician added the investigator to the database, matched tissue donations with tissue recipients, and expedited the harvesting process. The tissue harvesting program facilitated research, provided a useful service to investigators, and promoted prudent use of animal resources in what has become a more streamlined research process. This program represented the implementation of the Three Rs as well as a proposed fourth R: responsibility for humane care and commitment to the research animals under our care. Investigators were open to various research
alternatives, they simply needed the facility and a coordinated effort to ensure participation. As a result, interest in the tissue harvesting program has been high, and the program has been well-received.

P49 An Economical Enrichment Device for Nonhuman Primates

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Environmental enrichment to promote psychologic well-being of nonhuman primates is an essential component of modern animal care and use programs. To provide a form of environmental enrichment that would stimulate foraging behavior, but require a minimum of personnel time, we created an enrichment device from discarded 30-gallon plastic barrels. Barrels could be attached to the cage door, allowing a nonhuman primate to move freely between the enrichment barrel and its home cage. One to 2 lb of bedding was mixed with an animal’s daily ration and particulate forage and was added to the barrel. Nonhuman primates foraged in the enrichment barrel at all times of the day. Other benefits of the barrels included additional temporary cage space (1.77 m²), increased cage complexity, increased opportunities for nonhuman primates to observe nonhuman primates in the room, and increased interaction with animal technicians. Enrichment barrels have been used in our facility for 2 years and have been found to be durable, sanitizable in a standard rack washer, and inexpensive to make. Cost of materials is < $15/barrel.

P50 A Newsletter as a Training Tool for an Animal Care and Use Program

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Establishing effective communication as part of an institutional training program persons involved with animal care and use can be a difficult task when the research community is large, diverse, and geographically dispersed. Properly conceived and formatted, an institutional animal care and use newsletter can accomplish the following objectives: distribute information in a timely and efficient manner to a large number of animal care and use personnel; increase awareness of, and foster interest in, the many issues to be considered in the use of animals in research; and promote collaboration and cooperation among members of the institutional animal research community, including animal care, veterinary, research, safety, and regulatory personnel. We determined that a newsletter published quarterly could accomplish these objectives at our institution if it were useful and appealing to a wide group of readers. The newsletter was divided into the following 7 sections, each consisting of 1 to 4 regularly featured columns: cover story; the politics of animal research (existing and proposed animal research legislation, regulations, and policies, and information on the animal welfare/rights movements); strategies for coping with regulatory requirements (tips on completing an Institutional Animal Care and Use Committee (IACUC) application and advice on IACUC inspections); animals and research (interesting animal models, advice on anesthesia and analgesia, and complicating effects of animal husbandry practices and naturally developing animal diseases on research); people and animal research (health and safety issues, training information, and personnel highlights); sources of information on animal research from outside the institution (reviews of organizations, meetings, and publications); and a bulletin board (announcements and short communications from centralized service units, such as the husbandry staff, veterinary staff, diagnostic laboratory, technical service cores, and business office). Articles were kept brief to promote readability and maximize the number and variety of topics in each issue. Contributing editors for each column were recruited from the animal care and use community, particularly the centralized service units. In addition to the use of graphics and interesting column titles, a commercially available desktop-publishing software package was used to create an interesting and appealing format. The mailing list was developed from names garnered from IACUC applications and training session records and supplemented from mail-in subscription coupons placed in each issue. After 2 years, circulation of the newsletter increased from 1,000 to 1,500 readers. Comments from research and animal care personnel have been overwhelmingly positive. Requests for information as a result of articles published in the newsletter have been received regularly. The institutional animal care and use community is better informed about animal research issues. Our experience indicated that a well-conceived and formatted newsletter can be a valuable addition to an institutional training program for animal care and use personnel. It served to inform, educate, and engage the research community regarding legal, technical, educational, and administrative aspects of the use of animals in research.

P51 An Attempt to Design the Ever-Illusive, All-Encompassing Study Design Protocol for Animal Use

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Researchers at pharmaceutical companies and other institutions must submit protocols on animal studies to the Institutional Animal Care and Use Committee (IACUC) for review. In addition, studies are peer-reviewed by a committee for scientific merit and design. Ideally, it would be beneficial to design one animal study protocol form that addresses all issues concisely and conveniently for review by all concerned committees. We designed a protocol form that addressed the necessary issues and has been instrumental in promoting studies in an expedient and efficient manner. Within the form are areas for text detailing study rationale and purpose, preparation of materials that will be used in the study, administration of materials, study procedures, of necropsy and sample collection and analysis, and effects and devices of data analysis. Tables provided within the form prompt researchers to provide detailed information on personnel performing study procedures, information on test animals, animal identification, published reports, infectious agents, treatment design, and a schedule of events. Alternative models, nonduplication of experiments, a statistically valid number of animals per study group, and supportive literature citations are also addressed. Although the form could not initially conform to every type of study, it was in a format that allowed flexibility and could be customized by investigators. Additionally, the form was constructed as a word-processing program template, suitable for inclusion within electronic reports while conforming to an institution’s style requirements for reports. Information contained in the form consisted of personnel assignments, training verification, and study preparation (i.e., ordering materials, labeling vials, and quality assurance auditing). Through the development of this study design protocol for the use of animals, our institution has streamlined the review and reporting process.
P52 Benefits of Providing Supportive Care to Tumor-Burdened Mice Used for Chemotherapeutic Screening

