Center for Excellence Annual Report, 2000-2001

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CENTER OF EXCELLENCE IN LIVESTOCK DISEASES AND 
HUMAN HEALTH

A TENNESSEE HIGHER EDUCATION COMMISSION ESTABLISHED CENTER OF 
EXCELLENCE

ANNUAL REPORT
2000-2001

Dr. Leon Potgieter
Assistant Director, 2000-2001

Dr. Robert N. Moore
Director, 2001-2002

December 2001

COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF TENNESSEE
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PROGRAM REPORT

The Center of Excellence in Livestock Diseases and Human Health was created in 1984 to promote interdisciplinary activities designed to:

- Improve the quality of human life through better animal health.

- Augment livestock disease research capabilities in the College of Veterinary Medicine and the Institute of Agriculture.

- Identify and characterize animal diseases that are analogous to human diseases (animal models of human disease).

- Develop new strategies for the diagnosis, treatment, and prevention of disease.

Since its inception, the Center has successfully promoted these goals by serving to promote the development of programs that impact the understanding and treatment/prevention of livestock and human diseases. The objectives have evolved and now represent a predominant, contemporary concentration on molecular and cellular approaches to investigations supporting the general areas of infectious diseases/population medicine, toxicology, reproduction, host defense, molecular genetics, and carcinogenesis. All of the Center core faculty contribute to at least one of these areas of emphasis. The Center promotes the potential of new investigators to develop competitive research programs and facilitates the efforts of established investigators to maintain and expand their research efforts. In addition, the Center actively supports the training of graduate students, veterinary student interns, and postdoctoral research students.

Center accomplishments for the year 2000-2001 were excellent when measured both as benchmarks (Table 1) and, perhaps more significantly, as the increasing extramural funding base (Table 2). The return on the State’s investment in the Center as the ratio of expenditures from extramural grants and contracts to Center appropriation was 4.4. Grant and contract expenditures exceeded $2.5 million (Table 2). While this was impressive, the increase in total award funding supporting Center faculty increased from $10,863,253 the preceding year to $12,760,303, approximately $2,000,000. This increase was due primarily to significant new grants and contracts awarded to Drs. McEntee, Rouse, and S. Oliver, although other new valuable grants and contracts were awarded to Center faculty. The 16 Center faculty averaged approximately 5 scientific and scholarly publications (74 total) and 3 invited presentations (48 total) at regional and prestigious national meetings (Table 1).

This report features the accomplishments and activities of investigators and research teams that constitute the core of the Center. Their achievements serve a crucial function in promoting the College of Veterinary Medicine, the Institute of Agriculture, and the University of Tennessee. Furthermore, they continue to ensure that the Center maintains its strong competitive position and contributes to the fiscal health of our research
environment. The report endeavors to emphasize the strength of the Center and give a clear indication of how it meets its objectives.

Research Funding
Important goals of the Center of Excellence in Livestock Diseases and Human Health are to support researchers and to promote research by a variety of mechanisms. State fiscal restraints for several years have restricted our ability to recruit and hire competitive researchers. The Center has had a significant impact over the last three years in recruitment of researchers with ongoing research programs. The existence of the Center and its ability to contribute to start-up packages has made the difference in these recruitment efforts. The Center does not serve as a primary source of research funding for faculty. The main criteria used for funding proposals include scientific merit, likelihood of leading to extramural funding and relevance to the Center's objectives. Proposals submitted to the Center for funding are reviewed by the Research and Graduate Programs Advisory Committee. The latter has one representative from every department of the College of Veterinary Medicine. For 2000-2001, "seed" grant funds totaling $288,550 were awarded to promote research initiatives by Center faculty.

The following projects were supported this past year by the Center:

- **Dr. Frank Andrews.** Pathogenesis of acid injury in the non-glandular region of the equine stomach.

- **Dr. David Brian.** Genetic structural elements regulating RNA synthesis during coronavirus replication.

- **Dr. Mei-Zhen Cui.** Mechanisms of lipoprotein induction of tissue factor gene expression in smooth muscle cells.

- **Dr. James Godkin.** Retinoids in oocyte maturation and embryonic development.

- **Dr. Alan Mathew.** Characterization of bacterial resistance elements in swine herds.

- **Dr. Michael McEntee.** Cellular mechanisms of NSAID-mediated regression in intestinal tumors.

- **Dr. John New.** A pilot project to survey rodent populations in the Great Smoky Mountains National Park for the presence of hantavirus and *Borrelia* species.

- **Dr. Jack Oliver.** Studies of tall fescue toxin-induced aminoacidemia in cattle.
- **Dr. Steve Oliver.** Identification and characterization of streptococcal virulence factors.

- **Dr. Barry Rouse.** Role of chaperone-bound peptides in the induction of antiviral CTL responses.

- **Dr. Hildegard Schuller.** Effects of NNK on beta-adrenergic growth regulation of pulmonary adenocarcinoma in vitro.

- **Dr. Terry Schultz.** Quantification of the underestimation of toxic potency in micro scale testing.

- **Dr. Patricia Tithof.** Effects of components of cigarette smoke on endothelial cell and vascular smooth muscle cell arachidonic acid metabolism and apoptosis. (start-up grant)

- **Dr. Hwa-Chain Wang.** Novel intracellular signaling pathways leading to cell quiescence and apoptosis. (start-up grant)

- **Dr. Xuemin Xu.** Untitled start-up grant.

**Equipment and Facilities**

The Center promotes the research infrastructure of the CVM and the Institute of Agriculture by participating in the purchase and maintenance of essential research equipment and through maintenance and renovation of research facilities. Criteria considered in the allocation of these funds include justification of need, equipment availability, and the number of investigators who may benefit. Requests from 5 individual investigators for 5 pieces of equipment totaling $35,398 were funded by the Center this past year. Researchers benefiting from these equipment grants were Drs. Wang, S. Oliver, Bartges, Rouse, and Brian. In addition, Dr. Hildegard Schuller and other COE faculty secured a substantial equipment grant from the NIH ($150,000, Table 2) with matching funds ($111,448) provided by the Center. This grant supported the purchase of a state-of-the-art Fascan-Cell Sorter/Flow Cytometer which is being used in support of basic and clinical research projects. Moreover, the Center is providing technical support for this sophisticated instrument. Matching funds and technical support provided by the Center were critical in the success of this application. This "turbosorter" greatly enhances the research capacity and grant competitiveness of the Center faculty and is being used also by investigators not previously associated with the Center.

Renovations funded by the Center of Excellence included extensive repair and renovation of walk-in coolers in A329A and A307 laboratories in the Veterinary Teaching Hospital. These coolers are used primarily by investigators participating in the COE. Similarly, repair and maintenance of several pieces of research equipment were funded by COE funds. This included an ultracentrifuge and the transmission electron microscope.
Research Training and Student Awards

The College of Veterinary Medicine funds at least 10 positions for Ph.D. training of students with a professional medical degree. Some of these positions are based in the Department of Pathology (as part of their residency/PhD program) and some are awarded without restriction. Most of these students become linked with Center of Excellence faculty. These young investigators significantly bolster the achievements of the Center. Faculty benefiting from these graduate students include Drs. Brian, Tithof, Schuller and Wang.

