
The University of Tennessee College of Veterinary Medicine
CENTER OF EXCELLENCE
ANNUAL REPORT



P. O. Box 1071
Knoxville, TN 37901-1071

Dean's Office: 865/974-7262
Research Office: 865/974-5572
<http://www.vet.utk.edu>

TABLE OF CONTENTS

I.	Program Report – Dr. Leon Potgieter	2
II	Viral Immunology Laboratory – Dr. Barry T. Rouse	8
III	Mastitis Research Laboratory - Dr. Stephen P. Oliver	9
IV	Virus Molecular Biology Laboratory – Dr. David A. Brian.....	11
V	Antibiotic Resistance Laboratory – Dr. Alan G. Mathew	12
VI	Biophysiological Pathogenesis Laboratory – Dr. Patricia K. Tithof.....	14
VII	Reproduction Laboratory – Dr. James D. Godkin	16
VIII	Gastritis Pathogenesis Laboratory – Dr. Frank M. Andrews.....	17
IX	Tall Fescue Toxicity Laboratory – Dr. Jack W. Oliver	18
X	Biological Activity Testing and Modeling Laboratory – Dr. Terry W. Schultz.....	20
XI	Experimental Oncology Laboratory – Dr. Hildegard M. Schuller	21
XII	Anticancer Molecular Oncology Laboratory – Dr. Hwa Chain Wang.....	23
XIII	Cancer Modeling Laboratory – Dr. Michael McEntee	25
XIV	Cell Biology Laboratory – Dr. Xuemin Xu	26
XV	Dissemination of Research to the General Public – Dr. Nancy Howell.....	28
XVI	Table 1 – Benchmarks.....	29

PROGRAM REPORT

Dr. Leon N.D. Potgieter, Assistant Director of the Center of Excellence in Livestock Diseases and Human Health

It is my privilege to report on yet another productive year by the Center of Excellence in Livestock Diseases and Human Health for 1999-2000. Once again the Center has met most of its objectives admirably (see table 1). **The return of the State's investment in the Center was almost six fold, a record (see "Funding Levels" below). The number of scientific communications in the form of refereed publications by Center-based investigators continues to be excellent (this year an average of 5.24 per participant).** Research results are disseminated to fellow scientists in prestigious, refereed international journals and to stakeholders in popular journals and magazines distributed regionally and nationally. **I am particularly impressed by the numerous invitations extended to several faculty in the Center to make keynote/plenary presentations at national/international scientific meetings.** They include Drs. D.A. Brian, B.T. Rouse, H.M. Schuller and T.W. Schultz.

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 for Agriculture and the University of Tennessee. Furthermore, they continue to ensure that the Center maintains its strong competitive position and contribute 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.

Accomplishments

The accomplishments and activities of most core laboratories in the Center are summarized in this report in a manner that can be comprehended by a wide readership. It is evident from these synopses that the Center:

1. Improves the quality of human life by improving animal health.
2. Augments livestock disease research capabilities in the Institute of Agriculture.
3. Identifies and characterizes laboratory and animal models of important human diseases.
4. Studies animal/laboratory models for better understanding of human health.
5. Studies the mechanisms of disease development and characterizes causative agents of common diseases important to the State of Tennessee.
6. Improves the capabilities of the College of Veterinary Medicine, the College of Agricultural Sciences and Natural Resources and the Agricultural Experiment Station to manage these diseases.
7. Improves the facilities to enable the College of Veterinary Medicine to study more effectively infectious and toxic diseases of animals.
8. Disseminates through the Extension Service practical information required to reduce the incidence of livestock diseases.
9. Develops new strategies for the prevention of disease.

10. Improves facilities and expertise to enhance research training.
11. Develops innovative approaches to the treatment of human diseases.

Research Funding

An important goal of the Center of Excellence in Livestock Diseases and Human Health is 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 two 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 of Excellence emphasizes the following six specific areas: **Infectious Diseases/Population Medicine, Toxicology, Reproduction, Host Defense, Molecular Genetics and Carcinogenesis.** Each participant contributes to at least one of these areas of emphasis. **The Center's underlying philosophy is to enhance the capacity of young or new investigators to compete for extramural funding and to assist established researchers in maintaining extramural support.** 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 and is chaired by Dr. Hildegard Schuller.

