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Center of Excellence Annual Report, July 1992-June1993

College of Veterinary Medicine

Center of Excellence in Livestock Diseases and Human Health

Annual Report

July 1, 1992 - June 30, 1993

College of Veterinary Medicine
The University of Tennessee
Knoxville, Tennessee
Mike Shires, Dean

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THE UNIVERSITY OF TENNESSEE
COLLEGE OF VETERINARY MEDICINE

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July 16, 1993

The College of Veterinary Medicine, despite reduced state funding, has enjoyed another good year of scholarship. Our success can be greatly attributed to the flexibility this Center offers in supporting the best and most productive programs.

While a full search for an assistant/associate dean for research and graduate programs was completed this year, the College elected to forego filling the position for now. This decision was based on two major criteria: the current fiscal climate in higher education and information gathered during the search. In the interviewing process, it was recognized that while modestly funded, we have a firm research foundation and that our College is still recognizing growth. It was clear from our discussions with all the candidates that we distribute funds fairly and in a clearly identified manner. Moreover, the excellent scientific recommendations provided by both the external and internal advisory committees, coupled with the spirit of cooperation and commitment to quality fostered by the Center's administrators has, even in these financially troubled times, maintained excellent progress toward Center goals.

In view of the above findings, we feel that additional leadership in research and graduate programs, while desired, is not yet essential. The College is in the process of hiring a grants and contracts specialists to better oversee the research funds.

This report documents the success from careful management of COE funding. However, much scholarship is an indirect result of COE. For example, because of our successive growth, we are able to attract young researchers such as Dr. Steve Kania who comes to the College with his own funding. His grant entitled "Identification of C3 binding components that facilitate invasion by *Babesia bigemia*" is through the USDA. His research intertwines well with Dr. Leon Potgieter's and our effort in livestock diseases and population medicine.

Other excellent examples of how instrumental COE funds are to the College are:

- Dr. S. Orosz's project entitled "Evaluation of the pharmacokinetic disposition if Itraconzaole in Blue Fronted Amazon Parrots" was funded by the Association of Avian Veterinarians. Preliminary data secured with COE funds allowed her grant to be competitive enough to receive the funding.

- Dr. Rhea Morgan, a new faculty member, was able to capitalize on her COE seed monies by gaining funds from Schering-Plough Animal Health to "determine the effectiveness of cyclosporine ophthalmic ointment in dog."

As the College advances, COE funds will continue to be counted on to challenge excellence and reward diligence and dedication as we stay focused on our goals to maintain that cutting edge -- to strive for continual growth. These funds also provide much needed funding for some exposure to research as a career choice for some of our students.

Sincerely,

G.M.H. Shires
Dean

vjb

1992-93 CENTER OF EXCELLENCE MEMBERS
COLLEGE OF VETERINARY MEDICINE

Philip N. Bochsler, D.V.M., Ph.D.
Assistant Professor
Department of Pathobiology

Michael A. Breider, D.V.M., Ph.D.
Associate Professor
Department of Pathobiology

David A. Brian, D.V.M., Ph.D.
Professor
Department of Microbiology

Douglas A. Dawson, Ph.D.
Research Assistant Professor
Department of Animal Science

Donita L. Frazier, D.V.M., Ph.D.
Assistant Professor
Department of Environmental Practice

James D. Godkin, Ph.D.
Associate Professor
Department of Animal Science

Ted P. McDonald, Ph.D.
Professor
Department of Animal Science

Charmi Mendis-Handamama, Ph.D.
Assistant Professor
Department of Animal Science

Mark Miller, Ph.D.
Assistant Professor
Department of Pathobiology

Linda Munson, D.V.M., Ph.D.
Assistant Professor
Department of Pathobiology

Jack W. Oliver, D.V.M., Ph.D.
Professor
Department of Environmental Practice

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 Environmental Practice

Teresa K. Rowles, D.V.M., Ph.D.
Assistant Professor
Department of Animal Science

Barry T. Rouse, B.V.Sc., Ph.D.
Professor
Department of Microbiology

Hildegard M. Schuller, D.V.M., Ph.D.
Professor
Department of Pathobiology

Terry W. Schultz, Ph.D.
Associate Professor
Department of Animal Science

Robert M. Shull, D.V.M.
Professor
Department of Pathobiology

David O. Slauson, D.V.M., Ph.D.
Distinguished Professor and Head
Department of Pathobiology

J. Erby Wilkinson, D.V.M., Ph.D.
Assistant Professor
Department of Pathobiology

Programmatic Report

I. PROGRAMMATIC REPORT

I.A. REASSIGNMENT OF CENTER FACULTY

One of the four major research groups, Oncology, has been divided into two groups.