An important mechanism by which the Center of Excellence promotes biomedical research is to provide summer opportunities for veterinary students to do investigational work in research laboratories in the College of Veterinary Medicine. This past year the Center funded nine requests from first- and second-year students. The students are required to provide a summary of their work, which then is entered into a competition judged by Phi Zeta, the veterinary honorary society. This program is very successful. Several students have presented their work at national scientific meetings, and numerous manuscripts detailing results from work done by these students have been submitted for publication to refereed journals. In fact, over the past five years, this program has resulted in approximately 30 publications in refereed journals, several with the students as senior authors. The following veterinary student interns and their (mentors) and projects were supported by the Center during the summer of 2001:

- **Eric Rundlett** (Dr. Davis), "Determining Antibiotic Resistance in Cattle in a Developing Country".
- **Michael Owston** (Drs. Bemis and Kania), "Bacterial Gene Expression".
- **Lisa Miller** (Drs. Lane, Bartges, and Mawby), "Urinary Tract Disorders and Hypothyroidism in Doberman Pinschers, Golden Retrievers, and Labrador Retrievers".
- **Pam Wilkins** (Drs. Patton and Faulkner), "Diagnosis of Gastrointestinal Parasites in Free-ranging African Gorillas".
- **Jan McGinn** (Dr. Tithof), "Identification of Specific Components of Cigarette Smoke that Cause Vascular Smooth Muscle Death".
- **Shannon Jarchow** (Dr. Schultz), "Quantification of Thyroid Disruption by Micro scale Testing".
- **Kara Poucher** (Dr. Kennedy), "Feline Coronavirus in Nondomestic Felidae".
- **Adrienne Lickey** (Dr. Ramsay), "Disease Survey of Domestic Cats in Guatemala: Potential Health Risks to New World Felids".
- **Chuck Bane** (Dr. Reed), "Gross Anatomy of the Gray Short-tailed Opossum".
Personnel Changes

Dr. Michael Blackwell assumed the position of Dean of the College of Veterinary Medicine at the beginning of the fiscal year. He continued the practice of the two preceding Dean's by serving as the Director of the Center for the year. Operation of the Center remained under the supervision of the Assistant Director, Dr. Leon Potgieter, Professor and Head of Comparative Medicine, who also served as Director of the Comparative and Experimental Medicine graduate program (CEM). Effective at the beginning of the next fiscal year, Dean Blackwell appointed Dr. Robert Moore, Professor and Head of Microbiology, as the Director of the Center and of the CEM program. Dr. Potgieter led the Center ably for the past several years and is congratulated for promoting its highly successful status.

Recent recruitments of faculty with a significant research focus will benefit the Center of Excellence in the future. Over the past approximately three years we have recruited eight new researchers that will or are contributing to our Center of Excellence. They include Dr. Hwa Chain Wang (Comparative Medicine Department) who is well funded by NIH and Dr. Patricia Tithoff (Animal Science) who has received very competitive scores for her proposals and likely will be funded by NIH in the future. Dr. Joseph W. Bartges (Department of Small Animal Clinical Sciences) has received substantial funding from a variety of industry sources and the Morris Animal Foundation. Dr. David Slauson, former head of the Department of Pathology, recruited Drs. Xuemin Xu and Mei-Zhen Cui. They brought with them significant research funding from NIH (R01 grants), American Heart Association and foundations. Their research interests also fall within the Center’s focus. Dr. Hildegard Schuller, the current acting Head of Pathology, recruited Dr. Howard Plummer whose interest in cell regulation in carcinogenesis and asthma will complement other investigators in the Center. In addition a search is underway to fill a Pathology faculty position with an investigator with established interests in cancer research. Dr. Pam Small joined Microbiology this past year as an internationally recognized expert in the area of molecular pathogenesis of mycobacterial diseases, significant problems for human health and cattle industry. Dr. Small was awarded significant NIH grant support almost immediately and will join the Center as a core faculty member in 2001-2002. As her work expands into the area of tuberculosis research, she will do a large portion of her work in the biosafety level 3 core facility in the Veterinary Teaching Hospital.
Center Faculty Reports
Following are the reports from the core faculty of the Center. For organizational purposes the reports are grouped into the two umbrella aspects of the Center's interests, programs directly applicable to livestock health and population medicine and programs involving animal and laboratory models applicable to disease.

Livestock Diseases, Population Medicine, and Reproduction

Dr. Frank M. Andrews (equine gastritis)
Collaborators, staff and graduate students: Dr. A.G. Mathew, Dr. C.S. Patton, Dr. J.T. Blackford, Dr. A.M. Saxton, Dr. S. Murphy, Dr. J. Collins, Dr. R. Torres-Diaz, M. Sewell, A. Nadeau

Dr. Andrews studies the pathogenesis and mechanisms of gastric ulcer development in a horse model. Equine gastric ulcer disease (EGUS) is common in horses and has been implicated in poor performance with significant economic impact on the horse industry. Performance horses are commonly fed concentrated high-energy feeds that contain high levels of carbohydrates (starches). These carbohydrates undergo fermentation by resident bacteria in the stomach that may result in a low stomach pH and release of by-products such as volatile fatty acids (VFA; acetic, propionic, butyric, and valeric acids). Previous research in this laboratory established that high concentrations of VFAs are produced in the stomach of horses fed high-energy diets. These acids, due to their high lipid solubility, diffuse into the non-glandular gastric cells causing acidification and damage to sodium transport, which leads to cellular injury and gastric ulceration.

Current studies involve examination of fresh viable non-glandular tissue (most susceptible to gastric ulceration) from the stomach of horses. Tissues are placed in an Ussing chamber system, which allows measurement of tissue short-circuit current (sodium flow) and resistance across the tissue. A decrease in short-circuit current and resistance are the first indicators of tissue damage, and precede gastric ulcer formation. These tissues then are viewed under the microscope after special staining to determine the nature and extent of cellular damage.

Dr. Andrews' research suggests that low pH stomach acidity and volatile fatty acids, especially butyric, propionic and valeric acids, in contact with the stomach lining leads to cell damage. Also, valeric acid produced cell damage at a neutral pH. These cells lose their ability to transport sodium which leads to cellular swelling that, if exposure is long enough (6 hours) can lead to cell death and gastric ulceration. Examination of stomach tissues under light microscopy confirmed the presence of cell swelling in the non-glandular squamous mucosa, the area most prone to gastric ulceration in horses.

This research suggests that diets low in fermentable carbohydrates and high in protein and calcium (alfalfa hay) may be helpful in reducing stomach acid and VFAs aiding in the prevention of equine gastric ulcer disease. Also, diets could be developed to produce lower levels of these potentially harmful volatile fatty acids. In addition, the presence of
valeric acid in the stomach, since it causes damage at neutral pH, may be the reason why
the gastric ulcers in some horses are resistant to treatment.

Dr. David A Brian (coronavirus molecular biology/pathogenesis)
Fellows and Graduate Students: Dr. S. Senanayake, Dr. K. Nixon, Dr. A. Ozdarendelli,
G.D. Williams, S. Raman, C. Gay, H. Wu.

Dr. Brian’s interest in basic molecular biology of viruses has resulted in discoveries of a
fundamental nature for which his laboratory has received national and international
recognition. His research focuses on coronaviruses which cause some of the most
costly respiratory and gastroenteric diseases of livestock and fowl, and disabling diseases
of people. Efforts to control coronavirus infections have been frustrated by three major
obstacles:
1. An incomplete understanding of how coronaviruses replicate and persist in
animals.
2. The ability of coronaviruses to rapidly mutate into new pathogenic
variants.
3. The generally weak immune responses in animals to coronavirus
vaccination and the logistical problem of inducing protective mucosal
(local) immunity in the vulnerable newborn.

The primary research focus in Dr. Brian’s laboratory is the molecular biology of
coronavirus replication. With funding from the USDA and the NIH, and modest support
from the Center of Excellence, they are making an intense effort to understand how five
separate genetic elements in the coronavirus function to regulate production of viral
proteins and progeny virus. Research is being done also on a sixth genetic region, a hot
spot for variability, in an effort to understand the determinants of this process. Genetic
recombination (blending) at this site is a mandatory step used by the virus in the
generation of messenger molecules that encode portions of the virus’ genetic material. It
is anticipated that information from these studies will significantly impact the design of
new therapeutic strategies.

Of special interest is how a newly discovered element at one end of the virus gene
regulates replication of the genetic material. The element is a tRNA-like folded structure
(a pseudoknot), that may regulate virus replication by incorporating a cellular protein in
the virus replication machinery. Therapeutic interruption of such a virus-protein
interaction may lead to a cure of virus infection. Interestingly, one candidate protein in
this interaction is histidyl tRNA synthetase, a factor (autoantigen) in the human disease
polymyositis.

