



1994

Center of Excellence Annual Report, July 1993-June 1994

College of Veterinary Medicine

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The University of Tennessee
College of Veterinary Medicine

Center of Excellence in Livestock Diseases and Human Health

Annual Report

July 1, 1993-
June 30, 1994

G.M.H. Shires, Dean
College of Veterinary Medicine
The University of Tennessee
Knoxville, Tennessee

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A WORD FROM THE DEAN

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July 15, 1994

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The Center of Excellence in Livestock Diseases and Human Health has completed another productive year. This success can be traced to the Center's continuing efforts to focus on selected areas of emphasis, support the most productive projects, and invest in only the most promising young investigators and new projects. Again this year recommendations by both the external and internal advisory committees have provided the scientific basis for both policy and technical decisions made by the Center.

While the Center supported projects in the areas of Inflammation and Host Defense, as well as Infectious Diseases and Population Medicine, this past year particular emphasis was placed on Growth Factors and In Vitro Toxicology. With this emphasis two new faculty members will be part of the Center as of July 1994. Dr. Joyce Merryman will join the Growth Factor group. Dr. Daniel Ward will join the In Vitro Toxicology and Toxicokinetics group. Therefore, capital equipment purchases were targeted toward these groups.

A multiuser *in vitro* toxicology laboratory was the result of College Renovations. Equipment for this facility was purchased with Center funds. A large sterilization unit was also purchased. Although the sterilizer may be used by all members of the College, it will directly benefit the Growth Factor Group.

Continued support of the Center will assist us in research focused on livestock disease and human health which is important to the region, the State of Tennessee and the nation as a whole. This coming year we will continue to do the things we do with excellence as evident by the projects headed by Drs. Bochsler, Brian, McDonald, Potgieter, Rouse, Schuller, and Wilkinson.

We will continue to place a premium on projects using modern molecular biological techniques because they hold the greatest promise for extramural funding. We also plan to strengthen our research in the area of applied molecular genetics and our interaction with Oak Ridge National Laboratory. Therefore, we have renamed the Growth Factor Group the Growth Factor and Molecular Genetics Group.

We end this year and look forward to the next with a successful Center, in large part due to a diligent and competent core of faculty and staff dedicated to excellence in research in livestock disease and human health.

Sincerely,



G.M.H. Shires
Dean and Director of the Center

1993-94 CENTER OF EXCELLENCE MEMBERS

PHILIP N. BOCHSLER, D.V.M., PH.D.
Assistant Professor
Department of Pathology

DAVID A. BRIAN, D.V.M., PH.D.
Professor
Department of Microbiology

DONITA L. FRAZIER, D.V.M., PH.D.
Assistant Professor
Department of Comparative Medicine

JAMES D. GODKIN, PH.D.
Assistant Professor
Department of Animal Science

KEVIN A. HAHN, D.V.M., PH.D.
Associate Professor
Department of Comparative Medicine

TED P. McDONALD, PH.D.
Professor
Department of Animal Science

CHARMI MENDIS-HANDAGAMA, PH.D.
Assistant Professor
Department of Animal Science

MARK MILLER, PH.D.
Research Assistant Professor
Department of Pathology

LINDA MUNSON, D.V.M., PH.D.
Assistant Professor
Department of Pathology

JACK W. OLIVER, D.V.M., PH.D.
Professor
Department of Comparative Medicine

STEPHEN P. OLIVER, D.V.M., PH.D.
Associate Professor
Department of Animal Science

LEON N. D. POTGIETER, B.V.SC., PH.D.
Professor and Head
Department of Comparative Medicine

BARRY T. ROUSE, B.V.SC., PH.D.
Professor
Department of Microbiology

TERESA K. ROWLES, D.V.M., PH.D.
Assistant Professor
Department of Animal Science

HILDEGARD M. SCHULLER, D.V.M., PH.D.
Professor
Department of Pathology

TERRY W. SCHULTZ, PH.D.
Professor
Department of Animal Science

ROBERT M. SHULL, D.V.M.
Professor
Department of Pathology

DAVID O. SLAUSON, D.V.M., PH.D.
Distinguished Professor and Head
Department of Pathology

J. ERBY WILKINSON, D.V.M., PH.D.
Assistant Professor
Department of Pathology

Programmatic Report

I. PROGRAMMATIC REPORT

I.A. RENAMING OF A RESEARCH GROUP

One of the five major research groups, Growth Factors, has been renamed Growth Factors and Molecular Genetics. This name change reflects the Group's and Center's emphasis on molecular genetics and the expanded interaction with the Molecular Genetics group of the Biology Division of Oak Ridge National Laboratory. This interaction is coordinated by **DRS. E. WILKINSON** and **D. SLAUSON**. The area of molecular genetics focuses on the characterization of the role of specific genes in important diseases in mutants, especially mice. Involved in this is the phenotypic, cellular, and molecular analysis of spontaneous mutants, insertional mutants, saturation mutants, knockouts, and traditional transgenic animals. Studies such as these use all the tools of modern molecular biology, as well as the traditional tools of immunology, pathology, and developmental biology to completely evaluate the role of specific genes in specific diseases.

I.B. PERSONNEL CHANGES

1.B.1. Personnel Additions

DR. DANIEL A. WARD joins the "*In Vitro* Toxicology and Toxicokinetics" (IVTT) group. He received his D.V.M. from The University of Tennessee and his Ph.D. from the University of Georgia.

DR. JOYCE I. MERRYMAN joins the "Growth Factors and Molecular Genetics" (GFMG) group. She received both her D.V.M. and Ph.D. from The Ohio State University.

1.B.2. Personnel Deletions

None

I.C. CENTER GOALS AND MAJOR PROGRAMMATIC ACTIVITY

GOAL 1: TO IMPROVE THE QUALITY OF HUMAN LIFE THROUGH BETTER ANIMAL HEALTH.

Research performed by **DR. J. GODKIN** in conjunction with **DR. L. MUNSON** has focused on fetal-maternal interactions that contribute to the maintenance of pregnancy and growth and development of the embryo in domestic farm animals. This work has led to a new Department of Agriculture grant which began this year.. This past year, studies were completed on the fetal-maternal interactions between the embryonic protein

interferon tau (τ) believed to be the embryonic signal that mediates the maintenance of early pregnancy in ruminants. Two uterine cell culture systems were established, and it was demonstrated that recombinant bovine IFN τ inhibited, and oxytocin stimulated, uterine epithelial cell production of prostaglandins (PG) F $_{2\alpha}$ and E $_2$ but had no measurable effects on stromal cells. In addition, IFN τ had no effect on uterine cell protein production or PG synthase mRNA production. *Results demonstrate that IFN τ maintains early pregnancy by inhibiting uterine epithelial cell production of PGF $_{2\alpha}$.* This action maintains pregnancy through maintenance of progesterone production by the corpus luteum which is susceptible to regression mediated by PGF $_{2\alpha}$.

Expression of transforming growth factor-beta (TGF- β), a potent regulator of cell growth, was identified and characterized in ovine and bovine uterine endometrium and embryos. TGF- β 1 and β 2 was identified in tissues by immunocytochemical procedures and TGF- β 1, β 2 and β 3 mRNA's were quantitated by scanning densitometry of slot blots. Each TGF- β isoform exhibited different temporal and tissue specific expression suggesting unique functions. IFN τ was shown to diminish uterine TGF- β expression.

Retinol-binding protein (RBP), the vitamin A transport protein, was discovered to be a product of ovine and bovine blastocysts, placental membranes and uterine endometrium. Expression of RBP and its mRNA was identified in tissues by immunocytochemistry and *in situ* hybridization procedures. Changes in the level of mRNA expression in uterine and conceptus tissues were quantitated by scanning densitometry of slot blots. *Expression of conceptus RBP was shown to be developmentally regulated, while uterine RBP expression was modulated by ovarian steroids and the conceptus protein, IFN τ .*

**GOAL 2: TO AUGMENT LIVESTOCK DISEASE RESEARCH CAPABILITIES IN THE
INSTITUTE OF AGRICULTURE.**

DR. P. BOCHSLER and **DR. D. SLAUSON** continue their studies of the response of cattle to the presence of gram-negative bacterial pathogens, particularly one of the toxins released by these bacteria. Bacteria of this type are known to be responsible for several types of clinical disease of cattle, including forms of pneumonia, mastitis, and diarrhetic disease. The toxin they study is referred to as endotoxin or LPS, and it incites fever, depression, and other clinical symptoms in cattle, and may cause or contribute to causes of death. They have made significant progress in identification of some of the pathways and mediators that are important in the response of cattle to endotoxin. A few of the results of these studies include *identification of a bovine serum LPS-binding protein that is important in the response to endotoxin/LPS*, and study of mediators of inflammatory disease in cattle, such as TNF α and tissue factor.

**GOAL 3: TO IDENTIFY AND CHARACTERIZE ANIMAL DISEASES THAT ARE
ANALOGOUS TO HUMAN DISEASES.**

DR. S. MENDIS-HANDAGAMA has continued her basic research on the role of peroxisomes in the steroidogenic process that was investigated using primarily Leydig cells *in vivo* in adult rats. Previously, her research has shown that sterol carrier protein-2 (SCP₂), which binds to cholesterol in 1:1 molar ratio is highly concentrated in Leydig cell peroxisomes. Continuation of this line of research has recently revealed yet undiscovered aspects in the pathway of steroidogenesis in general, and particularly in Leydig cells. The results showed that with acute luteinizing hormone (LH) stimulation: (a) SCP₂ gets highly concentrated in Leydig cell peroxisomes (5-fold above control values); (b) this is due to redistribution and not due to *de novo* synthesis of SCP₂; (c) peroxisomes rich in SCP₂ fuse with Leydig cell mitochondria and, (d) this fusion of peroxisomes and mitochondria is associated with transfer of SCP₂ into mitochondria.

Whether SCP₂ delivers cholesterol at the inner mitochondrial membrane, or whether SPC₂ could travel into the mitochondria with cholesterol was an unresolved issue in the process of steroidogenesis. Though tissue differences are possible, her studies showed for the first time that *in steroidogenic cells in the testis, SCP₂ moves into mitochondria with LH stimulation, suggesting that SCP₂ delivers cholesterol into the inner mitochondrial membrane for side chain cleavage reaction to product pregnenolone.* Moreover, these studies revealed for the first time that *peroxisomes are responsible for this mechanism of cholesterol transport during steroid hormone biosynthesis in Leydig cells.* This work has lead to Dr. S. Mendis-Handagama receiving this year a National Science Foundation grant to continue her work on understanding the mechanisms involved in movement of peroxisomes towards mitochondria for cholesterol delivery.

During the past year **DR. R. SHULL** continued work on his long-term program aimed at discovering effective novel therapy for canine MPS I, a model of the same disease in children. A total of 6 gene transfer/gene therapy experiments were conducted utilizing 4 MPS I-affected dogs and 3 unaffected animals. The initial goal was to document survival of hematopoietic stem cells in long-term bone marrow culture after infection with a retroviral vector carrying the gene for the enzyme missing MPS I. In later experiments, transfected cells were returned to 2 affected dogs and evidence of *in vivo* gene expression sought. Initial results were not encouraging, as has been the experience at other institutions where similar work on gene therapy in large animal species is being conducted. In collaboration with individuals at the University of Toronto, Canada, a third dog was treated with cells modified genetically in a long-term culture system. Results of these studies are still being collected at this time, but evidence of at least intermittent enzyme production has been found. Six MPS I-affected dogs were

also treated by intravenous infusion of recombinant human enzyme. These trials were successful in documenting that human enzyme could be taken up by canine cells and that it could have dramatic effects in some tissues on the abnormal storage products that are typical of the untreated disease.

DR. T. SCHULTZ has completed studies aimed at predicting the toxicity of oxygen-containing aliphatic and aromatic industrial chemicals. This work was designed to develop mathematical models that predict the toxicity of chemicals from their molecular structure and, thus, eliminating the need to test them on animals. This past year, work centered on examining the toxicity of esters including benzoates and phthalates. The investigations have shown that in *animals with high esterase activity, esters are more toxic than in animals with low esterase active*. Moreover, when toxicity was corrected for this activity and a volume fraction analyses performed, a technique developed by Dr. Schultz's group, *toxicity can be attributed to the nonpolar narcosis mechanism of action*. This information goes a long way in explaining the highly variable toxicity data for esters reported in the literature.