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

Dr. Joe Bartges. Comparison of 24-hour urinary excretions of total glycosaminoglycans, individual glycosaminoglycans, citrate, and sulfate in the development of urinary calculi.

Dr. David Bemis. Antibody response to *Pasteurella multocida* toxoid expressed as a recombinant fusion with *Bordetella bronchiseptica* fimbrial protein.

Dr. David Brian: How two terminal genetic structural elements regulate replication of the coronavirus RNA genome.

Dr. Hugo Eiler. Evaluation of a bovine placentome culture system for tumor growth.

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

Dr. Alan Mathew. Effects of antibiotic management on shedding and resistance patterns of *Salmonella typhimurium* in swine.

Dr. Michael McEntee. Effect of polyunsaturated fatty acids on the development of prostatic cancer.

Dr. Jack Oliver: Pathophysiological studies of tall fescue toxins in cattle.

Dr. Steve Oliver: Identification and characterization of *Streptococcus uberis* virulence factors.

Dr. Sharon Patton. A rapid test for the diagnosis of *Toxoplasma gondii*.

Dr. Barry Rouse: Macrophage dendritic cell interaction during immune induction by DNA vaccines.

Dr. Hildegard Schuller: Growth control by beta-adrenergic receptors in breast cancer cells.

Dr. Terry Schultz: Molecular size and shape considerations for xenoestrogens.

Dr. Carla Sommardahl. Chromosomal localization of modifier genes that vary the phenotypic expression of the *orpk* gene between the FVB/N and C3H mouse inbred genetic backgrounds; a mouse model for polycystic kidney disease.

Dr. Patricia K. Tithof. Components of cigarette smoke augment arteriosclerosis by a mechanism that involves the arachidonic acid cascade.

Additionally, COE funds were distributed to Drs. Xuemin Xu and Hwa Chain Wang as part of a “start-up” package approved during recruitment of these investigators.

Equipment and Facilities

Requests from 14 investigators for 18 pieces of equipment were funded by the Center of Excellence this past year. Researchers benefiting from the Center grants were Drs. Henry S. Adair, Joseph Bartges, David Brian, Dennis Geiser, James Godkin, Alan Mathew, Jack Oliver, Stephen Oliver, Barry Rouse, Hildegard Schuller, Terry Schultz, Carla Sommardahl and Hwa-Chain Wang. Criteria considered in the allocation of these funds included justification of need, equipment availability in adjacent laboratories, and the number of investigators who may benefit. The latter is of particular importance; i.e. whether these pieces of equipment could be used in a “core” role benefiting as many researchers as possible.

Renovations funded by the Center of Excellence include extensive repair and renovation of a 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 was funded by COE funds. This included an ultracentrifuge and the transmission electron microscope.

With support from the Center of Excellence, Dr. Hildegard Schuller and other COE participants secured a substantial equipment grant from NIH. This grant supported the purchase of a Fscan-Cell Sorter which will be used for basic and clinical research. Matching funds provided by the Center was critical in the success of this application. It will greatly enhance the research capacity of the Center.

Research Training

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/Ph.D. 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.

In addition, The University of Tennessee's College of Veterinary Medicine is one of the few colleges of veterinary medicine to be chosen as a site for a National Institute of Health Institutional Training grant. This five-year training grant on the "Molecular and Cellular Pathobiology of Environmental Disease" is funded through the National Institutes of Environmental Health Sciences. The grant began in 1995 and is funded through the year 2000; past year expenditures were \$218,931. This training grant is centered in the Department of Pathology, but also involves scientists from other departments as well as important collaborators in the Life Sciences Division at the Oak Ridge National Laboratory. Dr. David O. Slauson has been Program Director for the grant and Pathology Department Head. The research training sponsored by this NIH grant emphasizes the basic molecular and cellular biology of disease, including environmental disease. Three DVM graduate students were supported by these NIH funds and worked in the laboratories of COE-associated scientists. They are Drs. Brian Jull, Steven Grubbs and Sharon Witonsky.