Dr. Brian’s laboratory has also discovered a small genetic variant of the bovine
coronavirus (a viral minigenome) that replicates in the presence of “normal” virus. This
minigenome is being experimentally engineered to carry many kinds of potential antiviral
molecules into cells. One molecule is an enzyme (a ribozyme) designed to destroy the
gene on which the virus depends for replication (the polymerase gene). This novel
therapeutic approach would, in theory, cure a virus-infected cell without killing it.
Dr. Alan G. Mathew (livestock enteric antibiotic resistance)
Staff and graduate students: R. Clift, S. Chattin, D. Arnett, P. Cullen, P. Ebner, K. Garner, G. Pulliam, J. Liu

Antibiotics are commonly used in livestock and pets in the US. Therapeutic use of antibiotics continues to play a major role in combating disease organisms, while subtherapeutic use in feeds increases animal performance, decreases the numbers of infectious organisms in the environment, and lowers the prevalence of organisms causing foodborne illness in humans.

In contrast to the above benefits, some evidence suggests that agricultural use of antibiotics may be partly responsible for the emergence of drug-resistant bacteria, which in turn may decrease the efficacy of similar products used in human medicine. However, little information is available on strategies for controlling of antibiotic-resistant organisms. In particular, almost no information is available with respect to modern livestock production facilities, management, environmental conditions, or drug therapies that affect resistance in organisms. Because resistance may be transferred to bacteria from a variety of resistant bacteria and associated hosts, it is important that factors involved are characterized so that more effective control strategies can be formulated.

A primary research focus of Dr. Mathew's group is to characterize genetic factors that lead to antibiotic resistance in animal and human pathogens. They also are investigating how different uses of antibiotics in livestock and pets affect antibiotic resistance patterns, concentrations, and shedding of food borne pathogens. They hope to determine the most effective antibiotic therapies and husbandry practices to maintain animal health, while at the same time limiting prevalence of food borne pathogens and antibiotic resistance of microorganisms.

A recent accomplishment of his group has been the determination that resistance to a widely used antibiotic, apramycin is controlled by genes found on bacterial chromosomes, particularly in non-pathogenic E. coli found in the GI tract of animals. Formerly, it had been assumed that such genes resided primarily on extrachromosomal plasmids which are highly mobile DNA elements frequently found in bacteria. This information indicates that resistance to apramycin is more persistent in some types of bacteria, and, thus, strategies for control will need to be revised. They have also developed a number of molecular techniques to quickly detect resistance genes and insertion points for those genes in bacterial genes and plasmids. This work has led to a more specific test for a particularly hazardous strain of Salmonella typhimurium, DT104, which carries resistance for at least 5 antibiotics. The PCR-based test can easily and quickly differentiate between non-typhimurium salmonellae, S. typhimurium, and S. typhimurium DT104 providing a powerful tool for epidemiological studies.
Other accomplishments of Dr. Matthew's group include the finding that various animal stressors, including heat and cold, increase the numbers of antibiotic resistant bacteria associated with livestock. In addition, investigations of the prevalence of resistant bacteria and food borne pathogens in pets are underway and have already provided important preliminary data that will ultimately lead to methods to reduce microbial risks associated with pets and companion animals. Additional work also continues to define sources and reservoirs of food borne pathogens in livestock units. Such information will be instrumental in the implementation of on-farm Hazard Analysis Critical Control Points (HACCP) strategies for control of specific food borne pathogens.

**Dr. Jack W. Oliver (bovine tall fescue toxicity)**

Co-investigators and staff: Dr. R. Linnabary, Dr. E. Schultze and Dr. B. Rohrbach, L.K. Abney, E.M. Bailey, M. Cottrell and J. Czarra

Tall fescue toxicosis continues to be the primary grass-related disease in the United States in terms of economic loss to animal producers, affecting over 8.5 million beef cows and 700,000 horses. Tall fescue toxicosis is also a costly disease to Tennessee cattle producers, resulting in an approximate $100 million dollar annual loss due to unrealized production. Tall fescue is an attractive forage species because of its ability to withstand drought, poor soil conditions and intensive defoliation from grazing. It is grown on more than 34 million acres of pasture, but 75% of the pastures are infected with the endophytic-fungus, *Neotyphodium coenophialum*, at a sixty percent or greater level. Most of the infested pastures are in the Southeastern United States.

The endophyte-grass association results in the production of alkaloid toxins produced by the fungus or by the plant in response to the fungus. The alkaloids are biologically active causing a decrease in appetite and impaired reproduction and growth in animals. Endophyte-infected tall fescue has greater forage and seed productivity than the non-infected variety and is more drought tolerant. At the same time, tall fescue toxicosis is a costly disease to animal producers, causing severe reductions in weight gains, milk production and fertility.

Results of studies by Dr. Oliver's group have established that vascular damage is a central event that occurs when herbivores consume infected tall fescue. As a consequence of injury to blood vessels, blood flow to tissues is impaired causing localized tissue damage and thereby affecting the function of body systems. The abnormalities in blood flow are integrally related to the economic losses encountered by the cattle industry in the United States. Dr. Oliver has been examining toxicity associated with purified alkaloids that are suspected of being the primary tall fescue toxins (i.e. ergine, ergovaline). The long range goal of these studies is to understand how the individual toxic alkaloids cause damage to tissues of cattle because little is known regarding which of the alkaloids in tall fescue cause(s) the lesions in this syndrome.

Studies have been completed on analyses of various parameters of blood and tissues in steers that grazed endophyte-infected tall fescue over a three-year period.
Markedly suppressed levels of serum copper were recorded in consecutive years, and copper deficiency may be the basis of the poor haircoats in these cattle. Gamma globulin (antibody) levels these cattle also were significantly reduced, suggesting that immunosuppression is an important aspect of the disease too. This information of tall fescue toxicity in cattle will be important in evaluating anti-fescue toxicosis treatments.

Continued research will be focused on the chronic effects of the two important alkaloids of toxic tall fescue, ergine and ergovaline. Cattle will be treated with each of these alkaloids to evaluate their effect on the function of a specific blood vessel receptor (alpha-2 adrenergic). Dr. Oliver determined that ergovaline administration at the rate of 0.2 ug/kg/hour caused the typical decrease in the hormone (prolactin) in blood that occurs in cattle grazing on fungus-infected tall fescue pastures. Since the lining cells of blood vessels (endothelial cells) are damaged by exposure to the alkaloids, several inflammatory mediators associated with endothelial cell injury will be measured in the serum of the cattle that are infused with ergine or ergovaline.

Laboratory studies with isolated bovine endothelial cells or smooth muscle cells, grown in culture were treated with various concentrations of ergovaline and ergine. Both of these alkaloids are toxic to endothelial cells but ergovaline was considerably more potent. Dr. Oliver’s results indicate that manipulation of the infection allowing ergine production in the plant but elimination of ergovaline presence would be beneficial. The ergine is necessary to convey insect resistance to tall fescue and the toxic effect would be minimized because the absence of ergovaline. Reducing the toxic effect of ergovaline in cattle will allow increased use of tall fescue, a forage with excellent nutrient quality, and root development that helps to control soil erosion. Dr. Oliver’s research has been supported by the Center of Excellence, but his primary support is from the USDA’s National Research Initiative.

**Dr. Stephen P. Oliver (bovine mastitis)**

Research conducted by Dr. Oliver focuses on mastitis in dairy cows caused by environmental organisms. Several kinds of bacteria are capable of infecting the udder causing mastitis. These pathogens invade the udder, multiply there and produce harmful substances that result in inflammation, reduced milk production and altered milk quality. Control of mastitis is extremely difficult because of the many types and sources of mastitis pathogens that can cause the disease. The National Mastitis Council estimates that mastitis costs U.S. dairy producers over two billion dollars annually. In Tennessee, losses due to mastitis may exceed $25 million annually. Thus, mastitis in dairy cows is likely the most costly disease affecting dairy producers in Tennessee, the U.S., and throughout the world.