GOAL 4: TO STUDY ANIMAL MODELS FOR BETTER UNDERSTANDING OF HUMAN DISEASE.

DR. H. SCHULLER's research programs in the area of lung tumors has continued to do very well. Among the findings of Dr. Schuller's laboratory is that *women who smoke during pregnancy genetically predispose their children to lung tumors as the children become adults*. Since not all lung tumors are the result of smoking, research is expanding to determine how other pollutants impact the development of lung cancer. Moreover, Dr. Schuller's research has been to develop a neuroendocrine lung carcinogenesis model in hamsters. She has *determined the etiology of the model*. Moreover, in collaboration with Byk Guldden Pharmaceuticals, she and **DR. K. HAHN** evaluated a novel calcium channel blocker, Dexniguldipine, which inhibits tumor cell growth. This agent is currently in animal and human clinical trials.

The main objective during the past year for **DR. B. ROUSE** was to understand the role of various cytokines in the immunopathogenesis of herpetic stromal keratitis (HSK). Previous studies from the laboratory have shown the predominant role of Th1 derived cytokines. This was done by isolating cells from the eye and stimulating them *in vitro* in various ways. While this could be a true representation of events *in vivo*, there is still a possibility that *in vitro* manipulations influencing the cytokine profile. Hence, in order to obtain a better picture of *in vivo* events, they examined the cytokine mRNA profile in total cellular RNA from cells isolated from the cornea. For this, they developed a highly sensitive and specific quantitative competitive reverse transcription polymerase chain reaction (RT-Q-PCR). The development of this approach was essential since the

cytokine mRNA *in vivo* is in low abundance and the number of cells that can be obtained from the mouse cornea is very limited. The detailed methodology has been published in an immunological journal. Essentially, the quantitation relies on the competition of target DNA and internal control DNA for all the reactants including the primer sets.

The cytokine gene expression study was extended to an extremely valuable but expensive model, namely the SCID mouse, which was reconstituted to generate HSK with T cells from immune or naive mice (2). Overall, the SCID mouse data was consistent with immunocompetent mice. In general, the *in vivo* cytokine gene expression analyses during HSK demonstrated a clear role for Th-1 derived cytokines and it appears that IFN- τ is the potent inflammatory mediator in the cornea.

Using this approach, they examined the inflammatory and T cell derived cytokine mRNA levels in both immunocompetent and reconstituted SCID mouse during the course of HSK development. Their data show that with regard to inflammatory cytokines, in both draining lymph nodes (DLN) and cornea, IL-1, IL-6, TNF α mRNA were expressed in relation to the clinical severity during the onset and clinical phases of HSK. There was a dramatic influx of TNF α in the more severe clinical lesions. The concentration of inflammatory cytokines were more in the ocular tissue over the DLN. With regard to the T cell derived cytokines, there were two surges in both DLN and ocular cells. One confined to early phase at 7-day post infection and other to the clinical phase. In the DLN, both Th1 and Th2 were seen, but Th1 predominated. In the ocular tissues, a transient Th0 type (IL-2, IFN τ , IL-4) was evident in the early phase. Later phase in the ocular tissue was confined to the Th1 pattern.

Presently, Dr. Rouse and his co-workers are working on *in situ* hybridization method in order to further characterize the cytokine profile (gene expression) and viral gene expression during the course of HSK. This is to identify and localize the cells involved in the immunopathology of HSK. Towards this end, they have developed specific probes for detection of viral genes, inflammatory cytokine genes and T cell derived cytokine genes. They have standardized the methodology for the detection of gB (viral gene), IL-2, IFN- τ , IL-6 mRNA in cells prepared by cytopsin. Studies are in progress in order to apply this procedure to sections of cornea obtained during various stages of the development of HSK.

DR. M. MILLER in conjunction with **DR. H. SCHULLER** has been examining the role of oncogenes in transplacental carcinogenesis. One goal of this research project was to adapt the polymerase chain reaction technique to amplify small quantities of DNA from paraffin-embedded tissues. This has been successfully done, and amplified sequences of the hamster Ki-ras gene obtained. In dose response experiments, a readily detectable signal was obtained by Southern blot analysis of the amplified DNA in less than 24 hours of autoradiographic exposure from as little as the equivalent of a 1 micron piece of tissue from embedded hamster lung samples. This technique was pivotal to Dr.

M. Miller's success this year in obtaining a National Institutes of Health grant to examine the role of oncogens in transplacental carcinogenesis in the mouse model. During this past year, Dr. M. Miller has trained Dr. Schaeffer and her technical staff on the proper way to perform the high i.p. injections on pregnant mice. They have *established a breeding colony and set up an excellent record keeping system to track the mice through the one-year study*. They have initiated the *carcinogenicity bioassay and have filled the first treatment group*. As noted earlier, his previous research on gene mutations were conducted in hamsters. Thus, he has also used COE support to purchase amplimers to the mouse *Ki-ras* gene and have amplified DNA by the standard procedures described in the literature and obtained very good results. His group are also *standardizing our research protocols to extract DNA from paraffin embedded mouse tissues*. Thus, when the mouse bioassay is completed and the mice are euthanized, they will be ready to begin the molecular biology analysis immediately. Use of the COE funds has thus allowed him to optimize the reaction conditions early in the study, which will save him a great deal of time later.

During the past year, **DR. E. WILKINSON** has continued to make great strides in characterizing the cells from Tg737 mice that are responsible for development of the kidney and liver lesion in this transgenic mouse model of autosomal recessive polycystic kidney disease. This work is in conjunction with investigators at the Biology Division of Oak Ridge National Laboratory. In general, studies continue to further *define normal structure and function of the Tg737 gene, determine how mutations in the gene result in the pathological changes seen in the disease and to identify other genes associated with expression of the disease*. Specific examples have been included. Dr. Wilkinson's laboratory has begun *an analysis of the ability of transformed cells from previously isolated cell lines to produce both hepatocellular carcinomas and cholangiocarcinomas in mice*. These studies involved both the transplantation of transformed oval cells into nude mice and the treatment of mutant mice with hepatic carcinogens. They have *generated transgenic mice that express the Tg737 gene from a different promoter and used these mice to correct the defect in the kidney but not in the liver of offspring of mutant mice bred to transgenic mice*. Moreover, they are now using these "phenotype rescued" mice to evaluate the long term effects of the liver lesion on the health of the animals and to utilize these animals for studies of the role of oval cells in liver cancer. In addition, collaborative efforts have also generated data to *suggest that the Tg737 gene may be involved in liver cancer*. Mutations in the Tg737 gene have been found in a high percentage of chemically induced liver tumors in rodents. Further work by Dr. Wilkinson's group this past year completed the analysis of the FVB TgN737Rpw mice. This work *described the detailed pathology of the kidney and liver lesions*.

In another collaborative project, **DR. B. ROUSE's** laboratory with **DR. E. WILKINSON's** laboratory have recently completed an analysis of the cellular basis for the

severe lymphoproliferative disease in scurfy mice. These investigations utilized a number of "knockout" mice that lacked important molecules involved in the normal immune response. The X-linked recessive mutation scurfy (*sf*) results in a phenotype characterized by a rapidly fatal immune disorder involving the skin and lymphoid systems. In order to understand better the immunobiology of the scurfy mouse, they have undertaken a series of experiments that investigate the role of the thymus and its components in the etiology and expression of scurfy disease. A few of the several important observations are noted. Results from experiments involving the transplantation of scurfy fetal thymi into H-2-compatible nude and SCID mice indicated that *scurfy disease acts upon the fetal thymic environment as early as day-14 in development*. Other experiments involving the selective transfer of wild-type or scurfy thymic components demonstrated that *both sf-derived T cell precursors, and a genetically sf thymic microenvironment were necessary for disease expression*. In addition, the roles of CD4 or CD8 single-positive T cells were evaluated. This was accomplished by treating scurfy neonates with monoclonal antibodies directed against the CD4 or CD8 molecules and by breeding the scurfy mutation onto mice that lacked either CD4+ or CD8+ T cells. *Results implicated CD4+ CD8- T cells as the critical effector cells in the pathogenesis of scurfy disease*.

**GOAL 5: TO UNDERSTAND THE PATHOGENESIS AND CHARACTERIZE THE
CAUSATIVE AGENTS OF COMMON DISEASES IMPORTANT TO TENNESSEE.**

Fescue toxicosis remains one of the most costly disease syndromes to Tennessee beef producers. Studies in **DR. J. OLIVER**'s laboratory during the past year have continued to focus on the mechanism(s) of toxic tall fescue alkaloids in cattle, using alkaloid effects on blood vessels to project cause-and-effect relationships to the animal as a whole.

During the past year, the mastitis research team in **DR. S. OLIVER**'s laboratory has developed a technique using Instagene purification matrix for DNA isolation. This technique is rapid, economical, and yields sufficiently pure DNA template for polymerase chain reaction from both gram-negative and gram-positive bacteria for randomly amplified polymorphic DNA (RAPD) fingerprinting. They also have evaluated 20 potential oligonucleotide primers for RAPD fingerprinting of 19 different reference strains belonging to the family *Enterobacteriaceae*, and the genera *Enterococcus*, *Staphylococcus*, and *Streptococcus*. Criteria for selection of potential primers were based on fingerprint patterns analyzed for number and size of RAPD fragments and absorbance of fragments using gel analysis software. *A proposed bacterial species identification scheme was developed. This scheme will be helpful in identifying particular species associated with a given mastitis case.*

Coronaviruses cause some of the most costly respiratory and gastroenteric diseases in domestic livestock and fowl. Yet vaccines to control their spread are often not effective because of the special challenges of inducing immunity at mucosal surfaces and because coronaviruses mutate rapidly. **DR. D. BRIAN**'s laboratory investigates coronaviruses that infect many animal species, including cows. By studying the molecular biology of bovine coronavirus replication, Dr. Brian's laboratory has *identified potential sites in the viral genome for targeted antiviral therapy and uncovered two potential mechanisms of persistent infection*. Most excitingly, they have *discovered a subviral replicon that they have cloned and engineered to carry viral immunogens and other potential antiviral or therapeutic molecules*.

GOAL 6: TO IMPROVE THE CAPABILITIES OF THE COLLEGE OF VETERINARY MEDICINE, THE COLLEGE OF AGRICULTURE, AND THE AGRICULTURAL EXPERIMENT STATION TO DEAL WITH THESE DISEASES.

Work continues on the molecular biology and immune response to bovine viral diarrhea virus. **DR. L. POTGIETER**'s laboratory has as their goal with BVDV research, the development of improved diagnostics and immunoprophylaxis. Investigation focuses on the structural proteins of this virus. These proteins are responsible for induction of the protective immune response. The three major structural protein-encoding genes have been cloned, analyzed, and expressed. Using these expression products, their *goal is to develop a sensitive and specific serological test that is very economical*. Further, these proteins may be subcloned into live virus vectors for use in recombinant live vaccines. Antibodies induced by these proteins in animals were found to have neutralizing capabilities. This pathogen is associated with respiratory and reproductive disease leading to considerable financial loss. *Improvement in these two areas of BVDV control, diagnosis and immunoprophylaxis, will have significant impact on the cattle industry*.

Additionally, work is ongoing to evaluate the ovine respiratory syncytial virus at the molecular level. The surface protein responsible for viral attachment and induction of protective immunity to this virus, the G glycoprotein, has been cloned and sequenced, and the nucleotide sequence has been extensively analyzed. The gene encoding the nonstructural proteins 1a, 1b, and 1c of ORSV has also been cloned, sequenced, and analyzed. *This is the only ruminant RSV 1A-C genes to be sequenced to date*. Cloning and sequencing of the G glycoprotein of ovine RSV adds to the information they have from the bovine RSV. This knowledge will be useful for the development of sensitive and specific diagnostic assays, as well as for the development of an effective vaccine. Further, *the analogy to the human RSV may prove to be very useful for development of a suitable animal model for this significant human pathogen*. This work is of interest to

NIH, and we anticipate significant extramural funding next year. The *sequencing of the IC gene will also be important for guiding vaccine and diagnostic assay development.*

GOAL 7: TO IMPROVE FACILITIES TO ENABLE THE COLLEGE OF VETERINARY MEDICINE TO STUDY MORE EFFECTIVE INFECTIOUS AND TOXIC DISEASES AFFECTING ANIMALS.