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Mice were transplanted with murine models of neoplasia that use allografts or xenografts typically are used to screen candidate chemotherapeutic agents for efficacy. The combination of tumor growth and chemotherapeutic-associated side-effects coupled with the requirement to measure median survival time often produces important detrimental effects in treated mice. Therefore, we examined the benefits of providing tumor-burdened mice with environmental and nutritional support while they underwent chemotherapy. Four groups of CD45 female mice (n = 40/group) were implanted intradermally with P388 murine lymphocytic leukemia cells. Mice in 2 groups were administered i.v. a single dose of liposomal encapsulated daunorubicin (LED) at the maximum tolerated dose, whereas mice in the 2 remaining control groups were treated intravenously with an 8.75% sucrose buffer solution. One group from each study was provided supportive care consisting of nesting material (environmental enrichment) and ration gruel (nutritional support) furnished in disposable petri plates placed on the cage bottom. Study duration was 46 days. Clinical condition of each mouse was evaluated by determining severity of 13 clinical signs, tumor volume, and body weight twice weekly for the first 2 weeks of the study and once weekly for the remainder of the study. Each clinical sign (lethargy, dehydration, dyspnea, tachypnea, pallor, distended abdomen, peripheral edema, muscle wasting, rough coat, hunched posture, impaired ambulation, ocular/nasal discharge, and barbering) was scored, and scores for each sign were summed to compute a total condition score. All data were represented as days prior to death (DPD) for group comparisons. The LED treatment resulted in a significant increase in median survival time and a significant decrease in tumor volume. Clinical signs were evident in all mice 10 to 1 DPD; however, they were 3 times more severe in LED-treated mice that did not receive supportive care. Clinical sign scores and total condition scores increased 10-fold in severity for all groups 3 to 5 DPD. By 2 DPD, significant differences were not detectable between groups. Supportive care did not have an effect on total condition score, body weight, tumor volume, or median survival time for control groups. In LED-treated mice, however, supportive care resulted in a significant reduction in the severity of lethargy, dehydration, rough coat, pallor, and barbering, and limited LED-induced loss of body weight by 50%. Supportive care did not alter LED-mediated reduction of tumor volume or prolongation of median survival time. Examination of total condition scores indicated that moribund mice could be identified within 2 days of death and euthanized to prevent unnecessary pain and suffering.

P54 Cellular Control of HgCl2-Induced Anti-Nucleolar Antibody Response in Mice

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Mice of the H-2b haplotype strains develop high anti-nucleolar antibody titers after receiving doses of HgCl2. The dominant autoantigen is fibrillarin, a specificity also seen in human scleroderma. This response pattern has been linked to the IA region of the major histocompatibility complex and has been reported to be co-dominant, suggesting that the effect is due to the IA molecule itself. However, in F1, intercrosses between the congenic strains B6.SJL/1Cy (B6.SJL; IgH, H-2b) and C57BL/6-J IgH; Thy1Gpi1* (designated B6.TC; IgH, H-2b), we found only 1 of 2 mice with anti-nucleolar antibodies after challenge with HgCl2, implying that regulation of this autoimmune response is complex. Therefore, we lethally irradiated F1 (B6.SJL x B6.TC) mice and reconstituted them with an equal mixture of bone marrow obtained from mice of the 2 parent strains. All mice had balanced reconstitution as evidenced by allotype-specific assays. Other F1 mice received only F1, B6.SJL, or B6.TC bone marrow. Five weeks after reconstitution, mice were challenged (1.5 mg of HgCl2/kg of body weight, s.c., 3 times/wk). By 5 weeks, 7 of 11 mice given a combination of bone marrow had a positive anti-nucleolar response. By allotype-specific testing, this was of the b allototype only and, therefore, derived from the susceptible B6.SJL donor. All mice given only B6.SJL or B6.TC bone marrow were positive and negative, respectively, whereas mice given F1 marrow were largely resistant. Thus, co-infusion of sensitive and resistant bone marrow did not cause cross-regulation of the anti-nucleolar response, nor could this response be prevented by nonhematopoietic cells of resistant host origin.
P55 Class-II Haplotypes Differentially Regulate Immune Response in HgCl2-Treated Mice

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One of the most striking features of exposure to low doses of mercury in mice is the high-titer haplotype-linked anti-nucleolar autoantibody response. The dominant autoantigen is fibrillarin, a specificity also seen in human scleroderma. On all backgrounds tested, mice of H-2b haplotype have been responders, whereas H-2a mice have been low responders. This pattern has been attributed to the class-II molecule itself, but the poor response of F1 mice that resulted from matings between high responders raised the possibility that the anti-fibrillarin specificity was actually due to a closely linked dominant negative gene. F1 crosses between congenic B6.SJL (H-2b) and C57BL/6 (H-2a) mice with a targeted deletion of IAα created F1 mice heterozygous for all major homocompatibility complex loci, but expressing only IAα. Compared with B6.SJL mice, we did not find a diminution of anti-nucleolar antibody titers, proving that IAα itself was responsible for susceptibility and I-Aα for downregulation. Unlike I-Aα, expression of the I-E class-II molecule could not downregulate the response in mice that were otherwise susceptible. These results suggested that a complicated role for class-II molecules in the regulation of a novel, environmentally-induced autoimmune response.