Dr. Oliver was the first to show that mastitis in pregnant dairy heifers occurred frequently near calving and that many of these infections persisted into early lactation. His research has resulted in a simple, effective and inexpensive method for controlling mastitis
in heifers. Intra-mammary antibiotic infusion before calving, was shown to be an effective procedure for:

1) eliminating many infections in heifers during late gestation
2) reducing the prevalence of mastitis in heifers during early lactation
3) for reducing the prevalence of mastitis in heifers throughout lactation.

He documented that a return of $12-$20 for each dollar spent was possible using this approach.

Several studies over the past 13 years at the UT Dairy Experiment Station involved collection of almost 200,000 milk samples for microbiological evaluation at intervals before calving, during lactation and during the dry period. Data from those studies have been computerized and this mastitis database may be the largest in the world. It now is being exploited for retrospective studies and will provide valuable information on the spread of mastitis pathogens, such as Streptococcus uberis and Streptococcus dysgalactiae, in high-producing dairy herds. Recently, they evaluated the influence of mastitis on reproduction in Jersey cows and found it profoundly impairs reproduction during early lactation.

Dr. Oliver has been actively seeking the identification of virulence (severity) factors produced by certain mastitis organisms (Streptococcus species) and implications of immunity to them. In many dairy herds Streptococcus uberis and Streptococcus dysgalactiae are responsible for a high proportion of mastitis with varying degrees of severity in lactating and non-lactating dairy cows. Strategies for controlling these mastitis pathogens are poorly defined and inadequate. This research focuses on:

1) genetic characterization of Streptococcus uberis and Streptococcus dysgalactiae
2) characterization of Streptococcus uberis and Streptococcus dysgalactiae with particular emphasis on factors involved in adherence and invasion into mammary epithelial cells
3) evaluation of immunity after immunization of dairy cows with components of Streptococcus uberis and Streptococcus dysgalactiae
4) effectiveness of experimental vaccines to Streptococcus uberis and Streptococcus dysgalactiae mastitis during the nonlactating period

Dr. Oliver's research group has determined that Streptococcus uberis and Streptococcus dysgalactiae readily adhered to and invaded cells lining the bovine udder. Chronic infections then may develop, and their intracellular location may protect these bacteria from anti-microbial drugs and host defense mechanisms. Mastitis pathogens cultured in the presence of mammary epithelial (lining) cells in the laboratory synthesize proteins not detected when bacteria are cultured alone. These unique proteins likely are involved in virulence of bacteria, including their capacity to adhere and invade mammary epithelial cells. Thus, culture of mastitis pathogens in the laboratory in the presence of mammary epithelial cells may result in expression of bacterial virulence factors similar to that which occurs in the animal. This important discovery will be exploited for the development of vaccines and management of mastitis.

Dr. Oliver's expertise in mastitis and milk quality has led also to a new research initiative in food safety. The primary goal is to provide comprehensive information on the
occurrence and distribution of *Salmonella*, *Escherichia coli O157:H7*, and *Campylobacter jejuni* in bulk tank milk, feces of cull dairy cows and the environment in dairies. Antibiotic resistance patterns and molecular characterization of foodborne pathogens is also being done.

Dr. Oliver has increased the awareness of scientists, extension specialists, dairy producers and pharmaceutical companies of the importance of environmental pathogens in bovine mastitis. Furthermore, he has discovered fundamentally important information that is critical for controlling the heterogeneous organisms that cause mastitis. Dr. Oliver's research philosophy is to design and conduct innovative and useful studies and to report to a wide variety of constituents. The ultimate goal of this research is to enable dairy producers in Tennessee, the U.S., and throughout the world to enhance the quantity and quality of milk produced and thus reduce the economic impact of mastitis.

Dr. Oliver's research has been supported for several years by the Center of Excellence, but his primary funding has been derived from substantial grants from foundations, FDA and the pharmaceutical industry.

**Dr. James D. Godkin (reproduction)**

Fellows, Staff and Graduate Students: Dr. D. Eberhardt, T. Livingston, H. King, S. MacKenzie, M. Roberts

The focus of Dr. Godkin's research group over the past few years has been the study of proteins that communicate fetal-maternal interactions and result in the successful maintenance of pregnancy. One important discovery was that interferon-tau, a placental protein, interacts with the uterus and alters maternal hormonal balance and maintains early pregnancy in ruminants such as cattle, sheep, goats, and buffalo. Another focus of Dr. Godkin's laboratory is reproductive efficiency with respect to the effect of growth factors and certain vitamin A-like proteins (retinoid-associated) and their genes on the early embryo, ovary, oviduct and uterus.

The major current focus of Dr. Godkin's laboratory is on factors that control development of the early embryo of domestic livestock. Recently, they made the remarkable discovery that treatment of animals with vitamin A-like compounds (retinoids), just prior to ovulation, results in improved viability of embryos that then develop following fertilization. In addition, his laboratory studies indicated that treatment of embryos with retinoids dramatically improved embryonic development. The goals of this research are to improve reproductive efficiency through the use of retinoid administration procedures, to develop more efficient assisted reproductive procedures and to determine the mechanisms by which retinoids affect oocyte (egg) maturation, embryonic development and survival.

This research has the potential to improve reproductive efficiency in livestock and improve assisted reproductive procedures in humans. It also has an application for the preservation of endangered species.
A patent application has been filed covering the use of retinoids in assisted reproductive procedures with the assistance of the UT Research Corporation. Dr. Godkin’s research has received support from the Center of Excellence which has been leveraged into substantial funding from the USDA’s National Research Initiative.

Animal and Laboratory Models of Disease

**Dr. Michael McEntee (cancer modeling)**
Collaborators, Staff and Graduate Students: Dr. J. Whelan, A. Cruikshank, N. Neilsen

Dr. McEntee’s research focuses on defining the relationship between tissue levels of polyunsaturated fatty acids, their metabolism as bioactive lipids (such as prostaglandin E$_2$), and forms of cancer to which they have been linked. In collaboration with a biochemist in the Department of Nutrition at UT, Dr. J. Whelan, he demonstrated recently that specific dietary polyunsaturated fatty acids can significantly protect against the development of intestinal cancer in a mouse model of the human disease. Non-steroidal anti-inflammatory drugs like aspirin inhibit the metabolism of polyunsaturated fatty acids into various prostaglandins. Their research involved the simultaneous pharmacologic and dietary manipulation of tissue polyunsaturated fatty acids concentrations.

Polyunsaturated fatty acids derived from fish oils reduced the incidence of this form of neoplasia by 50% in comparison to polyunsaturated fatty acids common in the U.S. diet (i.e. animal fat and vegetable oil). Their research suggested that this protective effect was specifically attributed to longer chain, highly unsaturated polyunsaturated fatty acids. Dr. McEntee also demonstrated for the first time in an animal that prostaglandins produced from the “bad” tissue polyunsaturated fatty acids specifically contributes to intestinal tumor growth. Inhibition of the metabolism of polyunsaturated fatty acids found in corn oil and red meat to prostaglandins significantly contributes to intestinal carcinogenesis.

The importance of prostaglandins in production of tumors was subsequently confirmed in experiments where tumors were eliminated following treatment with an antibody that specifically inactivates this polyunsaturated fatty acid product. Prostaglandin E$_2$ acts through specific cell receptors and they have shown that it is produced by the non-neoplastic part of intestinal tumors of their mouse model (as it is in humans). They are currently attempting to characterize the distribution of the prostaglandin E$_2$ receptors in these lesions in order to better understand the link between the production/target activity of this specific bioactive lipid and intestinal carcinogenesis. Recent data suggest that the molecular changes contributing to intestinal carcinogenesis in humans and their mouse model also occur in pet dogs that develop this form of neoplasia. This strongly implies that the beneficial effects of dietary and pharmacologic intervention demonstrated in the mouse model would directly translate to dogs, as well as humans.
In addition to the above experiments, studies have been initiated to investigate the contribution of polyunsaturated fatty acids and their metabolites to another common form of neoplasia that has been strongly linked to dietary fats in humans, prostatic cancer.