The COE research efforts are housed mainly in the Clyde M. York Veterinary Medical Teaching Hospital. This building which also contains clinical and teaching facilities faces a severe space shortage. While other collaborative research space is located in McCord Hall (Animal Science), Walters Life Science Building (Microbiology), and at The University of Tennessee Medical Center and Memorial Research Hospital (Medical Biology), research space everywhere is a premium. Long-term plans forecast a solution for this problem. However, in the short-term reorganization, and minor renovation are the stop gap measures. Research laboratories are multuser and generally organized around specific research approaches and are usually shared among different faculty with similar interests. Each laboratory contains state-of-the-art equipment necessary for conducting high quality research. While the past several years have been difficult ones from a fiscal standpoint and little funds have been available for the expansion of facilities, the College this past year did undertake the renovation of a laboratory to provide an up-to-date *in vitro* toxicology laboratory COE funds provide the new equipment for this facility. It was felt that this was pivotal to several of our younger faculty and was part of the Center's commitment to the IVTT group.

GOAL 8: TO DISSEMINATE THROUGH THE EXTENSION SERVICE THE PRACTICAL INFORMATION REQUIRED TO REDUCE THE INCIDENT OF LIVESTOCK DISEASES.

UTCVM has been featured in several publications over the course of the year. *UT Agriculture*, published by the Institute of Agriculture and encompassing a large statewide audience, featured articles by COE personnel. *Veterinary Medical Topics*, published semi-annually by the Extension Service, routinely features articles exploring livestock diseases. Regular features appear as well in the two UT alumni publications, *Context* and the *Torchbearer*. Moreover, COE personnel routinely speak to state commodity groups.

GOAL 9: TO DEVELOP NEW STRATEGIES FOR THE PREVENTION OF DISEASE.

Several projects whose overall objectives are to determine the factors that induce formation, maintenance and disruption of the blood-brain barrier and other organ specific

toxicity are being conducted by center members. **DR. T. ROWLES** has begun developing protocols for *in vitro* testing and kinetics of neurotoxicants. *Cytotoxicity assays in endothelial, glial, and neuronal cells were established.* Additionally, an *in vitro* model of the blood-brain barrier is being developed by co-culturing these cell types. Work this past year in collaboration with **DR. D. FRAZIER** suggests the *astroglia and brain capillary endothelial cells may interact in a complex biochemical network via mediators such as cytokines and growth factors to modulate maintenance of the barrier.*

Breast cancer is the leading cause of death in women ages 35 to 54. During the past year, COE has supported **DR. K. HAHN's** research project aimed at improving the therapeutic efficacy of chemotherapy in the clinical management of advanced stage breast cancer. The hypotheses of this study is that the cellular response of normal and malignant cells *in vitro* to alkylating cytotoxic drugs is influenced by differences in oxygenation, pH, and glutathione concentration and cytotoxicity can be potentiated by etanidazole sensitization. Last years work focused on determining whether differences in oxygenation in normal lymphocytes *in vitro* affected etanidazole sensitization of anti-neoplastic drugs such as bleomycin, doxorubicin HCl, and cis-diammine-dichloro-platinumII. It was concluded that *etanidazole sensitizes bleomycin, doxorubicin, and cis-platin cytotoxicity in hypoxic but not in aerobic G₀ lymphocytes.* Moreover, *bleomycin and doxorubicin induction of micronuclei in etanidazole-sensitized hypoxic cells and non-etanidazole-sensitized aerobic cells appears to be dose-dependent.* In contrast, *cisplatin induction of micronuclei does not appear to be dose-dependent.*

GOAL 10: TO IMPROVE FACILITIES AND EXPERTISE IN ORDER TO PROVIDE IMPROVED RESEARCH TRAINING.

SPECIAL MATERIALS AND EQUIPMENT

Equipment monies this year were spent on assisting with the establishment of IVTT laboratory. This multiuser laboratory will service Center members--**DRS. D. FRAZIER, K. HAHN, T. ROWLES, and D. WARD.** This mainly encompassed upgrade tissue culture and chemical handling capabilities. As well, several multiuser pieces of equipment were purchased, including a steam sterilization unit housed in the P-3 facility.

GRADUATE STUDENTS, POST DOCTORAL RESEARCHERS, AND RESIDENTS

Our training program remains small, but of high quality. **DRS. D. BRAIN, D. FRAZIER, T. McDONALD, J. OLIVER, S. OLIVER, B. ROUSE, AND E. WILKINSON,** each had a graduate student and/or post-doctoral researcher supported by the Center.

An institutional training grant has been resubmitted by **DR. D. SLAUSON,** Department of Pathology. If funded, it will support five graduate students/residents

selected from the Comparative and Experimental Medicine Graduate Program. These students will train in the area of "Cellular Pathobiology of Environmental Disease." The program will be a collaborative effort between the Department of Pathology and the Biology Division at Oak Ridge National Laboratory. *The program will seek to produce individuals who, by virtue of their training, will be uniquely equipped to address such important environmental research priorities as the molecular and genetic basis for disease, genetic and membrane events that may control differentiation and development, the role of receptor-mediated pathobiology including transmembrane signal transduction, molecular mechanisms of chemical carcinogenesis, and the molecular and genetic basis for immunologic susceptibility and predisposition.* The graduates of this program should then be able to contribute to an enhanced understanding of the environmentally-caused disorders of man and other animals, both in terms of the morphologic expressions of disease and in terms of its molecular and cellular pathogenesis.

Several young investigators continue to benefit from COE funds. Dr. Huda Al-Ansari continues her work on *the significance of strain variation within the ruminant respiratory syncytial viruses*. Dr. Steve Kannia is in the third year of his investigations of the protozoa *Babesia bigmina* which effects red blood cells of most domestic animals. Dr. Melissa Kennedy is involved with *examining the antibody response, viral clearance, and clinical parameters associated with cattle vaccinated against bovine viral diarrhea virus*. Dr. Jill Sackman is working on *a novel approach to vascular disease*. Dr. Jim Strickland has finished his second year on a *tall fescue toxicosis* project.

MINORITY RECRUITMENT

Through our minority internship/residency program, UTCVM has been successful in recruiting 5 minorities for the upcoming fiscal year.

The veterinary internship for African-American High School Students in Tennessee, developed with funding provided by a grant from the Tennessee Higher Education Commission, is now in its second year. This program is very competitive with applicants for the eight funded slots.

The Minority High School Apprentice Program sponsored by the National Institutes of Health is currently in its 13th year. It is directed by a Center member **DR. T. SCHULTZ**.

COLLABORATIVE RESEARCH PROJECTS

Collaborative research projects continue to be a hallmark of Center personnel, especially those in the GFMG group. The role of *genotype genomic imprinting and sex hormones in platelet and megakaryocyte production* is the topic of a new collaboration

between **DR. T. McDONALD** and Dr. Carl Jackson of St. Judes Medical Center in Memphis.

DR. L. MUNSON continues her collaborations with the University of Washington, Seattle to *evaluate placental development in the Patch mouse*. Molecular techniques are being used to identify homozygous mutant embryos so that the development of their placenta can be evaluated.

DR. R. SHULL continues his long-standing collaboration with Dr. Elizabeth Neufeld of the University of California at Los Angeles. This work involves *the development and evaluation of techniques in gene therapy*.

In collaboration with Dr. Ellis Avner of the University of Washington, Seattle, **DR. E. WILKINSON** is *characterizing the kidney cells involved in the production of the lesions in the Tg737 mice*. In collaboration with Dr. Steve Reeders of Howard Hughes Medical Institute, Yale University, Dr. Wilkinson is examining the structure of the Tg737 gene in over 100 human families with autosomal recessive polycystic kidney disease. In addition, Dr. Wilkinson has a collaborative study with Dr. Greg Dressler of the National Institutes of Health (NIH). This project *examines the nature of the congenital nephrotic syndrome in PAX-2 transgenic mice*. Dr. Wilkinson's laboratory previously established cultures of liver and kidney cells from Tg737 mice. To these have been added several lines of liver cells and have been developed from mice with different genetic backgrounds. Cooperative efforts between Oak Ridge National Laboratory and Proctor & Gamble *further characterize the putative liver stem cells of these cultures*.

Collaborative work is not limited to the GFMG group. **DR. T. SCHULTZ** of the IVTT group and researchers at the School of Pharmacy of John Moores-Liverpool University in England are working on modeling toxicity of bioreactive toxicants. *Bioreactive toxicants are characterized by having strong stereo-electronic interaction which appear to be best quantitated by molecular orbital quantum chemical parameters*. Moreover, Dr. Schultz in conjunction with Dr. Ovanes Mekenyan of the Higher Institute of Chemical Technology, Bourgas, Bulgaria is examining a "dynamic" approach to quantitative structure-activity relationships. By using high speed computers, they develop thousands of three-dimensional conformers for a single two-dimensional molecular structure. *They are evaluating which conformers best model the activity of the organic chemical*.

GOAL 11: TO DEVELOP INNOVATIVE APPROACHES TO THE TREATMENT OF HUMAN DISEASE.

During the past year, COE provided support for a post-doctoral fellow to work with **DRS. D. FRAZIER** and **DR. T. ROWLES**. The project looked to the development of

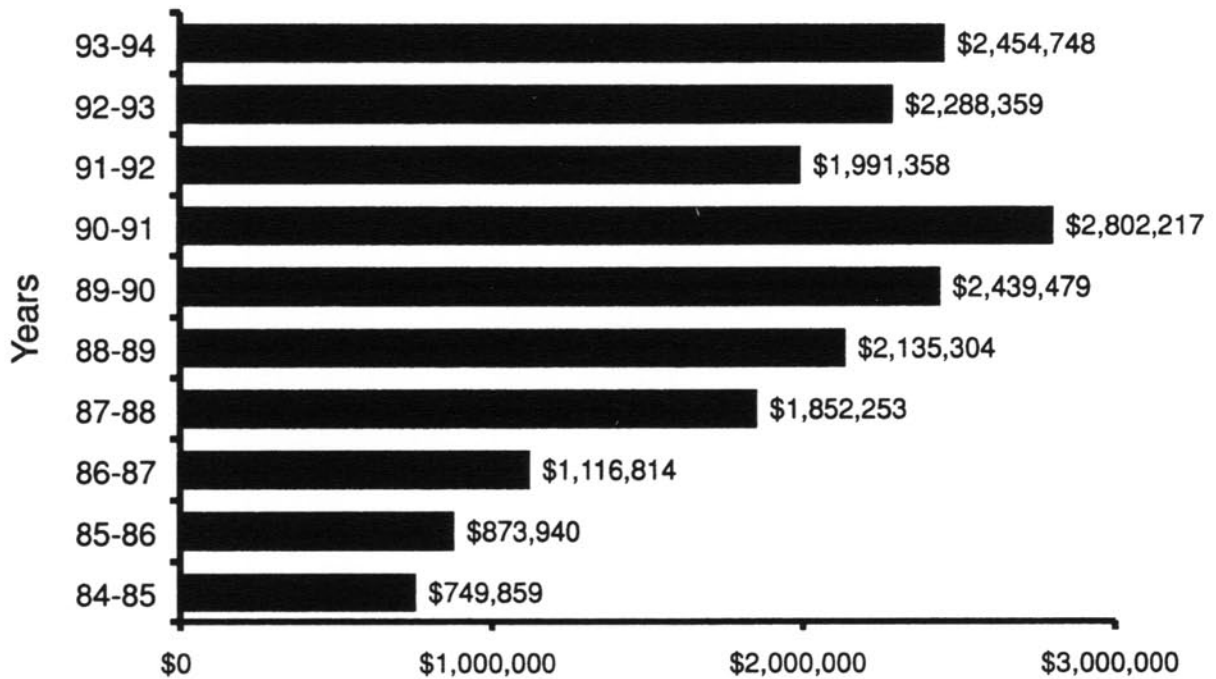
cell culture protocols, singly and in co-culture, as a means to evaluate organ specific toxicity of chemicals. Specifically *neuroblastoma cells*, *brain capillary endothelial cells* *astrocytes* and *kidney tubule cells*. COE continues to provide support for two doctoral students. The projects they are working on are complementary to others being worked on by the IVTT group.