One of **Dr. H. Schuller**'s accomplishments this year, has been to establish the "Carcinogenesis and Developmental Therapeutics (CDT) Program." This program, directed by **Dr. Schuller**, brings together basic cancer researchers and clinical oncologists to provide an incentive for interactive disciplinary collaborations and a forum for guidance and assistance of junior faculty in obtaining outside funds. Currently, active members of this program include: **Drs. K. Hahn** and A. Legendre (clinical veterinary oncology), T. Panella (human clinical oncology), D. McGavin (pathology), **M. Miller** (molecular biology), and **H. M. Schuller** (experimental oncology). As an initial success of this program, **Dr. Schuller** was able to obtain a contract with a pharmaceutical company for **Dr. K. Hahn**, for a 2-year clinical trial in dogs with osteosarcomas. *It is anticipated that this study will provide convincing preliminary data useful for the generation of larger grant funds in this area.*

Under the direction of **Dr. T. Schultz**, the "In Vitro Toxicology and Toxicokinetics (IVTT) Group" is comprised of **Dr. Schultz, Dr. D. L. Frazier, Dr. T. Rowles**, and Dr. D. Ward. The newly formed program will attempt to carve out a niche in the vast arena of toxicology by building on the strengths and interests of existing CVM faculty. **Dr. T. Schultz** brings to the group his long-standing interest in <u>in vitro</u> toxicity testing, structure-activity, and microscopy. **Dr. T. Rowles** brings her interest and expertise in mammalian cell/tissue culture techniques and neurotoxicity. **Dr. D. Frazier** brings to the group her interest and expertise in biochemistry and toxicokinetics. The initial focus of the groups will be on neurotoxicity. The basic research efforts will be aimed at elucidating the mechanisms of neurotoxic action. Specifically, the group will collaborate on the development, validation and use of a novel, complex co-culture system of endothelial, neuronal and neuroglia cells as an <u>in vitro</u> tool with which to model the blood-brain barrier (BBB) and its relationship to neurotoxicity. This model will provide a basis for studies investigating BBB transport and cytotoxicity induced by other photosensitizing drugs. This program will use as it spring boards the cooperative agreement with USEPA currently being finalized by **Dr. T. Rowles**.

These new groups will be structured into the preexisting Growth Factors Group, Infectious Diseases and Population Medicine Group, and the Inflammation and Host Defense Group.

I.B. PERSONNEL CHANGES

1.B.1. Personnel Additions

Dr. Kevin Hahn joins the "Carcinogenesis and Developmental Therapeutics (CDT) Program." He received both his D.V.M. and Ph.D. from Purdue University.

1.B.2. Personnel Deletions

Dr. Michael Breider joined Parke-Davis Pharmaceuticals in Ann Arbor, Michigan, on January 1, 1993.

Dr. Doug Dawson leaves the college on June 30, 1993, to accept a faculty position with Ashland University in Ashland, Ohio.

I.C. CENTER GOALS

Goal 1: **To improve the quality of human life through better animal health.**

Dr. L. Munson made significant progress in understanding the role of transforming growth factor-Bs in the development of the bovine placenta. She and her laboratory have determined that *TGF-βs 1-3 promote proliferation of bovine trophoblastic and endometrial epithelial cells in culture*. They also have found that this *atypical action of TGF-β may be due to synergism with platelet-derived growth factors (PDGF)*. PDGF-αβ is a potent mitogen for their bovine placental and endometrial cell line and for two primary human endometrial epithelial cell lines they developed from tissues obtained in collaboration with Drs. Nirmali B. Upadhyaya and Stuart Van Meter for the UTK Medical Center. She has also determined through collaboration with **Dr. J. Godkin** that TGF-β is synergistic with retinoic acid, a substance that also regulate PDGF transcription.