Dr. McEntee receives support from the Center of Excellence, but his primary funding is derived from the Department of Defense, American Institute of Cancer Research and Monsanto.

Dr. Barry T. Rouse (herpesvirus immunity and pathogenesis)

Dr. Rouse’s research deals with the recognition and interaction of the body with viral infections. This group has studied herpes simplex virus, an agent that affects the majority of humans. The virus persists indefinitely in infected individuals, and some suffer periodic lesions which are painful and inconvenient. When such lesions occur in the eye, they can lead to blindness. Dr. Rouse’s laboratory is involved in studies directed to understand the mechanisms by which herpes simplex infection causes blindness.

Dr. Rouse’s approach to understanding the interaction between herpes simplex virus and the immune system is to exploit model infections such as the mouse system as an animal model. Their aim is to understand how cells and molecular events set into play by herpes simplex virus lead to chronic inflammatory lesions or to resolution of disease. Ultimately, it may prove possible to manipulate the host defenses either to achieve protection by vaccines or resolution of injury by substances introduced by gene transfer technology and capable of influencing the immune response.

In the last year they have used mouse models which have been genetically manipulated to cause deficient immune systems and thereby to evaluate whether the ocular lesions are the consequence of an autoinflammatory (self-destructive) reaction. Their results do not support the latter hypothesis. Instead the evidence indicates that herpes simplex replication in the eye causes the output of molecules called cytokines and chemokines which activate certain invading cells of the immune system (T lymphocytes) to release inflammatory substances. This mechanism is referred to as bystander activation. Such a mechanism could represent an important component of any chronic inflammatory reaction.

The other major activity in Dr. Rouse’s laboratory is to understand the cell and molecular events that occur during an immune response to novel DNA vaccines. They have identified that a process called “cross priming” is the main event in this process and that it is mediated by a “chaperone molecule” bound to a protein (peptide). They are exploring the use of chaperone-bound peptides as a unique means of vaccination. This could have a major impact in the prevention of viral diseases in people and animals.

In studies of herpetic ocular disease, Dr. Rouse’s group has found that neovascularization is an essential event in the pathogenesis of this disease. Moreover,
they have determined that HSV-induced VEGF is involved in this process and that intentional diminution of the angiogenic factors production limits the severity of the viral-induced corneal lesions. Their data document for the first time the essential role of angiogenesis in the pathogenesis of herpetic stromal keratitis and also indicate that the therapy of this disease could benefit by procedures which diminish angiogenesis.

The Center of Excellence supports some aspects of this research which is funded primarily by substantial grants from the National Institutes of Health. Their work has generated national and international interest, and the laboratory is recognized as one of the premier viral immunology programs in the country. Dr. Rouse is one of a very select group of investigators in this country holding three R01 NIH awards simultaneously.

**Dr. Hildegard M. Schuller (experimental oncology)**
Fellows, Staff and Graduate Students: Dr. H. K. Plummer III, Dr. Brian A. Jull, Dr. Y. Cakir, N. Neilsen, and K. Walker

Lung cancer is the leading cause of cancer deaths in all industrialized countries. East Tennessee has one of the highest lung cancer rates in the United States. Although cancers at other organ sites are more than twice as common, their cure rate is considerably higher. The most common cancer in men is prostate cancer, with a cure rate of 84%. Breast cancer is the leading type of cancer in women with a cure rate of 74%. In contrast, 158,700 (89.3%) of the 177,700 patients diagnosed with lung cancer in the year 1997 died within 12 months of diagnosis.

Smoking and exposure to second-hand smoke are the most intensively studied and best-documented risk factors for the development of lung cancer. Contrary to cancers at other organ sites, the incidence of lung cancer continues to rise in all industrialized nations. Moreover, teen smoking in the U.S. has increased at an alarming rate, thus setting the stage for even higher numbers of lung cancer cases 30-40 years from now. Another important contributing factor to the rise in lung cancer cases is the growing number of lung cancers developing in individuals never exposed to primary or second-hand smoke. This trend, which has been globally observed during the last two decades in all industrialized countries, is particularly evident for pulmonary adenocarcinoma. Of the six types of lung cancer recognized by the World Health Organization classification, two (small-cell carcinoma and adenocarcinoma) accounts for 90% of all lung cancers, but 30% of these cases do not have a history of exposure to primary or second hand smoke.

The lung cancer “epidemic” is closely related to a globally observed rise in chronic lung diseases such as bronchitis, bronchiolitis, asthma, emphysema, and chronic obstructive pulmonary disease. This disease complex, which is often referred to as “allergies” has the same geographic distribution as lung cancer with which it shares some risk factors such as smoking and air pollution. Accordingly, East Tennessee, which has one of the highest lung cancer rates in the U.S., is also often referred to as “the land of allergies.” For all lung cancer types, chronic lung disease has been identified as a risk factor even without a history of exposure to smoke.
Dr. Schuller's research has been dedicated to the study of lung cancer for over 20 years. It is her belief that effective strategies for the prevention and therapy of this disease complex can only be based on an in-depth understanding of the regulatory mechanisms which govern the growth of normal lung cells and the cancers arising from such cells. In contrast to other laboratories that are searching for the "magic molecular event" responsible for the genesis of all lung cancers, she hypothesized that different lung cell types and different types of lung cancer may be governed by different regulatory mechanisms, which in turn may be affected differently by known risk factors for the disease.

Dr. Schuller’s achievements in lung cancer research have been recognized nationally and internationally. Her research has been supported by the Center of Excellence, but her primary support comes from substantial grants of the National Cancer Institute and the pharmaceutical industry.

Dr. Schuller’s group previously determined that the growth of small cell lung carcinoma and the cell of origin of this cancer type (pulmonary neuroendocrine cell) is regulated by a specific cell surface receptor (nicotinic acetylcholine) which has an important biochemical function (calcium channel). She found also that the tobacco-specific carcinogenic product (NNK) activates this receptor with high affinity. Binding of this product to the receptor causes a release of a biochemically active substance (serotonin) by these cells and that this substance markedly stimulates cell division when it is taken up by other cells. This is an important finding because it links, for the first time, the stimulation of a specific receptor by a tobacco-specific toxicant resulting in the activation of a series of cell-specific events that may result in uncontrolled growth. Experiments are now underway to test the hypothesis that substances which inhibit the re-uptake of serotonin will protect against the development and spread of small cell lung carcinoma. These drugs are already approved for the treatment of psychiatric diseases and migraine and could immediately enter clinical trials in smokers and small cell carcinoma patients.

Laboratory studies with various cultures of small cell lung cancer cells and pulmonary neuroendocrine cells suggest that smoking or chronic exposure to the product NNK, increases the concentration of the target receptors on these cells. Dr. Schuller now is working with Dr. Kabalka of the Department of Chemistry on the development of novel cancer imaging agents which selectively bind with high affinity to this receptor that will allow for a selective and highly sensitive detection of small cell carcinomas in people (by positron emission tomography). This will constitute an important clinical application of her research.

Dr. Terry W. Schultz (toxicity modeling)
Staff and Graduate Students: G. Sinks, B. Gregory, J. Seward, and E. Hamblen

Research in the Biological Activity Testing and Modeling Laboratory focuses on the development of databases for structure-toxicity modeling and the development and
validation of such models. The values of structure-toxicity models lie in their ability to predict toxic potency from molecular structure. This means that hazard assessment can be conducted while conserving time, manpower, resources, and animals.

Toxic potency is related to the uptake of the toxicant from the environment and its interaction with certain molecular sites of action. Since certain properties (such as hydrophobic, electronic, and steric factors) are related to molecular structure, Dr. Schultz's group focuses on identifying global descriptors of such properties that are best in modeling of uptake and interaction. Previous work by Dr. Schultz's group have shown that the site of action for toxic events is the cell membrane. In the case of covalent events, it is soft nucleophiles associated with membrane-bound proteins, whereas, in the case of non-covalent events it is the fatty acids of the membrane lipid bilayer.