P56 Early Changes in Oxygen Consumption in Endotoxemic Rabbits

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Sepsis-related decreases in oxygen consumption are believed to be due to microcirculatory defects resulting in ineffective delivery or to peripheral defects in utilization. We investigated the effect of endotoxin administration on oxygen delivery and utilization and 4-hour survival rates. Fourteen anesthetized rabbits were instrumented. Baseline values were obtained, and rabbits then were given endotoxin or saline solution. Baseline and 60-minute variables included Fick-derived oxygen consumption, delivery and extraction ratios, cardiac output, and mixed-venous oxygen saturation. Baseline values were the same for all rabbits. Control rabbits and endotoxin-treated survivors had values that were not significantly different for any variable, although survivors had increased oxygen consumption. Non-survivors had significantly (P < 0.01) lower values for all parameters, except for oxygen extraction ratio, which was significantly higher. Cardiac output and oxygen delivery decreased proportionately and similarly in non-survivors to 50% of the values for control rabbits. Decreases in cardiac output and oxygen delivery were detected only in non-survivors, and were partially compensated by increases in oxygen extraction. Survivors had a tendency to increase oxygen consumption without increasing cardiac output or oxygen delivery. Peripheral defects in oxygen utilization were not detected in any group. Early decreases in oxygen consumption in non-survivors appeared to be secondary to myocardial depression after endotoxemia and may have been an early indicator of a poor prognosis.

P57 Endotoxemia-induced Changes in Pulmonary Function in Anesthetized Dogs

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Pulmonary dysfunction occurs with scpsis, inhalation anesthe- sia, mechanical ventilation, thoracotomy and single lung ventilation. We investigated changes in pulmonary function in isoflurane-anesthetized mechanically ventilated dogs. Eleven dogs with surgically opened chests were intubated and instrumented for hemodynamic and pulmonary function measurements. After baseline measurements were obtained, endotoxin was infused, and subsequent measurements were made hourly. Pulmonary shunt fraction increased from 42% at baseline to 85% at 4 hours. Dead space-tidal volume ratio (Vd/ Vt) increased from 43% to 60%, without a change in tidal volume throughout the experiment. Arteriovenous O2 difference decreased to 47% by 1 hour and to 25% by 4 hours. Changes in shunt fraction, Vd/Vt, arteriovenous O2 difference, and arterial oxygen content were greatest during the first hour (74, 50, 72, and 74% of total change, respectively). Mean arterial pressure also decreased significantly during the first hour; systemic vascular resistance and cardiac output decreased during the second hour. The Vd/Vt and shunt fraction changes could not be accounted for by effects of mechanical ventilation, anesthesia, tidal volume, thoracotomy or cardiac output. These changes appeared to be primarily attributable to a ventilation-perfusion mismatch secondary to increased pulmonary dead space. Concurrent changes in oxygenation and pulmonary dynamics suggested the changes in pulmonary function after endotoxemia may be secondary to endotoxin-induced changes in pulmonary blood flow distribution.

P58 Persistent Increase of Pro-inflammatory Cytokine Concentrations in Mice With Subclinical Clostridium piliforme Infection (Tyzer's Disease)

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Results of serologic analyses suggest subclinical Clostridium piliforme infections (Tyzer's disease) are widespread in laboratory mice and rats. However, the effects of subclinical Tyzer's disease on mice and rats have not been characterized. Previously, we documented induction of hepatic cytokines in rodents with Tyzer's disease. However, systemic cytokine concentrations and the duration of increased cytokine expression remain unknown. Therefore, we inoculated groups of 10 susceptible (DBA/2) or resistant (C57BL/6) mice with a toxigenic or nontoxigenic isolate of C. piliforme and evaluated mice at 1, 3, 7, 14, and 28 after inoculation. Infection status was evaluated by histologic and polymerase chain reaction (PCR) analyses; expression of tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) mRNA and protein were assessed by reverse transcription PCR and enzyme-linked immunosorbent assays, respectively. Mice remained clinically normal throughout the study. Histologically, nontoxigenic bacteria were not evident in liver, nor were lesions detected. Toxigenic bacteria and bacterial-induced lesions were detected in the liver until day 14 after inoculation. When compared with sham-inoculated controls, IFN-γ concentrations were increased in mice infected with nontoxigenic and toxigenic
isolates until 14 days after inoculation; TNF-α concentrations were increased throughout the 28-day study. These data indicated that concentrations of proinflammatory cytokines were increased in *C. piliforme*-infected mice even when bacteria or lesions were undetectable. We suggest that research results obtained with *C. piliforme*-infected mice may be invalid.