Student Awards

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 eight 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 presented their work to faculty and staff of the Institute of Agriculture (see "Culture for Discovery") and at national scientific meetings. 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.**

Personnel Changes

Dr. G. Michael Shires, Dean and Director of the Center of Excellence in Livestock Diseases and Human Health retired at the end of the 1999-2000 fiscal year. The new Dean of the College of Veterinary Medicine, Dr. Michael Blackwell, will either serve as or appoint a new Director of the Center in the near future.

Recent recruitments of faculty with a significant research focus will benefit the Center of Excellence in the future (see discussion on "Funding Levels"). Over the past approximately two years we have recruited seven 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, head of the Department of Pathology in 1999, was able to recruit 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. Robert Moore, head of the Department of Microbiology, announced the recruitment of Dr. Pam Small from NIH, a well-known expert on tuberculosis. She will join the faculty in October 2000 and plans to do much of her research in the biosafety level three core facility in the veterinary teaching hospital. Tuberculosis is again an emerging disease and Dr. Small's research is likely to be highly competitive for extramural funding.

Dr. Monica Fann was recruited to fill the position of Associate Director of the Office of Laboratory Animal Care. This should have a significant impact in facilitating research at the College of Veterinary Medicine. Recently, Dr. Hildegard Schuller, interim head of the Department of Pathology, announced the approval of a search for an established researcher who would participate in the Center of Excellence of Livestock Diseases and Human Health.

Funding Levels

Extramural support for the Center of Excellence increased dramatically this year. Retirements and resignations have affected its growth for several years until this past year. Funding for the past ten years has been steady and recently, even declining, but **expenditures this year are the highest ever, surpassing by about \$300,000 the amount in 1990-91, the previous record.** Our investment in recruitment of researchers the past two to three years now is coming to fruition (see "Personnel Changes"). The State's investment in the Center of Excellence in Livestock Diseases and Human Health remains a healthy one. **Extramural funding expenditures this year totaled \$3,103,004 whereas the total funding level of all active grants and contracts supporting the Center amounted to \$10,863,253. The rate of return of State dollars for the year is 5.95!**

Our investigators brought in \$2,674,287 of new extramural funds in the past fiscal year (Drs. Godkin, Mathew, McEntee, S.Oliver, Rouse and Schuller). Also, I am very encouraged that since the end of the 99-00 fiscal year, several COE faculty recently appear to have secured new multi-year funding for COE-related research amounting to approximately \$2,605,000. They include Drs. Barry T. Rouse, Michael McEntee and Sharon Patton.

Culture for Discovery

In the past, the Center promoted a "Research Day" to showcase the achievements of participating faculty and to promote research at the College of Veterinary Medicine. Attendance at this event has declined significantly in recent years. It appears that the format of scheduling an entire day or two for this event was not appropriate for local

participation. **The past two years the Center has sponsored regular seminars, posters and presentations by participating investigators and students.** The program was advertised University-wide and has been very well attended and received. **It seeks to foster interest in research and to display the Center's role in promoting research at the Institute of Agriculture.**

The College hosted six visiting scholars in the "Biomedical Seminar Series" the past year. These scholars were selected, in part, to contribute to the Center's research emphasis.

External Review.

I look forward to another good year for the Center of Excellence in Livestock Diseases and Human Health. **I am confident that the support provided by the Center for some of our promising investigators will constitute the embryogenesis of established research programs. I anticipate that the extramural funding and other benchmarks will make an incremental jump again this year as a result of our strategic recruiting over the past two years (see "Funding Levels").** This should constitute an opportune time for reviewing our program. Therefore, the periodic external review of the Center is recommended for the coming year.

VIRAL IMMUNOLOGY LABORATORY

Dr. Barry T. Rouse

Fellows, Staff and Graduate Students: S. Chun, S. Deshpande, S. Kug Eo, U. Kumaraguru, S. Lee, T. Sobhani, M. Zheng

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.