The Biological Activity Testing and Modeling continues to make excellent progress in the development of a better understanding of chemicals that mimic estrogens. They have, with help from the Center, developed one of the largest single-endpoint databases for xenoestrogens in the world. From these data, Dr. Schultz and his collaborators have developed a series of molecular structural indicators that allow for the rapid screening of large sets of industrial chemicals for those that have the potential to cause endocrine disruption.

Work also continues on standardizing the *Saccharomyces cerevisiae*-based lac-Z reporter assay as an in vitro assay, which allows for the quantitative assessment of chemicals that alter estrogen receptor-mediated transcriptional activity. The estrogenic activities of 17-B-estradiol, biphenyl, chlorinated biphenyls (CB) and Aroclor mixtures have been examined. Results suggest that the use of plastic microtiter plates in toxicity testing with strongly hydrophobic chemicals may result in an underestimation of toxicity. Using the modified yeast estrogen assay, full agonist activity was observed for 4-CB, 2,4,6-CB, and 2,5-CB, while each of the Aroclor mixtures were only partial agonists. The equivalent EC50 values in ppm were in environmentally relevant concentrations of biphenyl (19 ppm), 4-CB (4.5 ppm), 2,5-CB (21 ppm), 2,4,6-CB (0.8 ppm), Aroclor 1221 (2.9 ppm), Aroclor 1242 (0.65 ppm), and Aroclor 1248 (2.3 ppm).

Other recent achievements by this group include improvement in descriptor selection for the prediction of toxic potencies of electrophiles. Specifically, Quantitative Structure-Activity Relationships (QSARs) relating toxic potency with hydrophobicity quantified by the 1-octanol/water partition coefficient and electrophilic reactivity quantified by the molecular orbital parameters, either the energy of the lowest occupied molecular orbital or maximum acceptor superdelocalizability reveals maximum acceptor superdelocalizability to be the better electrophilic parameter for modeling of industrial aromatic chemicals.

*Patricia K. Tithof (cardiovascular physiology and toxicity)*
Collaborators, Staff and Graduate students: Dr. M. Peters-Golden, Dr. H. Schuller, Dr. H.C. Wang, Dr. R. Donnell, M. Elgayyar, M.A. Barnhill
Dr. Tithof's research program in cardiovascular physiology concerns the effects of specific components of cigarette smoke on the biology of blood vessel lining (endothelial) cells and metabolism of a potent physiological chemical messenger, arachidonic acid. The latter is a fatty acid that is present in high quantities in the membranes of all cells and is a substrate for the production of eicosanoids, a family of biologically active lipid mediators. The latter have an important role in several diseases including asthma, arthritis, cancer and atherosclerosis.

Smoking, which greatly augments the process of atherosclerosis, increases the risk for heart attack or stroke by as much as 50%. Recent epidemiologic studies indicate that a high fish diet or frequent use of aspirin significantly decreases mortality rates due to heart attacks in heavy smokers. Fish contains high levels of certain fatty acids, which decrease the availability of arachidonic acid and also inhibit the production of arachidonic acid-derived eicosanoids. The protective effects of these fatty acids and aspirin against smoking-induced atherosclerosis suggest that components of cigarette smoke stimulate the arachidonic acid pathway. However, no previous studies have focused on the specific components of cigarette smoke responsible for this effect. Several compounds are contained in high concentration within the tar fraction of cigarette smoke. These include methylanthracenes (1,500 ng/cigarette), phenanthrene (362 ng/cigarette) and benzo(a)pyrene (25 ng/cigarette). Benzo(a)pyrene accelerates the development of atherosclerosis in animal models.

Endothelial cells form a single cell layer lining the blood vessel wall. Endothelial cell death and loss is a critical and important event in the early development of atherosclerosis. Loss of the endothelial cell layer results in inflammation of the vessel wall, vasoconstriction and clot formation; events important in the development of heart attacks and strokes. Previous studies indicate that cigarette smoking increases the rate of endothelial cell loss; however, neither the mechanism of cell death nor the specific components of cigarette smoke responsible have been elucidated.

These studies indicate that exposure of porcine aortic endothelial cells or human coronary artery endothelial cells to these compounds in cigarette smoke causes release of arachidonic acid. Furthermore, this release of arachidonic acid is associated with loss of endothelial cell viability through stimulation of a process known as apoptosis or programmed cell death. Apoptosis induced by these compounds can be inhibited by the fatty acid, eicosapentaenoic acid, which exists at high concentrations in fish. These results suggest that methylanthracenes, phenanthrene and benzo(a)pyrene may be important components of cigarette smoke that augment atherosclerosis through a process that involves arachidonic acid-mediated killing of endothelial cells.

Dr. Tithof also has initiated studies to investigate the effects of derivatives of nicotine on endothelial cell function. A derivative of nicotine, NNK, plays an important role in smoking-induced lung cancer. Recent studies in collaboration with Dr. Hildegard Schuller suggest that NNK induces tumor formation by a mechanism that involves release of arachidonic acid by stimulation of certain cell receptors (beta-adrenergic). The finding that NNK binds to beta-adrenergic receptors has important implications

The effects of NNK on endothelial cell viability were investigated by Dr. Tithof’s group. NNK, at very low concentrations (100 nM) caused apoptotic cell death of endothelial cells. Their results suggest that it was the result of NNK binding to beta-adrenergic receptors and that the arachidonic acid cascade was involved.

These studies provide novel findings concerning the effects of specific components of cigarette smoke on endothelial cell function and suggest a novel mechanism by which cigarette smoke augments atherosclerosis. These studies may contribute to the development of effective measures for preventing cardiovascular complications in smokers. Moreover, identifying specific components of cigarette smoke that augment disease may lead to the development of safer cigarettes. These strategies may be particularly important because the incidence of smoking continues to rise, despite intensive efforts to educate people about the hazards of smoking.

Dr. Hwa-Chain R. Wang (molecular oncology)
Fellows: K. Fecteau, J. Mei, Y. Sun, M. Tan

Dr. Wang’s long-term research goals concern tumor-specific intracellular molecular signaling networks and to uncover signaling pathways that can be induced by anticancer agents to lead cancerous cells into programmed cell death (apoptosis) or growth arrest (cell quiescence).

Short-term goals are to identify intracellular signaling elements whose activation is involved in induction of apoptosis or growth inhibition of cancer cells. A corollary to this is to identify novel anticancer agents, which may selectively induce apoptosis of cancer cells while sparing normal cells. Ultimately, he expects to apply the understanding of intracellular signaling control to anticancer therapeutics and prevention.

Currently, Dr. Wang focuses on three projects. The first is to understand the molecular and cellular function of a family of novel intracellular enzymes (Krs and QIK), which are induced in resting cells and cells undergoing programmed cell death. The second is to study molecular and biological activities of a novel natural anticancer agent (FR901228), which selectively induces programmed cell death of cancer cells. The third is to study changes in molecular and biological properties of human breast cells during development into cancerous stages induced by tobacco-related carcinogens.

Dr. Wang has identified a family of novel enzymes (kinases: SAMK/Krs1 and QIK) that are activated in normal resting cells, in quiescent cells, or in cancerous cells undergoing programmed cell death as a result of a variety of physiological, chemical or physical stresses. QIK is an active form of gene products of the Krs1 gene. Induction of QIK activity is involved in establishing cell quiescence but additional activation may result in cell death. He is investigating the molecular and biological roles of these
enzymes in cancer development and programmed cell death. Uncovering the apparent novel signaling pathway that cross-links cancer development of cells to programmed cell death should be directly exploitable for development of anticancer therapeutics.