**P59 Effect of Counter-Regulatory Stress Hormones on Development of Hyperglycemia and Insulin Resistance in Endotoxin Rabbits**

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Controversy exists over the correlation between release of stress hormones and the development of insulin resistance and hyperglycemia in the acute phase of sepsis. We investigated this correlation, using a rabbit model of the acute response to endotoxin challenge. Twelve anesthetized New Zealand White rabbits received endotoxin or saline solution. Glucose, insulin, cortisol, glucagon, norepinephrine, and epinephrine concentrations were measured at 0, 60, 120, and 180 minutes. Endotoxin was given immediately after 0 minutes, and insulin infusion was begun after 60 minutes. Glucose was not administered. Glucose concentration increased by 60 minutes and continued to increase despite a five-fold increase in insulin concentration by 120 minutes. Control rabbits had stable glucose concentrations and normal insulin responses throughout. By 180 minutes, epinephrine concentration was significantly increased in endotoxin-treated rabbits (4x baseline values) and decreased in control rabbits (0.25x baseline values). Norepinephrine concentration increased similarly in both groups, but was significantly higher in endotoxin-treated rabbits by 180 minutes. Hyperglycemia developed by 60 minutes in endotoxin-treated rabbits. Despite a five-fold increase in insulin concentration by 120 minutes, glucose concentration increased throughout the experiment, suggesting hyperglycemia may be secondary to insulin resistance. Stress hormones did not cause significant changes until after hyperglycemia and insulin resistance had developed. Clearly, release of stress hormones was not an initiating development of insulin resistance and hyperglycemia in the acute response to endotoxin.

**P60 Polyarteritis Syndrome in Anti-Myelin Basic Protein T-Cell Receptor Transgenic Mice**

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Lesions strongly resembling those of polyarteritis nodosa in human beings were found in young, immunocompetent, B6.B10.H-2b mice transgenic for an anti-myelin basic protein T-cell receptor used as a model of experimental allergic encephalomyelitis. Mice had lost weight, were ataxic, and had progressive paralysis. The primary histopathologic lesion was an arteritis of small-to-medium sized arteries with intimal collagen deposition, sclerosis, fibrinoid degeneration, and perivascular and intramural inflammation. Anatomically, lesions were detected most commonly in the coronary arteries, cerebral and thoracic vertebral arteries, and basilar artery. Coronary arteritis was not associated with apparent myocardial injury. Vertebral arteritis was associated with infarction and sclerosis of vertebral bodies. Basilar arteritis was associated with multifocal malacia and a moderate glial response in 1 mouse. Polyarteritis nodosa is classified as one of the systemic necrotizing vasculitides presumed to have an immunologic basis. Because these myelin basic protein transgenic mice were constructed as an allergic encephalomyelitis model of multiple sclerosis, the polyarteritis lesions may have been a specific manifestation of an induced systemic autoimmune reaction.

**P60.5 Pulmonary Inflammation in Mice Induced by Intranasal Instillation of Respiratory Syncytial Virus in Conjunction With Aerosolized Allergen**

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The lungs are the target organ in many infectious and allergic diseases. Therefore, there is a need to develop animal models of respiratory tract inflammation. Intranasal instillation and aerosolization are noninvasive techniques for administration of various therapeutic and infectious agents. Intranasal instillation of respiratory syncytial virus (RSV) was performed on mice anesthetized with isoflurane delivered via a precision vaporizer in an induction chamber. Anesthetized mice were removed from the chamber and held upright in a Biosafety Level 2 hood. The RSV (100 μl at 10^6 PFU) was administered into their nasal passages, using a micropipette instrument. Virus was inhaled during unassisted respiration. Immediately after instillation, mice were returned to their cages, and they recovered from anesthesia within 30 to 60 seconds. Starting 24 hours after RSV instillation, mice were exposed to aerosolized allergen. Aerosolization of dust mite allergen was achieved by using the Pari IS 2 Nebulizer. Unanesthetized mice were placed in a plexiglass pie chamber and exposed to the aerosolized allergen during one 20-minute session each day for 7 consecutive days. Using these inhalation techniques, we developed a respiratory tract disease model that caused substantial and reproducible inflammation in the lungs. Three groups of mice were studied: RSV instillation only, dust mite aerosolization only, and a combination of RSV instillation followed by dust mite aerosolization. Respiratory tract inflammation was defined by increased cellular infiltrate in bronchial alveolar lavage (BAL) fluid and evidence of pulmonary lesions. Mice exposed only to dust mite aerosolization consistently did not have a cellular infiltrate in BAL fluid and did not have pulmonary lesions. Mice exposed to only RSV did not have a cellular infiltrate in BAL fluid and had minimal lesions in the lungs. The combination group had a major increase in eosinophil and lymphocyte infiltrate in BAL fluid. The lungs had consistent and severe lesions in the vasculature and airways, including the alveoli. We established that RSV instillation followed by dust mite allergen exposure produced an effective and reproducible model of respiratory inflammation.