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.

MASTITIS RESEARCH LABORATORY

Dr. Stephen P. Oliver.

Fellows and Staff: R. Almeida, W. Fang, B. Gillespie, M. Lewis, D. Luther, S. Ivey, L. Coleman.

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.

VIRUS MOLECULAR BIOLOGY LABORATORY

Dr. David A Brian

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.

ANTIBIOTIC RESISTANCE LABORATORY

Dr. Alan G. Mathew

Staff and graduate students: R. Clift, S. Chattin, D. Arnett, P. Cullen, P. Ebner, K. Garner, G. Pulliam

Antimicrobial compounds are commonly used in US livestock systems. 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 production affect antibiotic resistance patterns, concentrations, and shedding of foodborne 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 foodborne pathogens and antibiotic resistance of microorganisms in livestock.

Recent work by the group has included a pioneering microbiological survey of swine farms that used or excluded antibiotics in the animals. They determined that pathogenic bacteria from farms that excluded antibiotics were more sensitive to antibiotics. However, resistant isolates occurred on both farm types, and young, recently weaned animals from all farms harbored the greatest number of resistant isolates. This may indicate that stresses associated with weaning affect bacterial antibiotic resistance patterns, regardless of whether antibiotics were used on the farm.

Dr. Mathew investigated also the effects of antibiotic treatment regimens on resistance patterns of *E. coli* and *Salmonella* bacteria in pigs. They inoculated pigs with a swine pathogen (K88 *E. coli*) and a foodborne pathogen (*Salmonella typhimurium*) and then treated with aminoglycoside, sulfonamide, and beta lactam antibiotics using various dosing schemes. Dosage methods, dosage levels and rotation with dissimilar antibiotics affected the genesis of resistance.

Another important discovery was that *E. coli* associated with livestock became resistant much more quickly than *Salmonella* in the same host, possibly indicating a greater ability to acquire resistance elements such as plasmids (R-factors), or a greater ability to generate chromosomal resistance elements. This finding will be very important as we assess the agricultural use of antibiotics and the risks of generating resistance in foodborne pathogens. They now are characterizing the bacterial resistance elements using various DNA-based techniques to determine the relative importance of R-plasmids and chromosomal elements in the acquisition and persistence of antibiotic resistance.

Dr. Mathew is continuing other work to determine how animal stressors such as crowding, low sanitation, and temperature stress affect the prevalence of antibiotic resistance in *Salmonella typhimurium* and *E. coli* in swine. Preliminary data indicate that such stressors increase the percentage of resistant isolates.

Work with Dr. Stephen Oliver has focused on the development of rapid methods for detection and differentiation of *Salmonella* variants. This will allow us to determine the origin and reservoirs of those bacterial variants that are most responsible for foodborne illness in livestock operations. They have determined that a linked PCR-ELISA assay is capable of rapid (less than 24 hours) differentiation of *Salmonella* bacteria from livestock samples. Such techniques will be instrumental in the implementation of on-farm Hazard Analysis Critical Control Points (HACCP) strategies for management of specific foodborne pathogens.

Dr. Mathew's research is supported by the Center of Excellence but primary funding for this work is derived from the International Life Science Institute and the National Pork Producers Council.

BIOPHYSIOLOGICAL PATHOGENESIS LABORATORY

Patricia K. Tithof

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 concerning a potential effect of NNK on the cardiovascular system. Evidence indicates a role for the beta-adrenergic system in the cause of atherosclerosis because beta-adrenergic blocking agents prevent endothelial cell injury. Beta-adrenergic blockade also reduces endothelial cell injury induced by cigarette smoke.

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.

REPRODUCTION LABORATORY

Dr. James D. Godkin

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.