Investigation into the molecular mechanisms of potential anticancer therapeutic agents on a variety of cancer cell types particularly human breast cancer cells is ongoing. Cancerous mouse embryo cell cultures and various human tumor cell cultures are used to screen anticancer agents. Studies on the molecular effects of a novel natural anticancer agent FR901228 on different intracellular metabolic signaling pathways should uncover the mechanism of this agent to selectively induce programmed cell death in cancerous cells. Dr. Wang determined that at least five important intracellular metabolic signaling pathways including the QIK pathway are affected. The information will be a basis for clinical trials using FR901228 in combination with other anticancer agents.

To mimic long term exposure of cells to the tobacco-specific carcinogen NNK, cultured human breast epithelial MCF10A cells were treated with low doses of NNK repeatedly. Cell clones which acquired tumor properties were isolated and subjected to studies of alterations in signaling pathways and cell growth. This is a pilot project to establish biochemical evidence of tobacco-specific carcinogens in the induction of human breast cancers.

Dr. Wang’s research is supported by the Center of Excellence, but his primary funding source is the National Institutes of Health (National Cancer Institute and National Institute of Dental Research).

**Dr. Xuemin Xu (molecular basis of neurodegenerative disease)**
Staff and Graduate students: G. Mao, Ph.D., Y. Shi, W. Gao, and E. Laag

The long-term goal of Dr. Xu’s research is to understand the molecular and cellular mechanism of Alzheimer's disease, and the formation of senile plaques, the pathological hallmark of this disease. His group is conducting two projects. One is to determine the pathological function of a substance (presenilin 1) in brain degeneration and the genesis of another substance (amyloid) observed in Alzheimer's disease. The other project is to determine the role of a certain protein (apolipoprotein E) in the formation and clearance of another protein involved Alzheimer’s disease (β-amyloid peptide).

Alzheimer’s disease is a progressive degenerative disorder, characterized by memory loss, confusion, and a variety of cognitive disabilities. An estimated four million American suffer from Alzheimer's disease. It is the fourth major cause of death in the United States following heart disease, cancer, and stroke. Alzheimer’s disease is the third most costly disease in the U.S. With the rapid growth of the senior population, Alzheimer’s disease poses, besides its tragic personal impact, serious problems to families, caregivers, government and health care in institutions.

Molecular genetic analysis of familial (heritable) Alzheimer's disease has led to the identification of three Alzheimer's disease-causative genes, those of β-amyloid precursor protein, presenilin 1, and presenilin-2. A fourth gene encoding apolipoprotein E has also been associated with Alzheimer’s disease as a risk factor but not as a causative gene for Alzheimer's disease.
Among these Alzheimer's disease-causative genes, mutations in presenilin 1 gene account for the majority of the known cases of familial Alzheimer's disease. Presenilin 1 has been implicated in two pathological events: (1) the generation of amyloid-β peptide, which is the building block of the toxic “plaques” characteristic of brain tissue from patients with Alzheimer's disease, and (2) programmed cell death, or apoptosis, a natural process in which unneeded or worn-out cells commit suicide. However, questions regarding the mechanisms by which the mutations in presenilin 1 proteins alter β-amyloid precursor protein processing and cause programmed cell death, as well as the normal function of presenilin 1, remain to be answered, which is the goal of their current research project.

Recent work resulted in the identification of a novel molecule (PSAP) which is capable of inducing programmed cell death, one of the mechanisms of neuronal cell death observed in Alzheimer's disease brains. It reacts specifically with presenilin 1. This finding established for the first time the molecular link between presenilin 1 and programmed cell death. Currently, we are conducting the experiments to determine the role presenilin 1 has in regulating PSAP-induced programmed cell death and with other Alzheimer's disease protein structures such as amyloid plaques and neurofibrillary tangles. This study will contribute to understanding the pathological function of presenilin 1 and will provide new insight into the mechanism of Alzheimer's disease. This study may also lead to the identification a new therapeutic target of Alzheimer's disease treatments.

Dr. Xu's studies in exploring the role of apolipoprotein E in β amyloid peptide formation suggests that these substances bind together to form complexes that interfere with the function of a critical enzyme α-secretase. The binding of apolipoprotein E to newly generated β amyloid peptide also may play a role in determining whether the latter is deposited or cleared. These studies will lead to a better understanding of the mechanism by which the apolipoprotein E is involved in Alzheimer's disease.

Dr. Xu's experiments are conducted with laboratory models, including brain (glial) cells containing the genes of apolipoprotein E and for amyloid precursor protein and in a yeast two-hybrid system. Because mutations in the presenilin 1 gene are associated with the majority of familial Alzheimer’s disease, these studies may provide important information for the early diagnosis and therapy of this disease.

Dr. Xu's work is funded by grants from the National Institutes of Health, Sigma Kappa and by the Alzheimer’s Association. He receives support also from the Center of Excellence.

**Dissemination of Research to the General Public**

**Dr. Nancy Howell**

One important function of the Center of Excellence is to provide information to the general public. This information may increase the public’s awareness of research and may provide individuals with valuable results that may improve their lives or their agribusiness.
To distribute information the College of Veterinary Medicine uses several methods. A general newsletter is distributed twice a year throughout Tennessee and beyond, highlighting research activities. Features concerning on-going research, in addition to results from concluded research projects, are included in the publication, *Veterinary News*, which is written for general audiences. Features also appear in other University of Tennessee publications, including *UT Agriculture*, *UT Alumnus* and *Tennessee AgriScience*.

In addition, news releases are distributed to state media and to regional and national media. Television and print publications produce numerous features about the College each year, many related directly to research conducted through the Center of Excellence. Public displays about the College frequently include highlights of COE research. Center of Excellence researchers are invited to share their research not only professionally, but as speakers to commodity groups, civic groups and other interested individuals.

Research is a component of the College’s web site, including COE projects such as the tall fescue toxicity research and other research projects.

Dissemination of research results through the news media helps inform the public and provides citizens with a better understanding of the practical applications of science in their daily lives.
FUTURE PLANS

Much of the Center's efforts during the present year (2001-2002) and next will concentrate on promoting the development of newly recruited investigators and in the promotion of initiatives to enhance the research capacity and direction of the Center. This year the Center is expending $322,500 to fund 13 projects proposed by investigators in the College of Veterinary Medicine and in the College of Agriculture and Natural Resources. In addition equipment grants totaling approximately $150,000 have been approved. Objectives for the two years are listed and explained as follows:

- The Center will actively seek areas for enhanced research possibilities to support its objectives. As an example, in December 2001 the Center sponsored a "brainstorming" session attended by representatives of the clinical departments of the College of Veterinary Medicine, biomedical engineering and material science faculty of UT's College of Engineering, and administrators from ORNL's Materials and Ceramics Division. Focus groups were formed to continue the interactions with the intent to determine areas for collaborative efforts. Plans include inviting a representative from the newly created National Institute of Biomedical Imaging and Biomedical Engineering to discuss areas of funding interest. Additional plans include a planning session with a DARPA official to explore funding opportunities in the bioterrorism arena.

- The Center will explore possibilities to interact cooperatively with other UT centers to promote joint research interests. As an example, the previously mentioned biomedical engineering focus groups involve faculty in the THEC Center for Materials Processing and Center for Environmental Biotechnology. In addition, the Center is currently supporting a project jointly funded with the Food Safety Center.

- The Center will participate conceptually and materially in strategic planning to develop areas of investigative strength in the College of Veterinary Medicine and the UTIA.