**P61 Use of Computed Tomography Lymphography Agent to Detect Metastatic Melanoma Disease in Sinclair Miniature Swine**

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Malignant melanoma is a heritable trait in Sinclair miniature swine and is an animal model that resembles familial melanomas in human beings. Cutaneous lesions develop at or shortly after birth in about half of the pigs, and these tumors readily metastasize to the lymph nodes, lungs, and liver. We character-
ized normal lymph nodes and nodes infiltrated with metastatic melanomas, with and without s.c. administration of iodinated nanoparticles, using indirect computed tomography (CT) lymphography. A 15% wt/vol iodinated nanoparticle suspension (1 to 4 ml) was injected s.c. into the distal part of the limbs of healthy Sinclair miniature swine (n = 4); 2 ml of contrast agent was injected s.c. in a ring pattern around each lesion in aged-matched swine that had cutaneous melanomas (n = 6). We obtained CT images through opacified regional lymph nodes prior to and 24 hours after injection. Cancerous lymph nodes with macro metastases were generally larger than opacified normal contralateral lymph nodes. Typical architectural changes in cancerous lymph nodes included incomplete opacification associated with small-to-large filling defects, disruption and irregularity of the opacified medullary zone, and irregular foci of opacification within the cortex. Altered lymph node architecture was evident in cancerous lymph nodes on indirect CT lymphographic studies after injection of iodinated nanoparticles. Correlation between indirect CT diagnosis and subsequent direct histologic examination was excellent. Results of these studies indicated that CT lymphography after s.c. administration of a nanoparticle contrast agent can potentially improve detection of tumor metastasis to regional lymph nodes, compared with non-contrast CT examinations, and that Sinclair miniature swine are a useful animal model for evaluating new methods of detection of metastatic disease.

P62 Identification of a 156-kDa Androgen-Regulated Heme Protein in the Harderian Gland of Golden Syrian Hamsters

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Golden Syrian Hamsters have accessory lacrimal glands (Harderian glands) located deep within the orbital cavity. Among rodents, this gland is large, often bilobed, and compound tubuloalveolar. Its functions remain unclear, although it is usually regarded as a source of lubrication for the nictitating membrane. It also has been proposed as a link in a retinal-pineal-gonadal system and as a potential source of pheromones. Harderian glands of Golden Syrian hamsters are characterized by a marked sexual dimorphism in morphologic features and secretory activity. Glands of males are heavier than those of females and possess 2 epithelial cell types (type I and type II). In contrast, the glands of females contain only type-I cells, store large amounts of porphyrins, and have a dark color, whereas glands of males are pale. Prepubertal and adult hamsters of both sexes were housed under a lighting schedule of 14 h light:10 h dark and given access to food and water. When required, they were bilaterally gonadectomized, using ether-induced anesthesia. There were 4 experimental groups: adult males euthanatized 7, 21, 28, 35, and 39 days after castration; adult males castrated 15 days previously and treated with testosterone, dihydrotestosterone, 3-trans-androstanediol, 3-trans-androstanediol, or estradiol-17β; adult females ovariecctomized 24 hours previously and treated with the same compounds as used in the males; and sexually intact untreated male and female hamsters. In all cases, androgens were administered 0.5 mg/d for 15 days, and estradiol-17β was administered at 10 μg/d for 15 days. Steroids were dissolved in corn oil and a 5% ethanol solution (vehicle) and were injected s.c. At the end of the experiments, hamsters were decapitated (body weight was < 250 g), and Harderian glands were dissected and used immediately. An abundant cytosolic protein was partially purified by ultracentrifugation and was analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This 156-kDa protein was detected in glands from males, but not in glands from females. Adult males castrated for differing lengths of time had a gradual disappearance of the protein throughout 5 weeks. Daily administration of testosterone, dihydrotestosterone, or 3-trans- or 3- cis-androstanediol to castrated males maintained the amount of protein. In females, the protein was induced after administration of these same compounds, but not after administration of estradiol-17β. These observations indicated androgen-mediated hormonal regulation. The results from this study revealed that Harderian glands of male hamsters produced a major heme protein whose expression was androgen-dependent. It is a cytosolic protein with an estimated sedimentation coefficient of 11s, as determined by ultracentrifugation analysis on sucrose density gradients. Remarkably, androgenic compounds, but not estradiol-17β, induced the expression of the 156-kDa protein in ovariecctomized females. The existence of an androgen-regulated heme protein in Harderian glands of hamsters may aid in the understanding of mechanisms whereby androgens modulate porphyrin biosynthesis in this organ. It also may be an excellent biomodel for pharmacologic studies involving the use of steroid compounds.

P63 Mutation in Thyroid Hormone Receptor β Causes Heart and Eye Defects in Transgenic Mice

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A transgenic mouse model was created to study the developmental role of a mutated thyroid hormone receptor gene. The mutated gene was isolated from a patient and was cloned with a β-actin promoter gene for overexpression. Standard pronuclear microinjection was used to produce the transgenic mice with FVB/N and FVBXCD1 background. Four mice were produced, and their offspring were used for developmental studies. All transgenic mice were observed to be small, reminiscent of cretinism, and some lagged behind in growth and the opening of their eyes. A few transgenic mice had narrow-slit eyes with small eyeballs. One became blind at 6 months of age. Transgenic mice had shorter lifespans than control mice; most died at 11 to 12 months of age. A large heart with an extremely large left atrium was associated with most deaths. On the basis of these observations, we believed it was likely that the mutant receptor interfered with normal development, retarding growth and mimicking hypothyroidism to produce smaller stature. In addition, because the nuclear receptors for thyroid hormone and retinoic acid are homologous, and knock-out of retinoic acid receptors in mice produced eye and heart defects, we are investigating the role of the mutant receptor in brain, eye, and heart development and function and the biochemical correlation/interaction of thyroid hormone with vitamin A.
P64 Stability of Analgesic Phenotypes in Recombinant Inbred Mice During Multiple Generations in an In-House Breeding Colony

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A comparison of second- versus ninth-generation CXBK/By mice was performed to detect generational differences in nociception and analgesic sensitivity to morphine. Male mice, 90 to 120 days old that represented 2 separate groups of mice from an in-house breeding colony were tested for nociception and for morphine-induced analgesia using a hot plate test. One group was the second generation from mice obtained as breeding pairs from Jackson Laboratory. The other group was the ninth generation from original breeding pairs obtained from Jackson Laboratory. Five or 6 mice were injected s.c. with saline solution or with varying doses of morphine and tested on a hot plate, using a cumulative dosing protocol. Results revealed that differences did not exist between the groups in baseline nociception [F (3, 27) = 1.395, F = .26] and also no difference in morphine-induced analgesia. The nociception and morphine-sensitivity phenotypes appeared to be stable over 9 generations in an in-house breeding colony, perhaps due to the recombinant inbred background of the mice and adherence to inbreeding protocols by technicians.