GASTRITIS PATHOGENESIS LABORATORY

Dr. Frank M. Andrews

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 genesis and mechanisms of gastric ulcer development in a horse model. Gastric ulcer disease is a common problem in performance horses. The latter 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 release of by-products such as volatile fatty acids (acetic, butyric, valeric and propionic acids). Previous research in this laboratory established that high concentrations of volatile fatty acids 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 volatile fatty acids, especially butyric and propionic acids in contact with the stomach lining of horses and in the presence of stomach acid (pH = 1.5 and 4.0) leads to damage to cell's sodium transport system, cellular swelling, irreversible tissue damage and ulceration. These findings suggest that dietary modification, reducing the formation of these acids, may be helpful in prevention or decreasing the incidence of gastric ulcer disease in horses. These results have implications for several animal species including humans.

This research is funded by the Grayson-Jockey Club Research Foundation, the Comparative Gastroenterology Society and the Center of Excellence.

TALL FESCUE TOXICITY LABORATORY

Dr. Jack W. Oliver

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.

BIOLOGICAL ACTIVITY TESTING AND MODELING LABORATORY

Dr. Terry W. Schultz

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.

This past year Dr. Schultz and his students have examined the ability of different molecular-orbital quantum-chemical parameters to quantify electrophilic reactivity. They have developed quantifiable chemical measurements for different types of interactions or bonding between the toxicant and its site of action.

Most recent work has focused on chemicals whose toxicity does not model well by this descriptor. Julie Seward, a senior doctoral student supported in part by COE funding, has shown that certain aromatic chemicals follow a unique model.

Other work in Biological Activity Testing and Modeling Laboratory has extended this approach to identify properties that best model toxic responses where interactions occur between the compound and a specific cell receptor. In the past year, this work focused on understanding the properties governing hormonal effects associated with the human estrogen receptor. Through an examination of the estrogenic activity of a series of selected chemicals using the *Saccharomyces crevice*-based *lac-Z* reporter assay, Dr. Schultz and his colleague were able to characterize and quantify the potency of estrogens that occur in the environment.

EXPERIMENTAL ONCOLOGY LABORATORY

Dr. Hildegard M. Schuller

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.

ANTICANCER MOLECULAR ONCOLOGY LABORATORY

Dr. Hwa-Chain R. Wang

Fellows: K. Fecteau, J. Mei, Y. Sun, M. Tan

Dr. Wang's long-term research goals concerns 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).

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 the molecular and biological roles of mutations in genes that control tumors (suppressor genes - transforming growth factor beta receptor genes, *Tbr-II* and *Tbr-I*) in the development of human oral cancers.

Dr. Wang has identified a family of novel enzymes (kinases: SAMK/Krs1 and QIK) that are activated in normal resting cells and in cancerous cells undergoing programmed cell death (as a result of a variety of physiological, chemical or physical stresses). 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.

The role of mutations in tumor suppressor genes (*Tbr-II* and *Tbr-I* genes) in the multi-stage development of human oral cancers will require the detection and analysis of

the mutations in these genes isolated from pre-malignant and malignant cells of the oral cavity. Specific mutations in the *TbR-II* and *TbR-I* genes will be identified by their functional inability to transmit signals to suppress tumor formation. This research is a part of a major collaborative program project to study molecular events in progression and prevention of oral cancer.

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).

CANCER MODELLING LABORATORY

Dr. Michael McEntee

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 E₂), 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 E₂ 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 E₂ 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.

CELL BIOLOGY LABORATORY

Dr. Xuemin Xu

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 presenelin 1. This finding established for the first time the molecular link between presenelin 1 and programmed cell death. Currently, we are conducting the experiments to determine the role presenelin 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.

TABLE 1**CENTER OF EXCELLENCE IN LIVESTOCK DISEASES
AND HUMAN HEALTH BENCHMARKS OF FACULTY ACCOMPLISHMENTS**

	1999-2000	
	<u>Actual</u>	<u>Average</u>
Number of:		
Articles	89	(5.24)
Books or Book Chapters	10	(0.63)
Published Proceedings	18	(1.13)
Total Publications	117	(7.31)
Abstracts	20	(1.25)
Invited Participation at:		
Regional Meetings	53	(3.31)
National Meetings	44	(2.75)
Faculty in Center	16	
Number of Visitors	6	