- The Center will increase its involvement in research training of veterinary students and graduate students through the introduction of matching travel grants and the increased use of stipend upgrades to help in the recruitment and retention of top quality graduate students.
TABLE 1

CENTER OF EXCELLENCE IN LIVESTOCK DISEASES AND HUMAN HEALTH BENCHMARKS OF FACULTY ACCOMPLISHMENTS

<table>
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<th>Number of:</th>
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<td>National Meetings</td>
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<td>FUNDING AGENCY</td>
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<td>Frank Andrews</td>
<td>The role of volatile fatty acids and calcium in gastric ulcer disease</td>
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<td>APR studies</td>
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<td>National Pork Producers</td>
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<td>Michael McEntee</td>
<td>Effect of n-3 and n-6 polyunsaturated fatty acids on growth and progression of prostatic cancer in vivo</td>
<td>DOD-Army</td>
<td>$312,938.00</td>
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<td>In vivo effects of resveratrol during early stages of intestinal tumorigenesis</td>
<td>American Institute of Cancer Research</td>
<td>$75,952.00</td>
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<td>Role of arachidonic acid and PGE2 as key mediators of intestinal tumorigenesis</td>
<td>American Institute of Cancer Research (Co-PI)</td>
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<td>Chemoprevention of intestinal tumorigenesis in the Apc Min/+ mouse by the inhibition of avB3 integrin</td>
<td>Pharmacia (Co-PI)</td>
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<td>Role of cyclooxygenase-1 and/or -2 in the mechanism of intestinal tumorigenesis</td>
<td>Pharmacia (Co-PI)</td>
<td>$245,398.00</td>
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<td>Jack Oliver</td>
<td>Reactivity of bovine vasculature to Ergovaline and Ergine of toxic tall fescue</td>
<td>USDA</td>
<td>$188,000.00</td>
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<td>Sex hormone concentrations in dogs with adrenal hyperplasia syndrome treated with melatonin</td>
<td>Morris Animal Foundation (Co-PI)</td>
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Stephen Oliver

Rapid, specific test for salmonella subtypes  
National Pork Producers’ Council  
$29,150.00  
06/01/99-07/31/00  
$2,925.55

Reproductive performance and reproductive disorders in Jersey cows  
American Jersey Cattle Club  
$543,663.00  
04/01/99-04/01/00  
$124,512.64

Development of chronic streptococcus uberis intramammary infections in lactating dairy cows  
Pharmacia and Upjohn  
$56,258.00  
01/01/99-12/31/00  
$36,086.69

Evaluation of the Uterine Environment During Clinical Mastitis in Lactating Jersey Cows  
American Jersey Cattle Club (Co-PI)  
$5,500.00  
04/01/00-04/01/01  
$3,342.89

Evaluation and use of BAM/FDA and rapid microbiological methods for on-farm surveys  
Food and Drug Administration (Co-PI)  
$475,610.00  
09/30/98-09/29/01  
$108,575.20

Evaluation of safety, specific immune responses, duration of immunity, and protection in dairy cows experimentally infected with Streptococcus uberis  
Pfizer  
$237,376.00  
10/25/00-10/24/02  
$99,280.56

Molecular genetics of Streptococcus uberis as it relates to its ability to cause mastitis in dairy cow  
Pharmacia and Upjohn  
$150,000.00  
08/01/00-07/31/05  
0

Efficacy of masticide for the treatment and prevention of teat lesions during the winter months  
Sporicidin International  
$33,738.00  
09/01/00-08/31/01  
$6,393.53

Evaluation of safety, specific immune responses and protection in experimentally infected dairy cows following administration of a S. uberis bacterin  
Pfizer  
$85,000.00  
07/12/00-04/12/01  
$81,573.89

Does clinical mastitis reduce steroidogenic function of the preovulatory follicle in lactating jersey cows  
American Jersey Cattle Association (Co-PI)  
$6,500.00  
04/01/01-04/02/02  
$29,254.00
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<th>Name</th>
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<td>Barry Rouse</td>
<td>Herpes zosterfilization</td>
<td>Smith-Kline Biological</td>
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<td>Immunity mechanisms in herpesvirus infections</td>
<td>National Institute of Allergy and Infectious Diseases – NIH</td>
<td>$1,656,250.00</td>
<td>01/01/01-12/31/05</td>
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<td>Mechanisms of herpetic stromal keratitis</td>
<td>National Eye Institute – NIH</td>
<td>$1,311,151</td>
<td>09/30/97-09/29/02</td>
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<td>Biodelivery sciences</td>
<td>Biodelivery Sciences</td>
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<td>HSP peptide complexes as a putative vaccine against herpes simplex virus</td>
<td>Antigenics Agency</td>
<td>$16,620.00</td>
<td>04/15/99-04/14/00</td>
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<td>Vaccination against herpes simplex virus</td>
<td>National Institute of Allergy and Infectious Diseases- NIH</td>
<td>$1,396,346.00</td>
<td>03/01/00-02/28/05</td>
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<td>Hildegard Schuller</td>
<td>Transplacental pancreatic carcinogenesis by NNI</td>
<td>National Institute of Health</td>
<td>$1,001,479.00</td>
<td>08/01/96-07/31/01</td>
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<td>FACS Vantage SE Cell Sorter/Flow Cytometer</td>
<td>National Center for Research Resources</td>
<td>$150,000.00</td>
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<td>Terry Schultz</td>
<td>Development of a bioremediation risk assessment scheme</td>
<td>US Environmental Protection Agency</td>
<td>$59,199.00</td>
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<td>Microbial transformation and molecular toxicology of estrogens</td>
<td>Water Resources Research Institute (Co-PI)</td>
<td>$295,334.00</td>
<td>12/07/98-12/06/00</td>
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<td>The Role of bioavailability in determining acceptable limits for the bioremediation of polychlorinated biphenyls</td>
<td>U.S. Department of Energy</td>
<td>$441,037.00</td>
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<td>Ecotoxicity of organic chemicals</td>
<td>Proctor and Gamble</td>
<td>$6,600.00</td>
<td>11/0199-10/31/01</td>
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<td>Degradation of natural estrogens in wastewater treatment facilities</td>
<td>University of Mississippi</td>
<td>$25,400.00</td>
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<td>Carla Sommardahl</td>
<td>Molecular analysis of PKD in the TGN737RPW mouse</td>
<td>NIH</td>
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<td>$96,897.45</td>
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<td>Name</td>
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<td>Amount</td>
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<td>Hwa-Chain Wang</td>
<td>Pathway leads to apoptosis in SCR-transformed cells</td>
<td>NIH</td>
<td>$517,520.00</td>
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<td>Biochemical evaluation of the functionality of mutated TBR-II and TBR-I receptors</td>
<td>Ohio State University</td>
<td>$192,453.00</td>
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<td>Biomedical applications of Conrex enhancer</td>
<td>Conrex</td>
<td>$14,910.00</td>
<td>11/15/00</td>
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<td>Xuemin Xu</td>
<td>Role of APOE in beta amyloid formation</td>
<td>Sigma Kappa</td>
<td>$30,000.00</td>
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<td>Role of apoE App processing</td>
<td>Alzheimer's Association</td>
<td>$80,000.00</td>
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<td>Role of apolipoprotein in AD amyloid formation</td>
<td>National Institute of Neurological</td>
<td>$677,968.00</td>
<td>05/01/99</td>
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<td><strong>TOTALS</strong></td>
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<td>Institution</td>
<td>College of Veterinary Medicine</td>
<td>Center</td>
<td>Livestock and Human Health</td>
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### Schedule 7

**CENTERS OF EXCELLENCE/CENTERS OF EMPHASIS**

**ACTUAL, PROPOSED, AND REQUESTED BUDGET**

<table>
<thead>
<tr>
<th>Institution</th>
<th>College of Veterinary Medicine</th>
<th>Center</th>
<th>Livestock and Human Health</th>
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<tbody>
<tr>
<td></td>
<td>Matching</td>
<td>Appropr.</td>
<td>Total</td>
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<tr>
<td>Expenditures</td>
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<td>265,250</td>
<td>530,500</td>
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<tr>
<td>Faculty</td>
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<td>Clerical/ Supporting</td>
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<td>Assistantships</td>
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<td>Total Salaries</td>
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<td>Non-Personnel</td>
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<td>Other Supplies</td>
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<td>Total Non-Personnel</td>
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<td>GRAND TOTAL</td>
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<td>Carryover State Appropriation</td>
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<td>32,178</td>
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<td>Total Revenue</td>
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<td>892,285</td>
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