P65 Effects of Sodium Hexametaphosphate on Dental Calculus Formation in Cynomolgus Macaques (Macaca fascicularis)

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Plaque accumulation, calculus formation, and gingivitis are important oral health problems in nonhuman primates in research facilities. Macaques at the Washington Regional Primate Center begin to have evidence of calculus formation and gingivitis by 5 years of age. Incidence of dental disease and extent and severity of lesions increase as the macaques age. Sodium hexametaphosphate (SHM), a chelating agent, is a practical method of preventing dental calculus in dogs when applied as a surface coating to dry dog food. Two groups of cynomolgus macaques (Macaca fascicularis) were included in a dental diet study for 6 months. The test group (n = 13) was fed chow with SHM applied to the outside of the biscuits, and the control group (n = 12) was fed the same diet, but without the SHM. Macaques had their teeth scaled and polished 2 weeks before the study began, then were evaluated on a monthly basis to determine periodontal probe depths, plaque accumulation, and evidence of calculus on central incisors. Macaques fed the test diet had an improvement in gingival health and a statistically significant decrease in plaque accumulation.

P66 Exertional Rhabdomyolysis in a Rhesus Monkey (Macaca mulatta)

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A 7-year-old sexually intact male rhesus monkey (Macaca mulatta) was examined because it was anorectic and reluctant to move. This monkey was not being used in a study and was not receiving medication at the time of clinical evaluation. He had a history of being extremely aggressive and easily excitable. On clinical examination, the monkey was bright, alert, and responsive, but approximately 5 to 10% dehydrated. He was reluctant to move around in the cage and vocalized intensely when restrained. He was sedated by using ketamine and was removed from his cage for physical examination. There was not any evidence of bloating, bruising, erythema, swollen areas, or local temperature variation detected during examination. Blood urea nitrogen (BUN) concentration was initially increased, along with the packed cell volume. These values returned to reference ranges once the monkey's hydration status was improved. Creatine phosphokinase, alanine aminotransferase, and aspartate aminotransferase activities were increased above the upper limits of our tests. Urine was light red and moderately concentrated. Red blood cells were not seen in the urine. Urinary protein concentrations were high and initially determined to be hemoglobin by an in-house ammonia sulfate test. On the basis of the dramatic increase in creatine phosphokinase activity, our differential diagnoses included exertional rhabdomyolysis, trauma, and myositis. Initial treatment included s.c. administration of 0.9% NaCl solution, naproxen sodium, and morphine. The major importance of skeletal muscle damage/necrosis is that myoglobin, which is released from degenerating muscle, causes acute renal failure when combined with other co-factors such as hypovolemia, acidosis, or ischemia. On the basis of our presumptive diagnosis, we doubted the result of the ammonia sulfate test. A more-sensitive test-tube filtration test for myoglobin was used and confirmed our suspicion of myoglobinuria. Water consumption and urine output were monitored to evaluate renal function, and furosemide was administered to induce diuresis. Biopsy specimens were obtained from the left biceps brachii and biceps femoris muscles 2 days after initial examination. Histologic examination of the specimens revealed marked multifocal myonecrosis with little inflammatory reaction. The monkey gradually resumed eating, and mobility increased to normal during the next 2 weeks. Examination of follow-up biopsy specimens of the identical muscles obtained 19 days after the initial biopsies revealed regenerating muscle and increased sarcomeral cells, indicative of normal healing. Two days prior to initial examination, another monkey had escaped from its cage and was loose in the colony room. We hypothesized that the loose monkey acted as a psychologic stress to the affected monkey. Because of the affected monkey's history of aggressiveness, we presumed that this event stimulated violent physical reactions such as jumping and cage shaking, which caused exertional rhabdomyolysis. Exertional rhabdomyolysis appears to be a relatively common sequela to unaccustomed exercise. It has been reported in man, horses, dogs, mice, rats, and wild caught animals. Exertional rhabdomyolysis has not been previously reported in a nonhuman primate.
P67 Ivermectin Toxicosis in Mice of Multiple Transgenic Lines

