I want to express sincere appreciation to the OLAC and IACUC Offices, the faculty and staff who oversee our animal care facilities and research education centers, the people who work with our campus animals, and our volunteer community members, who collectively help ensure the welfare of all our research and teaching animals. You have done a spectacular job this past year.

As many of you may know, our campus was recently visited by the Accreditation Council of the Association for Assessment and Accreditation of Laboratory Animal Care. The accreditation site visit included a comprehensive review of our entire animal use program and facilities, including onsite evaluations of our research education centers (RECs) located across Tennessee. I believe the accreditation review was exceptionally positive. As our IACUC Institutional Official, I am proud of the animal oversight provided by our OLAC and IACUC community.

The Accreditation Council has granted our UT campus and RECs full accreditation. Comments shared by the site visit team included statements emphasizing the clean facilities throughout the program, including the RECs where they noted the pastures, paddocks, and grounds were exceptionally well-maintained, all records meticulously documented, and laboratory materials appropriately stored. Comments related to the knowledgeable and engaged staff across the program were exceptionally appropriate. Everyone involved with our OLAC and IACUC responsibilities should be proud. Well done!
According to the USDA a painful procedure is a procedure that “would reasonably be expected to cause more than momentary or slight pain or distress in a human being”. The PHS policy has a similar definition of a painful procedure and the Guide for the Care and Use of Laboratory Animals has a detailed section on pain and distress in research and teaching animals. It is important to note that the definition of painful procedure includes those procedures which may primarily cause distress and not necessarily cause physical pain.

USDA has defined 4 pain/distress categories as they apply to the use of animals in research teaching and testing. The 4 pain classification categories are categories B, C, D, and E and these categories come from the USDA Annual Report form. Category B is used for any animal that is on a holding or breeding protocol, no experimental manipulations are done on this animal. Category C involves animals on a study that experience no pain nor distress or only momentary pain and/or distress. Category D is used for animals that experience more than momentary or slight pain and or distress that is alleviated by pharmacological means. Category E is for an animal that experiences pain and distress that is not alleviated.

Breeding protocols are usually category B unless painful procedures, such as tail tips done at or after 21 days of age with the use of analgesia, are performed. In this case, the breeding protocol would become a category D protocol. If tail tips are done prior to 21 days of age, the procedure is considered to be non-painful and the protocol is assigned a B category.

Category C protocols involve manipulations that cause no pain nor distress or only momentary pain and distress. Studies involving either percutaneous or intraperitoneal injections would be a category C as a needle stick would be expected to cause only momentary pain in a human. Also those studies that involve teaching auscultation techniques to veterinary students would also be assigned this category.

Those studies that involve pain or distress that is alleviated are considered to be in category D. Studies that involve surgical procedures with the use of appropriate anesthesia are a D protocol. Also studies that are disease models also are considered to potentially involve more than momentary pain or distress. Once the disease starts to manifest clinical signs, very precise endpoints need to be delineated with the appropriate time duration. An animal that is recumbent and unable to reach food or water would need to be quickly removed from study in order for the alleviation of pain and distress to be met. Usually a 12 hour time frame is sufficient. An animal that would need to go longer may very well be considered to be in unrelieved pain and distress.

Category E protocols are those in which animals have pain and or distress that is unrelieved. Protocols in this category are uncommon at University of Tennessee. Historically these protocols have involved pain or distress caused by the withholding of food for longer than 24 hours or have used the ascites method of monoclonal antibody collection. Some disease models that result in lesions and due to study parameters pain relieving drugs cannot be used may also be assigned a pain category of E.

USDA policy #11 provides specific guidance on how to assign pain/distress categories to animal studies. When considering the pain and distress classification, it is not...
uncommon for people to discount studies involving potential distress. However, procedures may cause only distress, not necessarily pain. Examples of these as listed in Policy #11 include food and/or water deprivation or restriction (beyond that used for pre-surgical fasting), paralysis in a conscious animal, and some disease models. This list is of course not all inclusive. Wildlife studies that involve live capture may also be studies that involve causing distress, especially if animals are left in the capture device for an extended amount of time.

If the study involves pain and/or distress, an alternative search must be done in accordance to the Animal Welfare Regulations. In short, all protocols that are assigned a D or an E must have a literature search performed that searches for alternatives to the painful procedures. This literature search has to search 2 databases. More information as to how to perform this search can be found on the IACUC website at [http://iacuc.utk.edu/policies-and-procedures/](http://iacuc.utk.edu/policies-and-procedures/).