Research performed in **Dr. J. Godkin**'s laboratory focuses on fetal-maternal interactions that contribute to the maintenance of pregnancy and growth and development of the embryo in domestic farm animals. Their efforts have been concentrated on proteins produced by the blastocyst, extraembryonic placental membranes and the maternal uterus. The conceptus interferon (INF) tau (τ), formerly called trophoblast protein-1 and acknowledged to be the embryonic signal for early pregnancy maintenance, was demonstrated to diminish uterine cell prostaglandin (PG) production in vitro. Additionally, interferon τ inhibited oxytocin stimulation of uterine cell PG production, illustrating that INFτ maintains early pregnancy by preventing production of $PGF_{2\alpha}$, the uterine luteolysin, which causes the regression of the corpus luteum in cyclic animals. Vitamin A (retinol) is essential for reproduction and retinoic acid, a natural metabolite of vitamin A, is believed to be the morphogen that dictates pattern formation in developing

embryos. They have demonstrated that retinol-binding protein (RBP), the retinol transport protein, is a product of all extraembryonic placental membranes and the maternal uterus. Embryonic cell growth, differentiation and cell migration are believed to be influenced by both humoral factors (such as growth factors and retinoids) and interaction with the extracellular matrix. It is anticipated that this project will receive major extramural funding next year. Recently, they have isolated from placental membrane cultures, a secreted precursor of collagen, an extracellular matrix protein. This protein has been cloned and sequenced. Currently studies are examining the role of humoral factors in collagen expression in placental and uterine cells and the influence of this collagen on cell behavior.

Goal 2: **To augment livestock disease research capabilities in the Institute of Agriculture.**

In **Dr. D. Slauson**'s studies on the mechanisms of interaction of endotoxin (LPS) with target cells, his laboratory found that *LPS-induced procoagulant activity in bovine lung macrophages was dependent on the important intracellular signalling kinase, protein kinase-C*. In more recent studies, they have expanded on this initial finding and have *obtained evidence that the proximal signalling pathway may involve a G-protein-linked receptor as well*. This G-protein-linked receptor appears to initiate signalling through a pathway that may not involve mobilization of Ca^{2+}. Additionally, recent research has clearly established the presence and a role for bovine lipopolysaccharide binding protein (LBP) in the interaction of LPS with crucial target cells. They are continuing to follow these leads regarding the transmembrane signalling pathways used by LPS and LPS/LBP complexes in bovine lung macrophages.

Dr. P. Bochsler has spent the year purifying and characterizing bovine lipopolysaccharide-binding protein. He has identified the bovine CD14 receptor (LBP) and *has characterized the response of bovine endothelial cells to endotoxin*. Further, he *has characterized the signal transduction in bovine endothelial cells in response to endotoxin or other stimuli*. He is also collaborating on elucidation of endotoxin-mediated signal transduction pathways in bovine macrophages with **Dr. D. Slauson**.

Goal 3: **To identify and characterize animal diseases that are analogous to human diseases.**

Dr. R. Shull studied the retroviral vector constructs LCIdSN and LHIdSN in canine marrow cultures and fibroblasts. Marrow cells from 2 Mucopolysaccharidosis I (MPS I)-affected dogs were infected by vector containing

supernatant in liquid culture on autologous, pre-established stromal layers. Non-adherent cells from liquid cultures produced iduronidase for 20 days at levels approximately twice that of endogenous iduronidase in similarly cultured cells from unaffected dogs. Fibroblasts from an affected dog and a normal control were similarly transfected with LCIdSN. Iduronidase production transfected MPS I fibroblasts was 2-10 times that of normal dog fibroblasts over a period of 50 days in culture.