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Pinworm (Syphacia obvelata) infection was detected on routine quarterly surveillance of a conventionally housed transgenic mouse colony. To eliminate the infection, an aqueous based ivermectin product (Eqvalan) was diluted to 22 μg/ml in tap water and administered via water bottles and by lightly spraying the adult mice. The day after treatment began, approximately a fifth of the treated mice were dead, and another tenth had seizures, weakness, tremors, incoordination, and parasyis. Ivermectin toxicosis was diagnosed on the basis of clinical signs and lack of lesions on necropsy of moribund mice. Affected mice were localized to cages on 2 of 4 racks in the room. The racks housed mice with 4 transgenic constructs from 3 background strains. Mice from a mixed background of SJ/129, CF-1, and FVB/N and mice from an SJ/129 x CF-1 background were affected. All other mice in the room were unaffected and had similar transgenes from an FVB/N background. Differential diagnoses for the toxicosis included an overdose from improper dilution and genetic hypersensitivity of affected mice. Samples of medicated water from the bottles of affected mice and from the sprayer used to treat the mice were sent to a reference laboratory for assay of ivermectin concentration. Both samples contained proper concentrations of ivermectin. Because it is believed that ivermectin degrades quickly after dilution, the reference laboratory prepared serial dilutions of Eqvalan and exposed them to the same time, temperature, and amount of light to which the medicated water samples had been exposed. Substantial degradation of ivermectin content was not evident after mimicking the conditions to which the samples had been exposed. On the basis of these findings, an overdose was ruled out as the cause of the toxicosis. Death of these mice was attributed to a genetic hypersensitivity to ivermectin from the SJ/129 x CF-1 background. This hypersensitivity is being investigated. Our standard treatment protocol for pinworm infections has been modified to include treatment of a small sample group from each transgenic line to detect hypersensitivity mice prior to treatment of an entire colony. Medicated water is left in the bottles for 3 days instead of providing fresh mixed medicated water daily.

P69 Genital Papilloma in a Juvenile Captive Cynomolgus Monkey (Macaca fascicularis)

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A juvenile, male cynomolgus monkey (Macaca fascicularis) was examined because of a thick and ulcerated prepuce and glans penis. Approximately three-fourth of the distal part of the prepuce was involved. Clinical examination, complete blood counts, and serum biochemical analysis as well as biopsy of the affected area were performed. Differential diagnoses at that time included trauma, papilloma, squamous cell neoplasms, and contact dermatitis. Complete blood counts revealed a moderate absolute eosinophilia. Serum biochemical values were considered normal. Examination of the initial biopsy specimen indicated papillomatosis. The condition was managed by using topical antibiotics containing corticosteroids, systemic antibiotics, and opioid analgesics. After the monkey was euthanatized, gross necropsy was performed, and the reproductropic organs and adjacent inguinal lymph nodes were submitted for histologic examination and screening for papilloma virus, using polymerase chain reaction techniques. Gross necropsy revealed a diffusely thickened prepuce with several foci of ulceration. Each inguinal lymph node had a small focus of reeding extending from the surface approximately 2 mm into the cortex. Histologic examination of the penis revealed a severely acanthotic epithelium with pegs protruding into the underlying dermis. In a few areas, there was a marked multifocal chronic dermal inflammation, presumably associated with the foci of ulceration seen grossly. There was marked chronic submucosal ulceration and lymphoid hyperplasia of the inguinal lymph nodes. Numerous eosinophils were evident in the foci of balanitis and in the medulla of the lymph nodes. Polymerase chain reaction conducted by using human papilloma primers under low stringency conditions failed to produce any product. Therefore, the final diagnosis was penile papilloma, balanoposthitis with ulceration, and urethritis. Although histologic evidence was consistent with papillomatosis, papilloma virus was not definitively identified as the etiologic factor.

A 20-year-old macaque (Macaca mulatta) was sedated with ketamine as part of an experimental procedure. While under anesthesia, investigators were unable to record the macaque’s blood pressure, using a pediatric arm cuff (Criticon), or obtain a clear electrocardiogram reading. On examination, the macaque was obese (22 kg) with a heart rate of 220 beats/minute and a gallop (split S2) sound. Pulse deficits were not detected. Thoracic radiography revealed mild cardiomegaly and interstitial lung disease. Electrocardiography and echocardiography were interpreted as atrioventricular dissociation with ventricular tachycardia. Results of serum biochemical analysis and complete blood counts were unremarkable. The macaque was again sedated with ketamine and given a bolus of lidocaine i.v. (1.5 mg/kg of body weight), and he converted to a sinus rhythm with a heart rate of 140 to 160 beats/minute. The macaque was started on mexiletine (5 mg/kg, p.o.) and rechecked 2 weeks later. He again was in ventricular tachycardia. The macaque was again converted to a sinus rhythm by administration of a bolus of lidocaine (1.5 mg/kg) and was placed on fluids containing 25 mg of lidocaine in 500 ml of normal physiologic saline solution, which was administered at a rate of 400 ml/h, i.v.). However, the macaque was not able to maintain a sinus rhythm and converted back to ventricular tachycardia despite the administration of 2 additional boluses of lidocaine (1 mg/kg) and increasing the fluid content to 30 mg of lidocaine in 500 ml of saline solution. After 1.5 hours, fluids were discontinued. Propranolol (5 mg, q 12 h) was added to the regimen, and 1 month later, the macaque was sedated with ketamine and examined. Again, he was in ventricular tachycardia. Mexiletine was gradually discontinued, and propranolol (10 mg/kg) was added to the treatment while maintaining the administration of propranolol. An examination 3 months later revealed a normal sinus rhythm with a heart rate of 145 beats/minute. The macaque is currently being maintained by administration of propranolol and propranolol.