Earlier work by **DR. T. McDONALD** showed that thrombopoietin significantly increases megakaryocyte sizes and platelet counts of sublethally irradiated mice, indicating that thrombopoietin will be useful in treating patients undergoing bone-marrow transplantation and/or patients with platelet production problems. Moreover, Dr. McDonald's group showed previously that C3H mice have higher average ploidy megakaryocytes than other mouse strains tested, but the mode of inheritance of the anomaly is unknown. Studies were carried out this past year in an effort to clarify the genetics of high ploidy megakaryocytes in C3H mice. They measured megakaryocyte DNA content for both male and female offspring from F1, as well as backcross matings. The data revealed that *polyploidy megakaryocytes DNA content distributions of the offspring from the matings showed that C3H mice have higher percentage of high ploidy megakaryocytes than did all other mice. Also, male mice had significant higher percentages of high ploidy megakaryocytes than did female mice.* The megakaryocyte DNA content for individual offspring of a given backcross appeared to form a single, continuous distribution, rather than segregating into 2 distinct groups, suggesting that *the higher megakaryocyte DNA content of C3H mice is caused by involvement of multiple alleles.* This conclusion is further supported by their findings that *the frequency of high ploidy megakaryocytes among offspring of the various matings was related to the proportion of C3H genotype contributed by the parents.*

DR. B. ROUSE continues his work to design delivery vehicles that optimize induction of cytotoxic T lymphocytes with viral proteins and peptides. This program, in collaboration with Dr. Leaf Huang, University of Pittsburgh, uses liposomes as the agent carriers. *This approach may prove a means to prevent virus infections in humans.*

Benchmarks

Figure 1
Center of Excellence in Livestock Diseases and Human
Health External Funding Levels Since Establishment



II. BENCHMARKS

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

**FACULTY MEMBERS ASSOCIATED
WITH THE CENTER OF EXCELLENCE**

	Year 5 <i>(Final Year of Initial Commitment Center)</i> 1991-92			Year 6 <i>(Year 01 as Accomplished Center)</i> 1992-93		Year 7 <i>(Year 02 as Accomplished Center)</i> 1988-89		Year 8 <i>(Year 03 as Accomplished Center)</i> 1991-92		Year 9 <i>(Year 04 as Accomplished Center)</i> 1992-93		Year 10 <i>(Year 05 as Accomplished Center)</i> 1994-95	
	Target	Actual	Avg	Actual	Avg	Actual	Avg	Actual	Avg	Actual	Avg	Actual	Avg
A. NUMBERS OF													
1. ARTICLES		74	(3.22)	68	(2.62)	97	(3.73)	83	(4.37)	78	(3.90)	95	(5.00)
2. BOOKS OR BOOK CHAPTERS PUBLISHED		7	(0.30)	17	(0.65)	14	(0.54)	6	(0.32)	7	(0.35)	9	(0.47)
3. PUBLISHED PROCEEDINGS		21	(0.91)	37	(1.42)	42	(1.62)	24	(1.26)	17	(0.85)	11	(0.58)
TOTAL PUBLICATIONS:	2.82	102	(4.43)	122	(4.69)	153	(5.89)	113	(5.95)	102	(5.10)	104	(5.47)
B. *NUMBER OF INVITED PARTICIPATIONS AT:													
1. REGIONAL MEETINGS	0.50	36	(1.56)	19	(0.73)	28	(1.08)	13	(0.68)	15	(0.75)	18	(0.95)
2. NATIONAL MEETINGS	1.25	55	(2.39)	28	(1.08)	44	(1.69)	36	(1.89)	47	(2.35)	47	(2.47)
C. ABSTRACTS	0.30	33	(1.43)	66	(2.54)	48	(1.85)	47	(2.47)	53	(2.65)	55	(2.89)
NUMBER OF FACULTY INCLUDED IN CENTER		23	...	26	26	19	...	20	...	19	...
NUMBER OF VISITORS	11	10	...	17	17	12	...	12	...	13	...

TABLE 2.
RESEARCH PROJECTS FUNDED EXTERNALLY
REPORT PERIOD 1993-94

PROJECT DIRECTOR	SOURCE EXPENDITURES	TOTAL AMOUNT AWARDED	ESTIMATED EXPENDITURES 7-1-93/6-30-94
BOCHSLER, P. N. <i>Bovine Lipopolysaccharide Binding Protein and Mechanisms of Macrophage Activation</i>	USDA 9/1/91-8/31/93 (Extension to 8/31/94)	140,000 -----	21,740
BOCHSLER, P. N. <i>Molecular Basis of Endothelial Cell Sensitivity to Lipopolysaccharide</i>	USDA 9/15/92-9/30/94	95,000 -----	47,496
BOCHSLER, P. N. <i>The Bovine CD14 Receptor: A Link in Endotoxin-mediated Macrophage Activation</i>	USDA 9/15/92-9/30/94	150,000 -----	75,000
BRIAN, D. A. <i>Coronavirus Structure and Replication</i>	NIH 9/1/89-8/31/94	549,224 -----	111,773
BRIAN, D. A. <i>Mechanism(s) of Coronavirus RNA Replication and Packaging</i>	USDA 9/15/92-9/30/95	200,000 -----	66,660
FRAZIER, D. L. <i>Transport of Photosensitizers Across the Blood Brain Barrier</i>	Beckman Laser SDI-MFEL Consortium 7/1/93-6/30/94	15,000 -----	15,000
FRAZIER, D. L. <i>Analysis of the Photosensitizer HPPH</i>	Beckman Laser Institute & Med Ctr 9/1/93-3/1/94	15,000 -----	15,000
GODKIN, J. D. <i>Retinoid Binding Proteins and Receptors in Bovine Placental Development</i>	USDA 9/1/93-8/31/96	212,000 -----	52,999
HAHN, K. A. FRAZIER, D. L. , Co-investigator <i>Phase II Evaluation of Doxorubicin in the Cat</i>	AAHA 7/1/94-6/30/96	10,000 -----	0
HAHN, K. SCHULLER, H. M. , Co-investigator <i>Contract--B859-035 Treatment of Dogs with Osteosarcoma</i>	BYK Gulden Pharmaceuticals 1/1/93-12/31/95	63,890 -----	21,300
MCDONALD, T. P. <i>Contract--Development of Assays for Thrombopoietin</i>	Genentech 3/1/88-2/28/93 (Extension to 2/28/95)	175,832 -----	25,116

PROJECT DIRECTOR	SOURCE EXPENDITURES	TOTAL AMOUNT AWARDED	ESTIMATED EXPENDITURES 7-1-93/6-30-94
MCDONALD, T. P. <i>Performance of Assays for Thrombopoietin</i>	Genentech 3/1/88-7/31/93 (Extension to 2/28/95)	112,700 -----	16,104
MCDONALD, T. P. <i>Characterization of Thrombopoietin</i>	AMGEN 10/1/92-12/31/95	73,671 -----	22,668
MCDONALD, T. P. <i>Thrombopoietin: Immunoassay & Characterization</i>	NIH 12/1/88-11/30/93 (Extension to 11/30/94)	548,681 -----	81,837
MCDONALD, T. P. Purchase of: <i>Nikon Labophot Fluor Dual Filter Microscope</i>	NHLBI Small Instrumentation Program 8/1/93-7/31/94	22,818 -----	22,818
MILLER, M. S. <i>Role of Oncogenes in Transplacental Lung Carcinogenesis</i>	NIEHS 5/1/94-4/30/97	343,804 -----	22,402
MUNSON, L. <i>The Pathological Effects of Melengestrol Acetate in Captive Wild Felids</i>	AAZPA Conservation Center 10/1/92-9/30/93	37,807 -----	9,453
MUNSON, L. <i>Effects of Progestagen Contraceptives on the Uterus and Mammary Glands of Exotic Felids</i>	Nixon Griffis Fund 3/1/91-2/28/94	2,843 -----	632
MUNSON, L. <i>Continuing Safety Assessments of Contraceptives for Non-domestic Felids</i>	AAZPA Conservation Center 3/14/94-3/14/95	38,087 -----	11,109
OLIVER, S. P. <i>Mastitis Research</i>	Alcide Corporation 12/90-12/94	57,752 -----	14,438
OLIVER, S. P. <i>Mastitis Research</i>	H. B. Fuller Co. 4/91-12/94	31,778 -----	8,664
OLIVER, S. P. <i>Immunization of dairy cows against <u>Streptococcus uberis</u>: Efficacy of an Experimental Vaccine during the Nonlactating Period</i>	Upjohn Company 1991-1994	54,225 -----	14,056
OLIVER, S. P. <i>Selenium and Vitamin E in Disease Resistance</i>	BASF 1992-1994	17,000 -----	5,000

PROJECT DIRECTOR	SOURCE EXPENDITURES	TOTAL AMOUNT AWARDED	ESTIMATED EXPENDITURES 7-1-93/6-30-94
OLIVER, S. P. <i>Use of Genetic Markers as Indicators of Mastitis Resistance and Milk Production in Jersey Cattle</i>	Amer Jersey Cattle Club 1993-94	6,000 -----	3,000
OLIVER, S. P. <i>Intramammary infusion of Alcide for treatment of clinical mastitis in dairy cows</i>	Alcide Corporation 1993-94	14,167 -----	7,085
OLIVER, S.P. <i>Macrophage phagocytosis of <u>Streptococcus uberis</u></i>	Upjohn Company 1994-95	8,000 -----	4,000
POTGIETER, L. <i>Significance of Strain Variation within the Ruminant Respiratory Syncytial Virus</i>	USDA 9/1/93-8/31/95	123,310 -----	51,380
POTGIETER, L. <i>Cloning and Sequencing of BRSV G Glycoprotein and Detection of Strain Divergence</i>	USDA Special Grant 8/1/90-7/31/93	149,974 -----	4,165
ROUSE, B. T. <i>Immunity Mechanisms in Herpes Virus Infections</i>	NIH 5/1/89-4/30/94	1,130,370 -----	197,670
ROUSE, B. T. , Advisor for D. Bouley <i>Herpetic Stromal Keratitis Pathogenesis: An Animal Model</i>	NIH Training Grant 7/1/93-6/30/95	110,168 -----	35,300
ROUSE, B. T. <i>Mechanisms in Herpetic Stromal Keratitis</i>	National Eye Institute (NIH) 9/30/92-9/29/97	774,745 -----	200,217
ROUSE, B. T. <i>Liposome Microencapsulation of Vaccine Antigens</i>	NIAID 6/1/90-5/31/95	828,289 -----	166,235
ROUSE, B. T. <i>Mucosal Immunity in Control of Herpetic Infection</i>	NIAID 8/1/93-7/31/96	562,404 -----	160,534
ROUSE, B. T. <i>Herpes Zosterification</i>	SmithKline Biological 12/15/89-12/31/94	124,746 -----	25,000
ROWLES T. K. <i>Characterization Isolated Rodent Microvessels/Astrocytes Co-cultures</i>	Environmental Protection Agency (EPA) 4/7/93-4/6/95	9,484 -----	4,740

PROJECT DIRECTOR	SOURCE EXPENDITURES	TOTAL AMOUNT AWARDED	ESTIMATED EXPENDITURES 7-1-93/6-30-94
ROWLES T. K. <i>Evaluation of Renal and Neural Function in Diving Mammals using <u>in</u> <u>vitro</u> and <u>in vivo</u> Techniques</i>	Office of Naval Research 6/1/94-5/31/95	36,833	-----3,069
SCHULLER, H. M. <i>Mechanisms of Neuroendocrine Lung Carcinogenesis by Nitrosamines</i>	Shannon Award Institute (NIH) 9/1/91-8/31/93	100,000	-----8,332
SCHULLER, H. M. <i>Characterization of Induced Neuroendocrine Lung Cancer</i>	National Cancer Institute (NIH) 2/7/92-1/31/95	464,975	-----170,144
SCHULLER, H. M. <i>Contract--Testing of Anti-carcinogenic Effects of Niguldipine</i>	Byk Gulden Pharmaceuticals 3/1/88-12/31/94	382,925	----- 52,497
SCHULTZ, T. W. <i>Photo-induced Toxicity of Substituted Anthracenes</i>	University of Minnesota 1/15/94-1/14/95	9,525	-----4,700
SHULL, R. <i>Molecular Study of MPS I: Gene Therapy in a Canine Model</i>	NIH 8/1/92-7/31/96	1,001,653	-----123,342
SLAUSON, D. O. <i>Leukocyte Function and Host Defense in Developing Calves</i>	USDA Competitive 8/15/90-8/30/92 (Extension to 8/30/93)	120,000	----- 35,499
SLAUSON, D. , Advisor for D. Dean <i>Signaling Pathways in LPS-Stimulated Lung Macrophages</i>	NIH Training Grant 11/30/91-11/30/94	97,500	----- 33,800
WILKINSON, J. E. <i>Directed Expression of the Agouti Gene Product in Transgenic Mice: A Potential Model for Obesity</i>	Glaxo Inc. 10/1/93-9/30/96	672,890	----- 72,747
WILKINSON, J. E. <i>Molecular Genetics of PKD in the Transgenic TG737 Mouse</i>	NIH 9/30/92-9/29/95	444,816	-----160,944
WILKINSON, J. E. <i>Immunobiology of the Scurfy Mouse</i>	NIH 9/30/92-5/31/95	633,875	-----249,083
	TOTAL		<u>\$2,454,748</u>

Plans For Next Year

III. PLANS FOR NEXT YEAR

CARCINOGENESIS AND DEVELOPMENTAL THERAPEUTICS GROUP

DR. H. SCHULLER will continue to pursue research in lung carcinogenesis. In particular, she will expand her studies in neuroendocrine tumors and anti-cancer therapeutics of the calcium channel blocker, Dexniguldipine.