Dr. D. Dawson, in conjunction with **Dr. T. Schultz**, has spent this year evaluating malformation of Xenopus embryos exposed to selected short-chain carboxylic acids. Valproic acid is a known human teratogen which causes malformation of the central nervous system. This acid has been tested in an effort to determine the structural requirement for the induction of malformation. They have found *that while branching effects metabolism, it is the presence of a 5- or 6-carbon chain that is important for teratogenesis.*

Dr. T. Schultz is embarking on the third year of a three-year project entitled "Predictive Toxicities of Selected Oxygen-containing Molecules." The project centers on examining the toxicity of oxygen-containing aliphatic and aromatic industrial chemicals specifically alcohols, aldehydes, and esters. Specific aims concerning toxicity testing and modeling of direct-acting bioreactive aldehydes, have been completed this past year. *This work has shown that testing protocol are directly correlated with toxicity and the toxicity of aldelydes cannot be extrapolated from one species to another.* Moreover, the test of unsaturated aliphatic alcohols, was also completed during the year. *Results on the relative toxicity of propargylic alcohols has shown these alpha-beta unsaturated alcohols are metabolized to highly toxic ketenes.*

Leydig cells in the testis interstitium are the primary source of testosterone in the adult male mammal. In the steroid synthetic pathway, cholesterol, which is an obligatory intermediate, needs to be transported into mitochondria to initiate this process. Despite the vast amount of research conducted on aspects of steroid hormone biosynthesis in many tissues (such a adrenal, ovary, testis) the mechanism of cholesterol transport into the inner mitochondrial membrane is poorly understood. As cholesterol is highly insoluble in water and cannot freely diffuse within the Leydig cells, existence of a carrier mechanism to perform this function is speculated. It is known that in liver and adrenal gland sterol carrier protein-2 (SCP_2), a 14 kDa protein is involved in this carrier mechanism. **Dr. C. Mendis-Handagama**'s previous studies have shown that *SCP_2 is highly concentrated in peroxisomes in Leydig cells and that intraperoisomal SCP_2 content is regulated by luteinizing hormone (LH), which stimulates steroid hormone production by Leydig cells.* During the past year, her research efforts have been to carry out preliminary

studies to investigate the mechanism of cholesterol transport during steroid hormone biosynthesis in Leydig cells in the testis in response to LH stimulation.

Goal 4: **To study animal models for better understanding of human disease.**

Over the past year, **Dr. B. Rouse** has succeeded in providing *further evidence in support of the hypothesis that stromal keratitis resulting from Herpes simplex virus represents an immunopathological reaction mediated by CD4+ T lymphocytes*. Their new data indicate that a single phenotype of CD4+ T cells, the Th1 phenotype, is involved in the lesions and that such cells might mediate pathology following their recognition of a host heat shock protein. Dr. Rouse will also evaluate the ability of recombinant attenuated Salmonella that express genes of Herpes simplex virus (HSV) to induce protective immunity against viral challenge in animal model systems. Special emphasis will be directed at the cytotoxic T lymphocyte response since it is hypothesized that for solid immunity to HSV this form of immunity must be included in the immune response. His research *will evaluate if a recombinant attenuated Salmonella vector system hold promise as a means of vaccinating against HSV in man.*

Dr. H. Schuller's research programs in the area of lung carcinogenesis and therapeutics and in the field of transplacental carcinogenesis have continued to flourish, resulting in an impressive number of publications and presentations. Among the highlights to Dr. Schuller's work was the *discovery that certain carcinogenic nitrosamines interact with specific signal transduction pathways in selected cell types thus resulting in continuous cell proliferation leading to cancer.* This work has now generated a new grant with the National Cancer Institute for a period of 5 years. Moreover, Dr. Schuller's *research in hamsters has shown, for the first time, that in utero exposure of fetuses to ethanol and tobacco-specific nitrosamines may cause a high incidence of pancreas cancer in the offspring.*

Another research project was to adapt the polymerase chain reaction technique to amplify small quantities of DNA from paraffin-embedded tissues. **Drs. M. Miller** and **H. Schuller** have been successful in obtaining amplified sequences of the hamster Ki-ras gene. In a recent dose response experiment, a readily detectable signal could be 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 should be readily adoptable for other gene sequences, and the development of this technique in their laboratories should greatly aid their research efforts. These results have been used to support NIH grant applications for both investigators.

During the past year, **Dr. E. Wilkinson** has made considerable progress in characterizing the phenotype of 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. The morphologic studies of the *in vivo* lesions including the transmission and scanning electron microscopy have been completed. Additionally, they have completed the lectin straining for both the liver and kidneys. Just recently, they have *determined that the Tg737 gene codes for a protein with tetratricopeptide repeats. This is the only gene with this structure yet identified and characterized in mammalian cells*. In lower eucaryootes, a number of such genes have been identified and are generally associated with regulation of the cell cycle.