P68 Treatment of Ventricular Tachycardia in an Aged Rhesus Macaque (Macaca mulatta)

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A 20-year-old macaque (Macaca mulatta) was sedated with ketamine as part of an experimental procedure. While under anesthesia, investigators were unable to record the macaque’s blood pressure, using a pediatric arm cuff (Criticon), or obtain a clear electrocardiogram reading. On examination, the macaque was obese (22 kg) with a heart rate of 220 beats/minute and a gallop (split S2) sound. Pulse deficits were not detected. Thoracic radiography revealed mild cardiomegaly and interstitial lung disease. Electrocardiography and echocardiography were interpreted as atrioventricular dissociation with ventricular tachycardia. Results of serum biochemical analysis and complete blood counts were unremarkable. The macaque was again sedated with ketamine and given a bolus of lidocaine i.v. (1.5 mg/kg of body weight), and he converted to a sinus rhythm with a heart rate of 140 to 160 beats/minute. The macaque was started on mexiletine (5 mg/kg, p.o.) and rechecked 2 weeks later. He again was in ventricular tachycardia. The macaque was again converted to a sinus rhythm by administration of a bolus of lidocaine (1.5 mg/kg) and was placed on fluids containing 25 mg of lidocaine in 500 ml of normal physiologic saline solution, which was administered at a rate of 400 ml/h, i.v.). However, the macaque was not able to maintain a sinus rhythm and converted back to ventricular tachycardia despite the administration of 2 additional boluses of lidocaine (1 mg/kg) and increasing the fluid content to 30 mg of lidocaine in 500 ml of saline solution. After 1.5 hours, fluids were discontinued. Propranolol (5 mg, q 12 h) was added to the regimen, and 1 month later, the macaque was sedated with ketamine and examined. Again, he was in ventricular tachycardia. Mexiletine was gradually discontinued, and propranolol (10 mg/kg) was added to the treatment while maintaining the administration of propranolol. An examination 3 months later revealed a normal sinus rhythm with a heart rate of 145 beats/minute. The macaque is currently being maintained by administration of propranolol and propranolol.
agent. Because the monkey was not from a breeding colony and did not have extensive contact with other monkeys, the condition was considered to be an isolated event and did not pose a risk to other members of the colony.

**P70 Use of Activated Clotting Time Values to Guide Effective Anticoagulation Treatment in New Zealand White Rabbits Undergoing Vascular Surgery**

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Perioperative administration of heparin requires monitoring to insure adequate anticoagulation for prevention of thrombus formation during cardiovascular surgery. Activated clotting time (ACT) is used as a measure of the ability of blood to clot. The ACT is used in lieu of the activated partial thromboplastin time as a coagulation test, because it responds in a linear fashion to increasing heparin dosages and correlates better with observed clinical anticoagulation. Activated clotting time values of > 250 seconds intraoperatively and 150 to 200 seconds postoperatively are considered representative of full anticoagulation in human patients undergoing cardiovascular surgery. New Zealand White (NZW) rabbits were used as an animal model for vascular surgery, including the use of autologous venous grafts in arteries. Jugular veins are excised, reversed in direction, and anastomosed end-to-end in cut sections of carotid arteries. As in human beings, rabbits were given heparin just prior to vascular surgery to block thrombosis. This required us to determine effective parameters for use of heparin as an anticoagulant in rabbits. Mean baseline ACT for male NZW rabbits were 116 seconds (n = 33). Using the ACT, we determined that the half-life of heparin in NZW rabbits after i.v. administration of a bolus of heparin was approximately 50 minutes. This compared to a half-life of 90 to 120 minutes in human beings. To achieve effective anticoagulation intraoperatively in rabbits, it was necessary to use an initial loading dose of 300 U of heparin/kg of body weight, i.v. This was twice the initial loading dose used in human beings. Rabbits were given this loading dose just prior to vessel anastomosis. At the conclusion of surgery, they were placed on a continuous infusion of heparin (25 U/kg/h). This regimen allowed us to maintain ACT values between 150 and 180 seconds for 7 days after surgery. We did not observe evidence of graft thrombosis by use of this treatment protocol. Therefore, the ACT appears to be an acceptable method for insuring adequate heparin anticoagulation in NZW rabbits.

**P71 An Evaluation of Heart Rates Derived From Holter Recordings of Cynomolgus Monkeys**

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Cynomolgus macaques are important for use in models evaluating preclinical toxicity of drugs intended for human use. There are only a few reports that describe normal electrocardiograms in macaques. Continuous Holter recordings of a group of 31 male and 31 female cynomolgus monkeys were examined. For each macaque, approximately 48 hours of Holter recordings were evaluated at 2-hour intervals for data on heart rate and arrhythmias. Mean heart rate during the recording period ranged from 141 to 174 beats per minute (BPM) in males and 157 to 188 BPM in females. Mean minimum heart rate ranged from 114 to 138 BPM in males and 128 to 152 BPM in females. There was a tendency for heart rate to be lower in males, compared with heart rate in females. Heart rate had a diurnal pattern of variation and tended to be lower during the second 24 hours of recording, compared with the first 24 hours. Mean heart rate of < 100 BPM was frequently recorded (284 in males and 120 in females). Analysis of this data indicated that the high heart rate seen in routinely recorded electrocardiograms are not representative of unrestrained macaques.