DR. M. MILLER's goals for the coming year are to work with Dr. D. Schaeffer in performing i.p. injections in pregnant mice and establish the breeding colony needed for his project on the role of oncogenes in transplacental carcinogenesis. Moreover, he will be standardizing the research protocol to extract DNA from paraffin embedded mouse tissues and begin the molecular biology analyses.

This coming year, **DR. K. HAHN** will continue his work, therapeutic efficacy of chemotherapy. Assisted by support from Bristol Laboratories and the National Cancer Institute with donated drugs, he will seek to determine if differences in cellular pH and/or glutathione concentrations in normal lymphocytes or mammary carcinoma cells in vitro quantitatively affect etanidazole (SR-2508), an oxygen-mimetic nitroimidazole, sensitization of cytotoxicity to selected chemotherapeutics including bleomycin, cisplatin, and doxorubicin.

GROWTH FACTORS AND MOLECULAR GENETICS GROUP

DR. E. WILKINSON plans a number of functional studies on the cultured Tg737 kidney cells to further define their characteristics. Further, the Tg737 gene will be replaced in the cultured cells by standard methods to precisely determine the effect of the Tg737 gene on cell structure and function. Additionally, they will use mammalian expression vectors with inducible promoters to turn the Tg737 gene on and off and study the function. A number of vectors containing mutations in specific regions of the Tg737 protein will be engineered as a basis for further studies of the structure and function of the protein. Finally, a number of in vivo studies using the cultured liver cells will be conducted.

DR. R. SHULL will continue the gene therapy and recombinant enzyme trials. Modifications of existing in vitro techniques for transferring the gene for the missing enzyme to several cell types will be investigated. In addition to bone marrow cells, he will attempt to use skin fibroblasts and myoblasts from skeletal muscle as targets for gene therapy. These cell types have the advantage of easier transfection with retroviral vectors and have been shown to secrete lysosomal enzymes. One or more dogs with MPS I will also be treated with injectible enzyme in trials lasting 6-12 months. The goal will be to see if beneficial effects can be realized in a broader range of tissues than in the first attempts that only lasted 13 weeks.

Work planned for the coming year in **DR. T. McDONALD**'s laboratory focuses primarily on investigating the mode of inheritance of the higher degree of megakaryocyte polyploidization in C3H mice. They will also examine the effects of large doses of TPO on platelet production in mice, complete work on the effects of vincristine on megakaryocyte complexes and other cytoplasmic abnormalities in megakaryocytes and platelets of rats, and investigate the role of genotype genomic imprinting and sex hormones in platelet and megakaryocyte production.

During the next fiscal year, **DR. C. MENDIS-HANDAGAMA** will continue to focus her efforts on understanding the mechanisms involved in movement of peroxisomes towards mitochondria for cholesterol delivery.

DR. J. MERRYMAN will join the GFMG group this coming year. Her work will focus on understanding the control of proliferation in cancer cells. Control of cellular proliferation is exerted at the G₁/S interface of the cell cycle by certain growth factors, cyclin-dependent protein kinases, and nuclear phosphoproteins such as the product of the retinoblastoma gene, p105-Rb. Parathyroid hormone-related protein (PTHrP) is a newly identified calcitropic hormone that causes the important paraneoplastic syndrome humoral hypercalcemia of malignancy. In addition PTHrP has also been shown to be an autocrine/paracrine factor in control of cellular proliferation in normal and malignant cells. The purpose of this investigation is to evaluate human tumor cell lines for production of PTHrP and expression of PTHrP receptors, and to examine the effects of PTHrP on cellular proliferation. These investigations will prove useful in understanding control of proliferation in neoplastic cells, a timely and important topic in the field of cancer biology, and will serve as a starting point for more in-depth investigations in this critical area of cancer research.

DR. L. MUNSON will continue her research on the role of PDGFs in bovine placental growth and the Patch mouse model. Studies examining the interaction of PDGF and retinoic acid will be continued. Moreover, collaborations with the UTK Medical Center include molecular analysis of normal, endometriotic, and malignant human endometrial epithelium to determine if PDGF-a and PDGF-b receptors are present as well as research into the effects of PDGF in endometrial hyperplasia and neoplasia.

DR. J. GODKIN plans to complete his investigations on the role of retinoids, binding proteins and receptors in embryonic development and uterine function. Work will continue on interaction of transforming growth factors and retinoids in uterine function and embryonic development. He also proposes to examine the role of retinoids in ovarian and oviduct function.

IN VITRO TOXICOLOGY AND TOXICOKINETICS GROUP

DR. T. SCHULTZ will continue his studies on predictive toxicology. Work will involve toxicity testing, molecular descriptor evaluation, and structure-toxicity relationship

development of bioreactive chemicals. Efforts will center on the development and validation of a computer aided structure evaluation (CASE) approach to identify toxicophores associated with bioreactivity.

For **DR. T. ROWLES**, next year's work will involve further development of protocols for the *in vitro* assessment of neurotoxicity and testing of selected toxicants.

DR. D. FRAZIER will be working to develop an *in vitro* model of the kidney. This model will be used for evaluation of transport and cytotoxicity of environmental toxicants and cancer chemotherapeutics.

DR. D. WARD, a new member of the Center this coming year, in conjunction with **DR. SCHULTZ** will continue his work begun in March of this year on the development and testing of blood ocular barriers.

INFECTIOUS DISEASES AND POPULATION MEDICINE GROUP

Work in **DR. L. POTGIETER**'s laboratory will continue on several projects. They will continue to investigate the protective nature of the structural proteins of BVDV individually and in combination with one another, as well as continue efforts to develop economical and accurate diagnostic assays. In the next year, they will also continue work on ruminant RSV. Specifically, they plan to identify subgroups within the RSVs using monoclonal antibodies in an ELISA system of identification and to assess the significance of strain variation within the RSVs. Moreover, they will be working on projects that involve immunostimulatory effects of ivermectin in dogs, immunology of dogs with generalized demodicosis, and development of improved methods for detecting feline immunodeficiency virus.

DR. S. OLIVER and his co-workers will continue to focus on development of RAPD fingerprinting which has the potential to be a routine bacterial species identification method. They plan on evaluating commercially available oligonucleotide primers and develop a tentative species identification scheme.

DR. J. OLIVER and associates will, in the next year, further characterize specific alkaloid effects on vascular biogenic amine receptors following chronic alkaloid infusion and endothelial cells. They also will examine adrenal function in cattle on endophyte-infected pasture. It is hoped that this next year will also see patent approval for an anti-fescue toxicosis vaccine.

DR. D. BRIAN's research over the next 12 months will focus on developing the coronavirus subgenomic replicon as a delivery vehicle for stimulating mucosal immunity and as a vehicle for other direct-hitting antiviral molecules.

INFLAMMATION AND HOST DEFENSE GROUP

DR. P. BOCHSLER will continue to investigate factors involved in the response of cattle to endotoxin produced by gram-negative bacteria. Their plans include further studies with

bovine lipopolysaccharide-binding protein that they have isolated and defining methods for the identification of the bovine CD14 receptor molecule. This receptor appears to be important in the response of cattle to endotoxin. In addition, they will examine the roles of interleukin-6, superoxide anion, and nitric oxide in bovine inflammation and host defense. Studies of endotoxin and important bovine immunoregulatory molecules will yield a better understanding of the bovine response to pathogens, and will eventually lead to improved methods of disease prevention and therapy.

The specific goals for **DR. B. ROUSE** include working on *in situ* hybridization methods in order to further characterize the cytokine profile (gene expression) and viral gene expression during the course of HSK. This is to identify and localize the cells involved in the immunopathology of HSK. Towards this end, he and his associates have developed specific probes for the detection of viral genes, inflammatory cytokine genes and T cell derived cytokine genes. Studies will also be conducted in an effort to detect selected cytokines and genes of cornea obtained during various stages of the development of HSK.

During the next year **DR. D. SLAUSON** will continue to dissect the signaling pathways used by LPS and LPS/LBP complexes for procoagulant induction and TNF- α release in bovine lung macrophages with special attention directed at the potential role of a G-protein linked receptor in the proximal pathway and a C-kinase as a terminal activator.

THE 1994-95 CENTER OF EXCELLENCE FACULTY ARE:

PHILIP N. BOCHSLER, D.V.M., PH.D.

*Assistant Professor
Department of Pathology*

DAVID A. BRIAN, D.V.M., PH.D.

*Professor
Department of Microbiology*

DONITA L. FRAZIER, D.V.M., PH.D.

*Associate Professor
Department of Comparative Medicine*

JAMES D. GODKIN, PH.D.

*Professor
Department of Animal Science*

KEVIN HAHN, PH.D.

*Assistant Professor
Department of Comparative Medicine*

TED McDONALD, PH.D.

*Professor
Department of Animal Science*

CHARMI MENDIS-HANDAGAMA, PH.D.

*Assistant Professor
Department of Animal Science*

DR. JOYCE I. MERRYMAN, D.V.M., PH.D.

*Assistant Professor
Department of Pathology*

MARK MILLER, PH.D.

*Research Assistant Professor
Department of Pathology
Surgery*

LINDA MUNSON, D.V.M., PH.D.

*Assistant Professor
Department of Pathology*

JACK W. OLIVER, D.V.M., PH.D.

*Professor
Department of Comparative Medicine*

STEPHEN P. OLIVER, PH.D.

*Associate Professor
Department of Animal Science*

LEON N. D. POTGIETER, B.V.SC., PH.D.

*Professor and Head
Department of Comparative Medicine*

BARRY T. ROUSE, B.V.SC., PH.D.

*Professor
Department of Microbiology*

TERESA ROWLES, D.V.M., PH.D.

*Assistant Professor
Department of Animal Science*

HILDEGARD M. SCHULLER, D.V.M., PH.D.

*Professor
Department Pathology*

TERRY W. SCHULTZ, PH.D.

*Professor
Department of Animal Science*

ROBERT M. SHULL, D.V.M.

*Professor
Department of Pathology*

DAVID O. SLAUSON, D.V.M., PH.D.

*Distinguished Professor and Head
Department of Pathology*

DANIEL A. WARD, D.V.M., PH.D.