The future of our animal care and use program looks bright so you better wear shades. We are getting a new vivarium for the first time in many years. The architects have been chosen, but the contractors have not. The location is known but the building footprint is not. The needs are known but how they will be met is not. This is a great group effort. We have spent the past year planning the new building for campus that will have classrooms, laboratory space for faculty from four different departments and a NEW VIVARIUM! Can you tell that we are excited? The building will be located at Cumberland and 13th avenue in the small parking lot next to the Jessie Harris building. Last year, Perkins and Will performed a detailed space summary to determine the needs for each group. They composed a detailed programming study that was the basis for the architects to work from.

Nine architectural firms provided proposals for the project. The top four firms were asked to make presentations to the committee. After the presentations were made, Lord Aeck Sargent and McCarty Holesaple McCarty were chosen to be the architects for this project. While it is too early to show any designs or floor plans, there will be areas for ABSL 1 and ABSL2 studies, quarantine, and barrier housing, more surgery and procedural areas and some special suites for nutritional studies and circadian rhythm studies.

Ground breaking is planned for December 2014 with occupancy in mid-2017. Once the facility is ready, the animals from the Walters Life Sciences and Jessie Harris dedicated animal facilities will be moved to the brand new vivarium.
What’s New in Technician Training?

Jane Czarra, BS, LATg

Dr. Julie Albright gave a two part series on dog and cat behavior. Part I dealt with body language and the cues they give their handlers. We learned that a wagging tail does not always indicate, “Happy to see you”. Recently she provided Part II which dealt with handling techniques related to the body language cues. She also discussed conditioning dogs in steps to be more tolerant of certain procedures so as to require less restraint. In the near future we hope to have a training to practice holds and wraps.

Dr. Albright’s talks inspired me to do some investigating into the senses of dogs and cats and how they gather information differently from humans. I hope to present this information to the technicians in the near future.

The animal care technicians from the College of Veterinary Medicine Lab Animal Facility participated in a rodent handling and restraint wet lab. This is a reminder that individuals and small groups are welcome to set up training sessions outside of the OLAC wet labs given three times per year.

Understanding Biosafety Cabinets

Jonathan Phipps, PhD

The Class II, Type A2 biosafety cabinet (BSC) is a mainstay in Biosafety Level-2 (BSL-2) labs. This cabinet protects the laboratory worker from exposure to potentially infectious agents while protecting the workspace from contamination. To achieve this feat, the BSC directs all air through High Efficiency Particulate Absorption (HEPA) filters within the cabinet prior to exhaust and entry to the internal work zone. These cabinets are engineered to maintain a minimum average inflow velocity of at least 75 linear feet per minute at the face opening of the cabinet. Airflow is directed into the filtration system through a front grille with 30% of the air passing immediately through the exhaust filter back into the room while 70% is directed through a supply filter to the work surface. Filtered air entering the cabinet is split between the front and rear grilles to be mixed with incoming room air and recycled through either the supply or exhaust filter. This serves to generate a laminar flow in which all air travels in a single direction across the workspace and minimizing the horizontal exchange of air.

The aforementioned features mean Class-II, Type A2 BSCs can operate without the need for hard-ducting to the building air supply; however, it does limit their uses with certain volatile chemicals as the recirculation of air can lead to a build-up of vapors in the cabinet and discharge into the room from the exhaust air. If your project requires the use of these compounds, please contact the Office of Biosafety to discuss alternative containment. In addition, our office is available to provide training in the operation of BSCs along with information regarding applications compatible with their use. Please see our website at http://biosafety.utk.edu/.
Madhu Dhar, PhD in Biochemistry with over 15 years of experience in cell and molecular biology is leading the bench work involving the isolation, identification, characterization and application of adult stem cells from equine bone marrow, fat, and peripheral blood. The college’s Veterinary Medical Center offers a supply of naturally occurring clinical cases, where stem cell therapy can be used to relieve pain and suffering and encourage healing.