In another collaborative project, **Dr. B. Rouse**'s laboratory with **Dr. E. Wilkinson**'s laboratory *will continue and expand their studies of the response of scurfy cells to stimulation*. Scurfy is an X-linked lymphoproliferative disease of mice that results from abnormalities in the development of T cells and depends on the genotype of the thymus. In these studies, quantitative mRNA expression, capture ELISAs, and ELISA-SPOT analysis will be done on stimulated scurfy and normal T cells and purified populations of T cell subsets. These *studies should provide convincing evidence for any imbalance in Th1 and TH2 CD4+ T cells in scurfy*.

Goal 5: **To understand the pathogenesis and characterize the causative agents of common diseases important to Tennessee.**

Coronaviruses cause the most economically important disease in chickens, avian infectious bronchitis, and are a cause of one of the most economically important disease syndromes in pigs and calves, neonatal scours. In pigs alone, the estimated annual cost in the U. S. due to coronavirus enteritis is 200 million dollars. **Dr. D. Brian**'s laboratory, which is entirely devoted to the study of coronaviruses, investigates coronaviruses that infect many animal species, including humans, and cause disease ranging from severe acute respiratory and gastroenteric disease to chronic disease of the central nervous system. *Recent evidence suggests that human coronavirus is causally related to multiple sclerosis*. Dr. Brian's laboratory has recently cloned a subgenomic replicon of the bovine coronavirus that is proving useful for establishing the mechanisms of coronavirus RNA replication and persistence. *The replicon may prove useful as an exciting new recombinant vaccine vehicle for stimulating mucosal immunity and for delivering medically therapeutic molecules.*

Over the past year, a major research focus in **Dr. S. Oliver**'s laboratory has been on Streptococcus uberis, an important mastitis pathogen that is very difficult to control. Results of these studies have shown that *both encapsulated and unencapsulated strains of S. uberis adhere to bovine mammary epithelial cells and to*

extra cellular matrix proteins such as laminin, fibronectin and collagen. Preliminary studies suggest that adherent bacteria become internalized or penetrate a bovine mammary epithelial cell line. If internalization occurs in vivo, this could allow S. uberis to evade host defenses, and gain access to stromal tissue. Studies are in progress to determine if epithelial cell internalization is a potential virulence factor in the pathogenesis of S. uberis mastitis. They showed that encapsulated strains of S. uberis were more resistant to phagocytosis by mammary gland macrophages than unencapsulated strains. Thus, the capsule which surrounds about 50% of S. uberis isolates may be a virulence factor by inhibiting phagocytosis. They also evaluated the use of new epidemiological markers to studying S. uberis mastitis in dairy cows. Results showed that *polymerase chain reaction-based DNA fingerprinting and bacteriocin-like inhibitory substance (BLIS) fingerprinting as epidemiological markers allowed tracing of an infection by the same clonal type in a cow over time*. New intramammary infections by different clonal types were also identified. Lastly, *a polymerase chain reaction-based DNA fingerprinting assay was developed as a method for identification of Streptococcus and Enterococcus species isolated from bovine milk*.

Several studies were initiated/completed in **Dr. J. Oliver**'s laboratory during the past year. The first project entitled "Lysergic acid amide (lysergamide) vasoconstrictor activity in the lateral saphenous vein and dorsal metatarsal artery of cattle" encompassed lysergamide induced contraction in these blood vessels which has considerable biological implications to the fescue toxicosis syndrome. *USDA researchers identified this alkaloid in 1991 as being one of the major alkaloids present in endophyte-positive fescue grass*. The alkaloid was determined to have affinity for the serotonergic-2 ($5\text{-}HT_2$) receptor, which is present in many organ systems, including the brain, kidneys, and gastrointestinal tract in particular. Thus, the alkaloid may ultimately be proven to have important biomedical uses in addition to contributing to the toxic fescue syndrome. Work has also been completed in describing the use of the Alzet™ osmotic pump system for dosing animals with purified alkaloids found in toxic tall fescue. The technique will continue to be used in studies designed to determine the chronic effects of toxic fescue alkaloids on tissues. Further, HPLC methodology has been developed and published to determine the content of lysergamide in cattle serum. Dr. J. Oliver*'s group was the first to report detecting this important new alkaloid of toxic fescue in the sera of cattle grazing endophyte-positive fescue pastures*. Finally, efforts continue to secure funds from pharmaceutical firms to develop an anti-fescue toxicosis vaccine, for which Dr. Oliver has applied for a patent.