*Assistant Professor
Department of Small Animal Med. &*

J. ERBY WILKINSON, D.V.M., PH.D.

*Associate Professor
Department of Pathology*

Budget Forms

SCHEDULE 1
 TENNESSEE HIGHER EDUCATION COMMISSION
 CENTERS OF EXCELLENCE
 1993-94 BUDGET AND PROPOSED 1994-95 BUDGET

	1993-94 Actual Expenditures			1994-95 Proposed Budget		
	Matching	Appropriations	Total	Matching	Appropriations	Total
REVENUE						
With State Appropriation						
Carryover From Previous Appropriation		506,000	506,000		506,000	506,000
With Matching Funds	253,000	34,942	34,942			
Carryover from Previous Matching	10,600		253,000	253,000		253,000
TOTAL	263,600	540,942	804,542	253,000	506,000	759,000
EXPENDITURES						
Salaries						
Faculty	47,619	95,381	143,000	45,161	90,459	135,620
Other Professional	56,196	112,560	168,756	35,669	71,441	107,110
Clerical/Supporting	20,960	41,982	62,942	17,034	34,122	51,156
Assistantships	8,387	16,800	25,187	13,269	26,578	39,847
Students	10,586	21,205	31,791	9,990	20,010	30,000
TOTAL SALARIES	143,748	287,928	431,676	121,123	242,610	363,733
fringe Benefits	30,462	61,017	91,479	31,635	63,365	95,000
TOTAL PERSONNEL	174,210	348,945	523,155	152,758	305,975	458,733
Travel	413	826	1,239	1,914	3,086	5,000
Software						
Books and Journals	206	414	620			
Other Supplies	36,062	85,179	121,241	43,378	86,889	130,267
Equipment	37,394	74,903	112,297	49,950	100,050	150,000
Maintenance	13,640	27,320	40,960	5,000	10,000	15,000
Scholarships	1,459	2,921	4,380			
Consultants	216	434	650			
Renovation						
Other (Specify)						
TOTAL NON-PERSONNEL	89,390	191,997	281,387	100,242	200,025	300,267
GRAND TOTAL	263,600	540,942	804,542	253,000	506,000	759,000

Appendices

Curriculum Vitae for Joyce I. Merryman

<u>Name</u>	<u>Position Title</u>	<u>Birthdate</u>
Joyce Irene Merryman	Assistant Professor	October 28, 1953

<u>Education</u>	<u>Degree</u>	<u>Year</u>	<u>Field of Study</u>
Hocking Technical College	A.D. Nursing	1978	Nursing
The Ohio State University	B.S.	1983	Zoology
The Ohio State University	D.V.M.	1987	Veterinary Medicine
The Ohio State University	Ph.D.	1993	Pathobiology

Professional Experience

1993-Present Assistant Professor, Department of Pathology, University of Tennessee College of Veterinary Medicine.

1989-1993 National Research Service Award, National Cancer Institute, studying the role of humoral factors in the pathogenesis of humoral hypercalcemia of malignancy.

1989-1993 Graduate Research Fellow, The Ohio State University, Department of Veterinary Pathobiology pursuing advanced research training leading to completion of the Ph.D degree in Experimental Pathobiology.

1987-1989 Schering Plough Corporation Fellow/Resident, The Ohio State University, Department of Veterinary Pathobiology pursuing research training leading to a Ph.D. degree in Experimental Pathobiology and specialty training leading to Board Certification by the American College of Veterinary Pathologists.

1985-1986 Student Research Associate, The Ohio State University, Department of Veterinary Pathobiology, in the laboratories of C.C. Capen and T.J. Rosol

1980-1985 Registered Nurse, Grant Hospital, Columbus, Ohio, Surgical and Trauma Critical Care

1978-1980 Registered Nurse, The Ohio State University Hospitals, Cardiovascular Surgical Nursing

Professional Teaching Experience

1987-1992 Graduate Teaching Associate in applied Veterinary Pathology, (Necropsy training for professional students).

1991 Graduate Teaching Associate in Dermatopathology (Dermatology core curriculum course for professional students).

Professional Honors

National Research Service Award, National Cancer Institute, "Humoral Factors and Hypercalcemia of Malignancy", 1989-1992

Schering Plough Corporation Fellow, 1987-1989

Finalist, Graduate Research Forum, The Ohio State University, 1989

Phi Zeta National Veterinary Honorary, 1986-present

Phi Zeta Research Award, The Ohio State University, College of Veterinary Medicine, 1987

Graduated *summa cum laude*, The Ohio State University, College of Veterinary Medicine, 1987

Second Place, American College of Veterinary Pathologists Young Investigator Award, 1993

Professional Societies

Phi Zeta, 1986-present

American Veterinary Medical Association, 1981-present

American Society for Bone and Mineral Research, 1989-present

Publications in Peer-Reviewed Journals

Merryman, J.I., Rosol, T.J., Brooks, C.L., and Capen, C.C.: Separation of parathyroid hormone-like activity from transforming growth factor (TGF) $-\alpha$ and $-\beta$ in the canine adenocarcinoma (CAC-8) model of humoral hypercalcemia of malignancy. Endocrinology, 124:2456-2463, 1989.

Rosol, T.J., Merryman, J.I., Nohutcu, R.M., McCauley, L.K., and Capen, C.C.: Effects of transforming growth factor- α on parathyroid hormone- and parathyroid hormone-related protein-mediated bone resorption and adenylate cyclase stimulation *in vitro*. Domestic Animal Endocrinology 8:499-507, 1991.

McCauley, L.K., Rosol, T.J., Merryman, J.I., and Capen, C.C.: Parathyroid hormone-related protein binding to human T-cell lymphotropic virus type I-infected lymphocytes. Endocrinology 130:300-306, 1992.

Rosol, T.J., Steinmeyer, C.L., McCauley, L.K., Merryman, J.I., Werkmeister, J.R., Gröne, A., Weckman, M.T., Swayne, D.E., and Capen, C.C.: Characterization of chicken polyclonal anti-peptide antibodies specific for human parathyroid hormone-related protein (1-36). Veterinary Immunology and Immunopathology 35:321-338, 1993.

Merryman, J.I., Rosol, T.J., Werkmeister, J.R., McCauley, L.K., Suter, M.M., and Capen, C.C.: Regulation of parathyroid hormone-related protein production by a squamous carcinoma cell line *in vitro*. Laboratory Investigation, 69:347-354, 1993.

- Fenger, C.K., Bertone, J.J., Biller, D., Merryman, J.I.: Generalized medullary infarction of the long bones in a horse. Journal of the American Veterinary Medical Association, 202:621-623, 1993.
- Werkmeister, J.R., Merryman, J.I., McCauley, L.K., 1, Horton, J.E., Capen, C.C., Rosol, T.J.: Parathyroid hormone-related protein production by normal human keratinocytes in vitro. Experimental Cell Research, 208:68-74, 1993.
- Merryman, J.I., Rosol, T.J., Capen, C.C.: Effects of gallium nitrate in nude mice bearing a canine adenocarcinoma (CAC-8) model of humoral hypercalcemia of malignancy. Journal of Bone and Mineral Research, 9:725-732, 1994.
- Merryman, J.I., Dewille, J., Werkmeister, J.R., Capen, C.C., Rosol, T.J.: Effects of transforming growth factor-beta on parathyroid hormone-related protein production and RNA expression by a squamous carcinoma cell line *in vitro*. Endocrinology, 134:2424-2430, 1994.
- Okada, H., Merryman, J.I., Rosol, T.J., Capen, C.C.: Effects of humoral hypercalcemia of malignancy and gallium nitrate on thyroid C-cells in nude mice: immunohistochemical and ultrastructural investigations. Veterinary Pathology, 31:349-357, 1994.
- Okada, H., Merryman, J.I., Rosol, T.J., Capen, C.C.: Ultrastructural and histomorphometric evaluations of the effects of gallium nitrate on bone in nude mice bearing a canine adenocarcinoma (CAC-8) model of humoral hypercalcemia of malignancy. Veterinary Pathology, In Press, 1994.
- Grooters, A.M., Miyabayashi, T., Biller, D.S., Merryman, J.: Sonographic appearance of uremic gastropathy in four dogs. Veterinary Radiology and Ultrasound, 65:35-40, 1994.
- Capen, C.C., Okada, H., Merryman, J.I., Rosol, T.J.: Effects of gallium nitrate in nude mice bearing a canine adenocarcinoma model of humoral hypercalcemia of malignancy: biochemical, histomorphometric, and ultrastructural investigations. Journal of Bone and Mineral Metabolism, In Press, 1994.

Published Abstracts

- Rosol, T.J., Capen, C.C., Merryman, J.I., and Carpenter, G.: Inhibition of *in vitro* bone resorption by a hypercalcemia adenocarcinoma (CAC-8) by a PTH receptor antagonist (8,18Nle,³⁴Tyr bPTH[3-34]). In: Abstracts of American Society of Bone and Mineral Research Meetings, Indianapolis, IN, June 1987; and published in Journal of Bone and Mineral Research 2 [Suppl 1], (1987): 86A.
- Merryman, J.I., Rosol, T.J., and Capen, C.C.: Transforming growth factor activity in a canine tumor line derived from an anal sac adenocarcinoma maintained in nude mice. In: Abstracts of Annual Phi Zeta Research Day, Columbus, 15 May 1987.
- Rosol, T.J., Capen, C.C., Merryman, J.I., Danks, J.A., Hayman, J., Ebeling, P.R., and Martin, T.J.: Investigations on parathyroid hormone-related protein in canine anal sac adenocarcinoma and infusion of PTHrP in nude mice. In: Abstracts of 39th Annual Meeting of American College of Veterinary Pathologists, Kansas City, MO, 31 October-4 November, 1988, p. 13.

- Merryman, J.I. and Capen, C.C.: Separation of parathyroid hormone-like activity from transforming growth factors- α and - β in a canine adenocarcinoma (CAC-8) model of cancer-associated hypercalcemia. In: Abstracts of 14th Annual Forum for Graduate Students in the Biological and Health Sciences, The Ohio State University, 1989.
- Merryman, J.I., Rosol, T.J., Capen, C.C.: Separation of parathyroid hormone-like activity from transforming growth factor (TGF)- α and - β in a canine adenocarcinoma model (CAC-8) of humoral hypercalcemia of malignancy (HHH). In: Abstracts (No. 805) of American Society of Bone and Mineral Research/International Conference on Calcium Regulating Hormones First Joint Meeting, Montreal, Quebec, 9-14 September 1989, and published in Journal of Bone and Mineral Research 4 (Suppl. 1): S319, 1989.
- Merryman, J.I., Rosol, T.J., and Capen, C.C.: Separation of parathyroid hormone-like activity from transforming growth factors- α and - β in a canine adenocarcinoma model of humoral hypercalcemia of malignancy. In: Proceedings of 40th Annual Meeting of American College of Veterinary Pathologists, Baltimore, MD, 1-4 November 1989, p. 45.
- Rosol, T.J., Merryman, J.I., Martin, S.L., and Capen, C.C.: Adenocarcinoma derived from specialized cutaneous diverticula (anal sac) in dogs associated with persistent hypercalcemia: Clinical, pathologic, ultrastructural, immunohistochemical, and *in vitro* studies. In: Abstracts of First World Congress on Veterinary Dermatology, 28-30 September, 1989, Dijon, France, p. 28; and published in Advances in Veterinary Dermatology, Vol. I. (C. von Tscharner and R.E.W. Halliwell, Editors), Baillière Tindall, London, 1990, p. 445.
- Rosol, T.J., Merryman, J.I., McCauley, L.K., Steinmeyer, C.L., Swayne, D., and Capen, C.C.: Characterization of polyclonal and monoclonal antibodies to parathyroid hormone-related protein. In: Abstracts (No. 481) of American Society for Bone and Mineral Research, Atlanta, GA, 28-31 August 1990; and published in Journal of Bone and Mineral Research 5 (Suppl. 1, 1990): p. S194.
- Rosol, T.J., Merryman, J.I., McCauley, L.K., and Capen, C.C.: Effects of transforming growth factor alpha on parathyroid hormone- and parathyroid hormone-related protein-mediated bone resorption and adenylate cyclase stimulation *in vitro*. In: Proceedings of 41st Annual Meeting of American College of Veterinary Pathologists, Phoenix, AZ, 10-14 December, 1990, p. 52.
- Rosol, T., Merryman, J., McCauley, L., Werkmeister, J., Grone, A., Steinmeyer, C., Swayne, D., Capen, C.: Pathogenesis of cancer-associated hypercalcemia of malignancy: Characterization of polyclonal antibodies to parathyroid hormone-related protein in chickens. In: Abstracts of the Eighth International Workshop on Vitamin D. Paris, France 5-10 July 1991, p. 51 and Published in: Vitamin D - Gene Regulation, Structure-Function Analysis, and Clinical Application. (A.W. Norman, R. Bouillon, and M. Thomasset, Editors). Walter de Gruyter, Berlin/New York, 1991, pp. 919-920.
- Rosol, T., Merryman, J., McCauley, L., Werkmeister, J., Swayne, D., and Capen, C.: Pathogenesis of humoral hypercalcemia of malignancy (HHM): Production of polyclonal antibodies to parathyroid hormone-related protein (PTHrP) in chickens. In: Abstracts (No. 187) of 80th Meeting of U.S. and Canadian Academy of Pathology, Chicago, 17-22 March, 1991; and published in Laboratory Investigation 64, (1991): 33A.