Stem cells are undifferentiated or progenitor cells that are capable of differentiating into specialized cell types. Commonly, stem cells come from either embryonic or adult sources; however in an effort to avoid ethical, political, and legal issues, the large animal clinical and research endeavors do not utilize stem cells from an embryonic source. The adult, somatic, stem cells we utilize are isolated from any adult tissue. Adult stem cells are commonly isolated from bone marrow, peripheral blood, fat, and umbilical cord blood and are also referred to as adult mesenchymal stem cells (MSCs). Because of their biological properties and their ease of collection, MSCs are routinely being used in cell-based therapies. Dr. Dhar’s group not only provides adult stem cells to the clinic but is also engaged in studying their molecular and cellular properties “to make them better and suitable” for improved clinical outcomes. In the large animal research laboratory, equine stem cells isolated from bone marrow, adipose tissue, and peripheral blood can be expanded in a tissue culture dish and cryopreserved for future use. Cryopreserving these cells in a special media ensures that the cells are living and will grow when they are taken out of the cryopreservation. Lab personnel in a dedicated stem cell laboratory, process, culture and cryopreserve bone marrow-derived adult stem cells and make them available to the clinic as the need arises. For example, conditions have been optimized such that roughly 10 million stem cells can be made available in as little as 1-2 hours, to a clinician for one injection into a tendon with superficial digital flexor injury. The ability for stem cells to assist in the patient’s recovery is highly dependent on the disease state and should not be considered a “miracle drug”. When we provide stem cells for clinical application, it is our responsibility to make sure that we provide well characterized and well-studied stem cells. Another major aspect of stem cell biology being actively studied in the laboratory is the choice of autologous versus allogeneic (genetically different) stem cells. Even though the thrust in veterinary and human medicine is in using stem cells isolated from a patient itself (autologous) and reintroducing them into the same patient, technically that can be challenging. For example, whenever stem cells are used in therapy, adequate numbers of these cells are required, and they can only be obtained by growing them in a tissue culture laboratory in presence of a defined medium. Our research shows that if we start with roughly 100,000 cells ideally, 6-8 million cells should be available after 1 week. As a result, we have to wait at least for 1 week to obtain enough cells for one injection. Hence, we isolate stem cells from horses in our teaching herd; characterize them completely with respect to specific stem cell properties, and then decide their clinical use. The main focus of the research lab is to provide adult MSCs as “off the shelf” therapy for the large animal clinic. Dr. Dhar’s research has been supported internally by the UTIA Center of Excellence and externally by Morris Animal Foundation.
When conducting research, investigators want experimental and control groups to be exactly the same, with the exception of the variable they are interested in. However, laboratory animals are influenced by many non-experimental factors that might potentially affect the outcome of the study. Those factors should be identified and accounted for at all stages to ensure they do not increase the variability or adversely affect the outcome of the experiment. Non-experimental variables that can influence animal research outcomes are those related to the animal itself. Animal related factors can be divided into age, sex and reproductive status, genetic makeup, immune status, nutritional status, and circadian rhythm.

The age of the research animal is an important variable to control since younger animals can have different responses than older animals. For example, neonates have immature body systems compared to adults. Older animals may develop deficiencies as body systems begin to fail. Gender can also be a variable. Liver biotransformation of certain chemicals can be slower in females, thus toxicity of certain compounds may differ between sexes. Reproductive status is a variable, since the physiology of females that are pregnant or lactating is different than those that are not.

The genetic makeup is also an important consideration. Both inbred and outbred animals are widely used in biomedical research. For outbred animals, the breeding colony must be large enough to maintain genetic variability. For inbred strains, the genetic purity of the strain must be monitored. Genetic differences of the same inbred strain can arise from mismatings or spontaneous mutations. Additionally, strains can differ in response to treatment and susceptibility to disease. C57BL/6 mice are highly susceptible to Plasmodium berghei with all mice developing erythrocytic infection following intravenous injection of 50 sporozoites. The same level of infection could only be established in BALB/c with 10,000 sporozoites.

Immune dysfunction including allergy, autoimmunity, and immunodeficiency can influence an experimental outcome. There are a wide variety of agents that alter the immune function, including chemical, food additives, metals and microbes. Most research animals carry a mixed microbial population that depends in part on housing conditions, diet, exposure to microbes, and other factors. These microbes may be a source of variability between animals housed in different facilities and can influence parameters such as nutritional requirements and drug metabolism. Immune deficient models such as nude mice are very susceptible to organisms such as Corynebacterium spp. which are not normally pathogenic in immune competent models. Special caging and care procedures are vital to minimizing such infections. Inadvertent infections in these special models can not only cause serious disease, but they can also interfere with the animal’s immune response during research.

An animal’s nutritional status is dependent on the type of food provided, method and amount of feeding, appetite, and age. Various dietary conditions such as mineral, vitamin, protein, lipid, and composition of the diet can have an effect on experimental outcomes. Many natural ingredient diets contain soybean and/or alfalfa meal. Both ingredients contain phytoestrogens which have been shown to alter mammalian physiology including cancer growth, atherosclerosis, metabolism, obesity, behavior, reproduction development, and immunology.

Circadian rhythms can also influence research results. Many behavioral, biochemical and physiologic parameters vary according to the day and night. It is very important to perform research manipulations at the same time of day for all animals.

Animal research involves the collection of data from carefully designed experiments. In order to obtain consistent and reliable results, the best attempts should be made to control or standardize all known variables when conducting experiments involving animals.

References:
CCAC Training Module on Laboratory Animals Used in Biomedical Research. Animal Related Factors. Available at: http://www.ccac.ca/Documents/Education/Modules/Vivaria/Biomedical_research/Companion-notes.pdf