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 1C 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 in the Clyde M. York Veterinary Medical Teaching Hospital which contains clinical and teaching facilities as well as modern research laboratories. 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).

The center enjoys excellent, but not expansive, research and graduate education facilities. The past several of years have been difficult ones from a fiscal standpoint, and little funds have been available for the expansion of facilities. We regard talented people as more important than facilities, and in the past few years, we have had to focus our attention on not losing any important human resources. Funds have recently been made available for the planning phase of a new Biotechnology Building on the Institute of Agriculture Campus. If USDA and State of Tennessee matching funds can be secured, such a building would provide at least some additional research space for COE researchers and graduate students.

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 an article on **DR. T. SCHULTZ** pertaining to toxicological issues as they relate to birth defects. *Veterinary Medical Topics*, published semi-annually by the Extension Service, routinely features articles exploring livestock diseases. *Channel 10* featured the genetic mouse model that is part of **DR. E. WILKINSON**'s research. This same feature was later picked up and shown nationally on *CNBC*. Regular features appear as well in the two UT alumni publications, *Context* and the *Torchbearer*.

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

During the past year, COE has supported **DR. K. HAHN**'s two Cytogenetics Laboratory pilot investigations in genetic toxicology. Project 1 entitled "Quantification of in vitro induced sublethal DNA damage by photodynamic therapy," determined if chloroaluminum-sulfonated phthalocyanine (A1SPc) photodynamic therapy (PDT) caused sublethal DNA damage in EMT-6 mouse sarcoma cells treated in vitro. The second project titled "Micronuclei assay: A predictive indicator for tumor response to treatment" determined if anti-neoplastic drugs such as cyclophosphamide, doxorubicin HC1, and cis-diammine-dichloro-platinumII induced sublethal DNA damage in normal circulating canine peripheral blood lymphocytes cultured in vitro and if the degree of damage was of predictive value for in vivo treatment response in tumor-bearing dogs. The *endpoint of study for both projects was evaluating the formation of cellular micronuclei. Micronuclei are fragments of DNA packaged independently during cellular division and are*

quantitatively significant as an indicator of sublethal DNA damage. With this prelinary data, Dr. Hahn plans to submit a first investigator's grant to NIH.

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.* Toxicant testing is planned for next year. Additionally, an in vitro model of the blood-brain barrier will be developed by co-culturing these cell types.

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 laboratories of three new Center members--**Drs. K. Hahn, C. Mendis-Handagama,** and **T. Rowles.** This mainly encompassed upgrade tissue culture and molecular biology capabilities. As well, several multiuser pieces of equipment were purchased, including three centrifuges, a gas chromotograph, and a tumor analysis system.

Graduate Students, Post Doctoral Researchers, and Residents

Dr. David Dean, sponsored by **Dr. D. Slauson,** was the first student in the college to receive an individual NIH research training grant. He is studying the "Signalling pathways in LPS-stimulated lung macrophages."

Dr. Donna Bouley became the second student in the college to receive an individual NIH research training grant. Under the direction of her advisor, **Dr. B. Rouse,** Dr. Bouley will study "Herpetic stromal keratitis pathogenesis: An animal model."

These NRSA Fellowship Awards are very competitive at a national level, so it is very gratifying that these proposals have been selected for funding. COE monies are used to support the research of our NRSA fellows.