- Werkmeister, J., Rosol, T., McCauley, L., Merryman, J., Horton, J., and Capen, C.: Regulation of parathyroid hormone-related protein in normal human keratinocytes. In: Abstracts of 20th Annual Meeting of IADR/AADR (International/American Assn. of Dental Research), Acapulco, Mexico, 17-21 April, 1991; and published in Journal of Dental Research 70, (1991): 312.
- Merryman, J.I., Rosol, T.J., McCauley, L.K., and Capen, C.C.: Production of parathyroid hormone-related protein by two human squamous cell carcinoma cell lines in vitro. In: Abstract (No. 594) of American Society for Bone and Mineral Research, San Diego, CA, 24-28 August, 1991; and published in Journal for Bone and Mineral Research 6, (Suppl. 1, 1991): S232.
- Werkmeister, J.R., Rosol, T.J., Merryman, J.I., McCauley, L.K., Horton, J.E., and Capen, C.C.: Regulation of parathyroid hormone-related protein in normal human keratinocytes in vitro. In: Abstract (No. 586) of American Society for Bone and Mineral Research, San Diego, CA, 24-28 August, 1991; and published in Journal for Bone and Mineral Research 6, (Suppl. 1, 1991): S230.
- McCauley, L.K., Rosol, T.J., Lairmore, M.D., Merryman, J.I., and Capen, C.C.: Expression of parathyroid hormone-related protein in human T cell leukemia virus type (HTLV-1) infected lymphocytes. In: Abstract (No. 568) of American Society for Bone and Mineral Research, San Diego, CA, 24-28 August, 1991; and published in Journal for Bone and Mineral Research 6, (Suppl. 1, 1991): S226.
- Rosol, T., Merryman, J., McCauley, L., and Capen, C.: Transforming growth factor-alpha effects on parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP)-mediated adenylate cyclase stimulation and bone resorption in vitro. In: Abstracts of Eighth International Workshop on Vitamin D, Paris, France, 5-10 July, 1991, p. 169.
- Merryman, J.I., Capen, C.C., and Rosol, T.J.: Characterization of polyclonal and monoclonal antibodies to parathyroid hormone-related protein (PTHrP). In: OSU Phi Zeta Research Forum, 28 May, 1991.
- Rosol, T.J., Merryman, J.I., McCauley, L.K., Werkmeister, J.R., Gröne, A., Swayne, D., Steinmeyer, C., and Capen, C.C.: Characterization of polyclonal antibodies to parathyroid hormone-related protein (1-36). In: Proceedings of 42nd Annual Meeting of American College of Veterinary Pathologists, Orlando, FL, 8-13 December, 1991, p. 57.
- Merryman, J.I., Rosol, T.J., Werkmeister, J.R., McCauley, L.K., Suter, M.M., and Capen, C.C.: Production of parathyroid hormone-related protein by normal and malignant keratinocytes in vitro. In: Proceedings of 42nd Annual Meeting of American College of Veterinary Pathologists, Orlando, FL, 8-13 December, 1991, p. 56.
- McCauley, L., Rosol, T., Lairmore, M., Merryman, J., and Capen, C.: Modulation of parathyroid hormone-related protein mRNA expression in T-lymphocytes. In: Abstracts of Annual Meeting of American Association of Dental Research, Boston, MA, 11-15 March, 1992; and published in Journal of Dental Research 71, (1992): 212.
- Werkmeister, J., Rosol, T., Merryman, J., McCauley, L., Horton, J., and Capen, C.: Regulation of parathyroid hormone-related protein (PTHrP) in normal human keratinocytes. In: Abstracts of Annual Meeting of American Association of Dental Research, Boston, MA, 11-15 March, 1992; and published in Journal of Dental Research 71, 1992: 199.

- Merryman, J.I., Werkmeister, J.R., Rosol, T.J., McCauley, L.K., Suter, M.M., and Capen, C.C.: Parathyroid hormone-related protein production and receptor expression by normal and malignant keratinocytes. In: Abstracts (No. 213) of 81st Meeting of U.S. and Canadian Academy of Pathology, Atlanta, GA, 14-20 March, 1992; and published in Laboratory Investigation 66, 1992: 37A.
- McCauley, L., Rosol, T., Lairmore, M., Merryman, J., and Capen, C.: Polymerase chain reaction amplification of parathyroid hormone-related protein cDNA from high and low HTLV-1 viral antigen-positive lymphocytes. In: Abstracts (No. 487) of 81st Meeting of U.S. and Canadian Academy of Pathology, Atlanta, GA, 14-20 March, 1992; and published in Laboratory Investigation 66, 1992: 83A.
- Werkmeister, J.R., Merryman, J.I., Rosol, T.J., McCauley, L.K., and Capen, C.C.: Parathyroid hormone related protein production and receptor expression by normal and malignant keratinocytes in vitro. In: Abstracts of the XIth International Conference on Calcium Regulating Hormones, Florence, Italy, 24-29 April, 1992, and published in: Journal of Bone and Mineral 17(suppl. 1), 1992, p 101.
- Merryman, J.I., Rosol, T.J., DeWille, J.W., Farmer, S.J., Werkmeister, J.R., Capen, C.C.: Stimulation of parathyroid hormone-related protein production by transforming growth factor- β_1 in a squamous carcinoma cell line. Abstract of the 14th Annual Meeting of the American Society for Bone and Mineral Research, Minneapolis, Mn., 1992, and to be published in Journal of Bone and Mineral Research, 1992.
- Werkmeister, J.R., Rosol, T.J., Merryman, J.I., Capen, C.C.: Parathyroid hormone-related protein production by normal human keratinocytes in vitro is associated with a less differentiated phenotype. Abstract of the 14th Annual Meeting of the American Society for Bone and Mineral Research, Minneapolis, Mn., 1992, and published in Journal of Bone and Mineral Research, 1992.
- Merryman, J., Rosol, T., Dewille, J., Farmer, S., Werkmeister, J., Capen, C.: Stimulation of parathyroid hormone-related protein production by transforming growth factor- β_1 in a canine squamous carcinoma cell line. Abstract of the 43rd Meeting of American College of Veterinary Pathologists, San Diego, Ca., 1992, and published in Proceedings of the 43rd Meeting of the American College of Veterinary Pathologists.
- Merryman, J.I., Rosol, T.J., Dewille, J.W., Werkmeister, J.R., Capen, C.C.: Effects of transforming growth factor- β on production of parathyroid hormone-related protein production by a squamous carcinoma cell line. Abstract of the 82nd Meeting of the US and Canadian Academy of Pathology, 1992, and published in Laboratory Investigation, 66:1992.
- Merryman, J.I., Okada, H., Capen, C.C., Rosol, T.J.: Effects of gallium nitrate in nude mice bearing a canine adenocarcinoma (CAC-8) model of humoral hypercalcemia of malignancy. Abstract of the 15th Annual Meeting of the American Society for Bone and Mineral Research, 1993, and published in Journal of Bone and Mineral Research, 8:suppl1, 1993.
- Okada, H., Merryman, J.I., Rosol, T.J., Capen, C.C.: Effects of cancer-associated hypercalcemia and gallium nitrate on thyroid C-cells in nude mice. Abstract of the 15th Annual Meeting of the American Society for Bone and Mineral Research, 1993, and published in Journal of Bone and Mineral Research, 8:suppl1, 1993.

- Gröne, A., Merryman, J.I., Werkmeister, J.R., Capen, C.C., Rosol, T.J.: Parathyroid hormone-related protein (PTHrP) production in malignant and normal keratinocytes. Abstract of the 44th Meeting of American College of Veterinary Pathologists, San Antonio, TX., 1993, and published in Veterinary Pathology, 30:A155.
- Merryman, J.I., Okada, H., Capen, C.C., Rosol, T.J.: Effects of gallium nitrate in nude mice bearing a canine adenocarcinoma model of humoral hypercalcemia of malignancy. Abstract of the 44th Meeting of American College of Veterinary Pathologists, San Antonio, TX., 1993, and published in Veterinary Pathology, 30:A77.
- Okada, H., Merryman, J.I., Rosol, T.J., Capen, C.C.: Effects of cancer-associated hypercalcemia and gallium nitrate on thyroid C-cells in nude mice. Abstract of the 44th Meeting of American College of Veterinary Pathologists, San Antonio, TX., 1993, and published in Veterinary Pathology, 30:A158.
- Capen, C.C., Okada, H., Merryman, J.I., Rosol, T.J., : Effects of gallium nitrate in nude mice bearing a canine adenocarcinoma model of humoral hypercalcemia of malignancy. Abstract of the 3rd International Conference on New Actions of Parathyroid Hormone, Tokyo, Japan, 1994, and to be published in Proceedings of the 3rd International Conference on New Actions of Parathyroid Hormone, 1994.

CURRICULUM VITA

NAME Daniel Austin Ward
ADDRESS 828 Pintail Road
Knoxville, TN 37922
BIRTHDATE October 23, 1960
FAMILY Wife - Sherry L. Ward, AHT
Children - One son, Garrett D. Ward

EDUCATION

Christian Brothers College; Memphis, TN; 1978-1981
Chemical Engineering Major
No degree conferred

Middle Tenn. State University; Murfreesboro, TN; 1981-1982
Pre-veterinary major
No degree conferred

University of Tennessee; Knoxville, TN; 1982-1985
Veterinary Medicine Major
DVM conferred June 1985

University of Georgia; Athens, GA; 1986-1989
Pharmacology major
PhD conferred June 1990

PROFESSIONAL ACTIVITIES

Oct. 1990-present
Assistant Professor, Dept. of Urban Practice
College of Veterinary Medicine
University of Tennessee
Knoxville, TN 37901-1071

Sept. 1989-June 1990
Pharmaceutical Manufacturer's Association Foundation
Postdoctoral Fellow in clinical Pharmacology
University of Georgia
Athens, GA 30602

1986-1989
Ophthalmology Resident
College of Veterinary Medicine
University of Georgia
Athens, GA 30602

1985-1986
Small Animal Clinician
East North Veterinary Clinic
Greenville, SC

BOARD CERTIFICATION

Board certified by the American College of Veterinary Ophthalmologists 1991.

PROFESSIONAL ORGANIZATIONS

American College of Veterinary Ophthalmologists
American Society of Veterinary Ophthalmology
Association for Research in Vision and Ophthalmology
American Veterinary Medical Association
Phi Zeta Society

HONORS

Pharmaceutical Manufacturer's Association Foundation Postdoctoral Fellowship in Clinical Pharmacology, 1988

American Academy of Veterinary Pharmacology and Therapeutics Travel Award, 1988

Phi Zeta - University of Tennessee, 1985

American Society of Animal Science Scholarship Award - Middle Tennessee State University, 1982

Alpha Chi (Theta Chapter) - Christian Brothers College, 1981

PUBLICATIONS

Articles

Burgess H, Ward DA. The effects of topical 1% aproclonidine hydrochloride on the intraocular pressure of normal canine subjects, **in preparation 2/94**.

Ward DA. Blood-aqueous barrier stabilizing effect of flurbiprofen, diclofenac, tolmetin, and suprofen. *Arch Ophthalmol*, **submitted 2/94**.

Ward DA. Oculomycoses. In Kirk RW (ed): Current Veterinary Therapy X. Philadelphia: WB Sanders Co., **in press**.

Ward DA, Lakritz J, Bauer RW. Scleral mastocytosis in a horse. *Eq Vet J* 25: 79-80, **1993**.

Ward DA, Ferguson DC, Ward SL, Green K, Kaswan RL. Comparison of the blood-aqueous barrier stabilizing effects of steroidal and nonsteroidal antiinflammatory agents in the dog. *Prog Vet Compar Ophthalmol* 2: 117-124, **1992**.

Ward DA, Ferguson DC, Kaswan RL, Green K. Leukotrienes and sensory innervation in blood-aqueous barrier disruption in the

dog. *J Ocular Pharm* 8: 69-76, 1992.

Ward DA, Latimer KS, Askren RM. Squamous cell carcinoma of the corneoscleral limbus in a dog. *J Am Vet Med Assoc* 200: 1503-1506, 1992.

Sims MH, Ward DA. Response of transient pattern-electroretinograms (PERG) in dogs to alterations in the spatial frequency of the stimulus. *Prog Vet Comp Ophthalmol*, 2: 106-112, 1992.

Martin CL, Kaswan R, Gratzek A, Champagne E, Salisbury MA, Ward DA. Ocular use of tissue plasminogen activator in companion animals. *Prog Vet Compar Ophthalmol* 3: 29-36, 1992.

Ward DA, Clark ES. Ocular Pharmacology. *Veterinary Clinics of North America: Food Animal Practice* 7: 779-791, 1991.

Ward DA, Ferguson DC, Kaswan RL, Green K, Bellhorn RW. Fluorophotometric evaluation of experimental blood-aqueous barrier breakdown in dogs. *Am J Vet Res* 52: 1433-1437, 1991.

Salisbury MA, Kaswan RL, Ward DA, Martin CL, Ramsey JM, Fischer CA. Topical application of cyclosporine in the management of keratoconjunctivitis sicca in dogs. *J Am Anim Hosp Assoc* 26: 269-274, 1990.

Ward DA, Martin CL, Weiser I. Band keratopathy associated with hyperadrenocorticism in the dog. *J Am Anim Hosp Assoc* 25:583-586, 1989.

Ward DA. New trends in veterinary ocular pharmacology. *Georgia Veterinarian* 41:8-9, 1989.

Martin, CL, Ward DA. Medical therapy of glaucoma. In Kirk RW (ed): Current Veterinary Therapy X. Philadelphia: WB Sanders Co., 1989, pp. 647-651.

Kaswan RL, Salisbury MA, Ward DA. Spontaneous canine keratoconjunctivitis sicca. A useful model for human keratoconjunctivitis sicca: Treatment with Cyclosporin eyedrops. *Arch Ophthalmol* 1989; 107:1210-1216.

Abstracts

Ward DA, Ferguson D, Ward S, Green K, Kaswan R. Comparison of the blood-aqueous barrier stabilizing effects of steroidal and nonsteroidal antiinflammatory agents in the dog. *Vet Pathol* 1992; 29:480.

Ward DA, Kaswan RL, Green K, Bellhorn RW. Fluorophotometric evaluation of experimental acute anterior uveitis in the dog. *Trans Am Col Vet Ophthalmol* 1988; 19:113.

Ward DA, Kaswan RL, Martin CL. Ocular granulomatous meningoencephalitis in a dog. *Tran Am Col Vet Ophthalmol* 1987; 18:307.

Sims MH, Ward DA. Response of transient pattern-electroretinograms (PERG) in dogs to alterations in the spatial frequency of the stimulus. *Proc Am Coll Vet Internal Med*, May 1993.

Martin CL, Kaswan R, Gratzek A, Champagne E, Salisbury MA, Ward DA. Ocular use of tissue plasminogen activator. *Vet Pathol* 1992; 29:472.

Kaswan RL, Salisbury MA, Ward DA, Martin CL, Fischer CA, Ramsey JM, Kemp DT. Topical application of cyclosporine: A new method to increase tear production for keratoconjunctivitis sicca. *Trans Am Col Vet Ophthalmol* 1987; 18:296.

Kaswan RL, Fischer CA, Ward DA, Martin CL, Ramsey JM. Clinical trials of ophthalmic cyclosporine in chronic keratitis. *Trans Am Col Vet Ophthalmol* 1987; 18:276-295.

PRESENTATIONS

National and Regional Meetings

American College of Veterinary Ophthalmologists, Fort Worth TX. "Ocular granulomatous meningoencephalitis in a dog," November 1987.

American College of Veterinary Ophthalmologists, Las Vegas NV. "Fluorophotometric evaluation of experimental acute anterior uveitis in the dog," October 1988.

American College of Veterinary Ophthalmologists, San Diego CA. "Comparison of the blood-aqueous barrier stabilizing effects of steroidal and nonsteroidal antiinflammatory agents in the dog," November 21, 1992.

Johns Hopkins Center for Alternatives to Animal Testing, Baltimore MD. "In vitro model of the blood-ocular barriers as an aid in oculotoxicity testing," February 23, 1994.

Continuing Education Seminars

Greenville Veterinary Medical Association, Greenville, SC. "Canine glaucoma," 2 hours, January 1988.

East Tennessee Veterinary Medical Association, Gatlinburg, TN. "Equine keratitis," 3 hours, October 3, 1992.

University of Tennessee College of Veterinary Medicine Continuing Education Conference, Knoxville TN. "Update on ophthalmic

drugs," 1 hour, December 7, 1992.

University of Tennessee College of Veterinary Medicine Feline Club, Knoxville TN. "Case presentations in feline ophthalmology," 1 hour, September 15, 1993.

**University of Georgia College of Veterinary Medicine:
Physiology/Pharmacology Seminar**

"Anterior segment slit-lamp fluorophotometry in the dog" - February 1988.

"Experimental blood-aqueous barrier breakdown in the dog" - November 1988.

University of Georgia College of Veterinary Medicine: Continuing Education Program.

"Inherited and Congenital diseases of the ocular fundus" - February 1987.

University of Tennessee Intern/Resident Seminar Series

8 lectures, various ophthalmological topics, Spring 1991.

"Ocular Emergencies" - July 1992.

"Keratitis" and "Anterior Uveitis" - February 1993.

"Ocular Emergencies" - July 1993.

"Corneal Opacification" and "Case Discussions" - February 1994

"Biostatistics" - 5 hour series 2/94 - 6/94

University of Tennessee Grand Rounds Series

"Odontogenic Keratocyst in a Dog" - April 5, 1992.

"Ocular Blastomycosis in a Dog" - 1992.

"Myasthenia Gravis Presenting as Blepharoptosis in a Dog" - Sept.10, 1993

Lay Audience Presentations

"Ocular emergencies" - Greater Atlanta Boxer Association, February 1988.

"Ocular diseases affecting the Bulldog" - Atlanta Bulldog Association, September 1989.

"Feline Ophthalmology" - Knoxville Feline Club, February 20, 1992.

RESEARCH EXPERIENCE

Grants

"Chemomodulation of the canine blood-aqueous barrier:
Evaluation by fluorophotometry"

Principle author: Ward DA

Principle investigator: Kaswan RL

Co-investigators: Ward DA, Kaswan RL, Martin CL, Ferguson DC

Funding: Funded by the University of Georgia Veterinary
Medical Exp Station, \$5,100, June 1987

- a. Grant renewal funded by the University of Georgia
VMES, \$2,000, June 1988.
- b. Grant renewal funded by the University of Georgia
VMES, \$4,600, June 1989.

"A quantitative model of uveitis in the dog"

AVMA Foundation Research Grant Program

Principle author: Ward DA

Principle investigator: Kaswan RL

Co-investigators: Ward DA

Funding: Ranked in top 25% of 214 proposals submitted and
approved funding, but not funded due to limitation
of available funds.

"Comparison of ocular anti-inflammatory drugs in the dog:

Evaluation by fluorophotometry."

Solvay Resident Grant Competition, 1989

Principle author: Ward DA

Principle investigator: Ward DA

Funding: Selected as finalist in competition but not
selected as an awardee.

"Pharmacologic management of blood-aqueous barrier disruption
in dogs."

Principle investigator: Ward DA

Funding: UT Centers of Excellence, \$5,000; Small Animal
Research, \$3,000.

"Characterization of PGE₂ receptors in canine iris-ciliary body
membrane preparations."

Principle investigator: Ward DA

Co-investigator: Frazier DA

Funding: UTCVM Venture Grant, \$2,900; UTCVM Department of
Urban Practice, \$1,000.

"Evaluation and comparison of two surgical techniques for
the treatment of canine glaucoma."

Principle investigator: Ward DA

Co-investigator: Morgan RV

Funding: Companion Animal Fund, \$3,560.

"In vitro model of the porcine blood-aqueous barrier."

Principle investigator: Ward DA

Co-investigators: Rowles T, Frazier D, Schultz T

Funding: UTCVM Venture Grant, \$3,400.

"In vitro model of the blood-ocular barriers as an aid in
oculotoxicity testing."

Principle investigator: Ward DA

Co-investigators: Rowles T, Frazier D, Schultz T

Funding: Johns Hopkins Center for Alternatives to Animal
Testing, \$30,000, 2/1/94.

"Isolation of and indirect immunofluorescent testing for
Chlamydia psittaci in cats with conjunctivitis."

Principle investigator: Ward DA

Co-investigators: Kennedy M, Legendre A, Potgeiter L, Morgan R,
Grove C

Funding: Intervet, Inc., \$5,000.

"Effect of topical demecarium bromide on systemic
acetylcholinesterase levels in dogs."

Principle Investigator: Ward DA

Co-investigators: Abney K, Israel J

Funding: UTCVM Companion Animal Fund, \$382.10.

"The effects of topical 1% aproclonidine hydrochloride on the
intraocular pressure of normal canine subjects."

Principle Investigator: Ward DA

Co-investigator: Burgess H

Funding: UTCVM Small Animal Research, \$1,760.

"In vitro model of the blood-ocular barriers as an aid in
oculotoxicity testing."

Principle Investigator: Ward DA

Funding: **Pending** -- UTCVM Centers of Excellence, \$15,681
requested 3/94.

"In vitro model of the blood-aqueous barrier as an aid in
oculotoxicity testing."

Principle Investigator: Ward DA

Co-investigator: Rowles TA, Frazier D, Schultz TW

Funding: **Pending** -- Fight for Sight, the Research Division of
the National Society to Prevent Blindness, \$12,000 requested
3/94.

Current Investigations

Evaluation and comparison of two surgical techniques for the
treatment of canine glaucoma.

Comparison of 3 surgical techniques for the treatment of canine
distichiasis.

Isolation of and indirect immunofluorescent testing for *Chlamydia psittaci* in cats with conjunctivitis.

CONTINUING EDUCATION COURSES ATTENDED

American Society of Veterinary Ophthalmology, Atlanta, GA, June 1986.

American College of Veterinary Ophthalmologists Annual Meeting, Fort Worth, Texas, November 1987.

Basic Sciences Course in Ophthalmology, Stanford University School of Medicine, Palo Alto, CA, July 1988-September 1988.

Phacoemulsification/Intraocular Lens Implantation Short Course, University of California School of Veterinary Medicine, Davis, California, June 1989.

American College of Veterinary Ophthalmologists Annual Meeting, New Orleans, Louisiana, October 1989.

American College of Veterinary Ophthalmologists Annual Meeting, San Diego CA, November 1992.

TEACHING EXPERIENCE

Small Animal General Surgery - SMS 540 (University of Georgia)
Assistance in ophthalmology portion of student surgery laboratory, Spring Quarter 1987 & 1988

Studies in Small Animal Clinical Medicine - SM 590 (University of Georgia)
Clinical rotation in ophthalmology - Participation totaling 60 weeks during the period September 1986-September 1989

Small Animal Medicine - SMS 520 (University of Georgia)
Limited participation in didactic ophthalmology lectures, 1988 & 1989

Small Animal Medicine - SMS 521 (University of Georgia)
Limited participation in didactic pharmacology lectures, 1989

Special Senses - VM856 (University of Tennessee)
Didactic ophthalmology lectures (11) and laboratories (5), Fall 1990, Winter 1992, Winter 1993, Winter 1994.

Veterinary Medical Technology - AS496 (University of Tennessee)
Technology used in the practice of veterinary ophthalmology (1 hr), 4/15/93, 3/31/94.

COMMITTEE ASSIGNMENTS

College of Agriculture Advisory Council, Student Development Subcommittee, member, 1991 - present.

Postdoctoral/Resident Committee, member, 1992-present.

Educational Computing Committee, member, 1993-present.

Committee to Formulate Guidelines for Visiting Foreign Veterinarians, chairman, 1993.

MISCELLANEOUS

Mentor for ophthalmology section of UTCVM Open House, 1991, 1992, 1993.

Presentation on careers in veterinary medicine, Farragut High School Career Day, Spring 1991.

Participant in UT Academy for Teachers of Science and Mathematics program at UTCVM, July 8, 1992.

Arranged Departmental Christmas party, December 1992.

Participant in UTCVM/Knoxville Zoo "Teddy Bear Picnic," July 1993.

Participant in UTCVM Field Day for West High School Physiology Class, 3/3/93.

Tour of UTCVM for Blount County elementary school students, 1/28/94.

Active involvement in use of computer generated teaching methods: Gave continuing education course using this format December 1992, presented one lecture for VM856 using this format March 1994.

Presenter, 1993 Last Lecture Series on behalf of the Class of 1994.

Advisor, Student Chapter of the AVMA, 1993-present.

Tour of UTCVM for Leadership Education conference, 3/18/94.

Publication #R180101-10-001-95

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