Our training program is small, but high in quality. An institutional training grant has been submitted by **Dr. D. Slauson,** Department of Pathobiology. 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 Pathobiology 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 research associates and residents have benefited directly from the use of COE funding to provide for preliminary data via venture grants. A few examples include Dr. Huda Al-Ansari, a post doctoral research associate, with **Dr. L. Potgieter** who received funding from USDA for their project entitled "Significance of strain variation within the ruminant respiratory syncytial viruses." This funding will enable them to determine the seroprevalence of the prototype ORSV in cattle, to determine variability among BRSV isolates using RNase A mismatch analysis, and to establish the number of RSV subgroups infecting cattle. These *goals could provide important data that would help determine which RSV strains should be incorporated into a multivalent vaccine to effectively control the disease in cattle.*

Dr. Wendy Davies-Dean has received a grant from the American Animal Hospital Association (AAHA) for $6,100 for her study entitled "Evaluation of intraosseous administration of total parenteral nutrition in cats." Nutritional support of injured or ill dogs and cats is recognized as a vital component of case management. Her *goal is to provide an option for practitioners in cases where parenteral nutritional support is necessary but delivery by traditional routes has failed or is impossible.* Hopefully by making parenteral nutrition more accessible, this potentially lifesaving therapy will be used more routinely.

Minority Recruitment

To compliment the Minority High School Apprentice Program sponsored by the National Institutes of Health (now in its 12th year), a new veterinary internship for African-American High School Students in Tennessee has been developed with funding provided by a grant from the Tennessee Higher Education Commission. While the students participating in the NIH sponsored program spend 8 weeks at the college, the veterinary internship program participants sponsored by THEC, will be working with a veterinarian in their home towns for eight weeks during which one week they will be introduced to the UTCVM and UT campus.

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

COLLABORATIVE RESEARCH PROJECTS

To further determine if absence of PDGF-α receptors interferes with placental growth, **DR. L. MUNSON** is now collaborating with the University of Washington-Seattle to evaluate a spontaneous PDGF-α receptor-deficient mouse model, the Patch mouse. To evaluate placental development in the Patch mouse, they have developed riboprobes and initiated in situ hybridization techniques in their laboratory. These new molecular techniques will be used to identify homozygous PDGF-αR mutant embryos so that the development of their placenta can be evaluated.

In collaboration with Dr. Ellis Avner of the University of Washington, Seattle, **DR. E. WILKINSON** has begun to characterize the kidney cells involved in the production of the lesions in the Tg737 mice. Their preliminary data suggests that *the cells are principal cells and that the lesion develops as a result of a failure of maturation of the principal cells and the differentiation into the specialized intercalated cells*. Similarly, the liver lesion appears to arise from a failure of differentiation of the liver stem cells into mature biliary epithelium.

In other studies, they have cloned and sequenced the human homolog of the mouse Tg737 gene and established the molecular structure of the intron-exon boundaries. In collaboration with Dr. Steve Reeders of Howard Hughes Medical Institute, Yale University, they are *examining the structure of the Tg737 gene in over 100 human families with autosomal recessive polycystic kidney disease*. Further, Dr. Wilkinson has also completed collaborative studies with Dr. Greg Dressler of the National Institutes of Health (NIH) on the *nature of the congenital nephrotic syndrome in PAX-2 transgenic mice*.

As well, Dr. Wilkinson's laboratory *has established cultures of liver and kidney cells from Tg737 mice*. Several lines of liver cells have been developed and from mice with different genetic backgrounds. A Cooperative Research and Development Award has been established through the Oak Ridge National Laboratory and Proctor & Gamble to further characterize the putative liver stem cells.

Collaborative work was initiated between **DR. J. OLIVER** and researchers at the University of Arkansas *to characterize the activity of a purified ergovaline preparation developed by them using Dr. Oliver's isolated bovine blood vessel model.* Further, collaborative work has also been initiated between Dr. Oliver with other UTCVM researchers and Clemson University, to characterize in utero biochemical changes in placental fluids in mares on endophyte-infested and endophyte-free tall fescue pastures. Two graduate students, one resident and five faculty members between the two institutions are involved in the project.

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

One aspect of **DR. B. ROUSE**'s work involves designing delivery vehicles that optimize induction of cytotoxic T lymphocytes with viral proteins and peptides. This program, which proceeds collaboration with Dr. Leaf Huang, University of Pittsburgh, uses liposomes as carriers of autogens and adjuvants. *This approach to vaccinate may prove valuable to prevent certain human virus infections.*

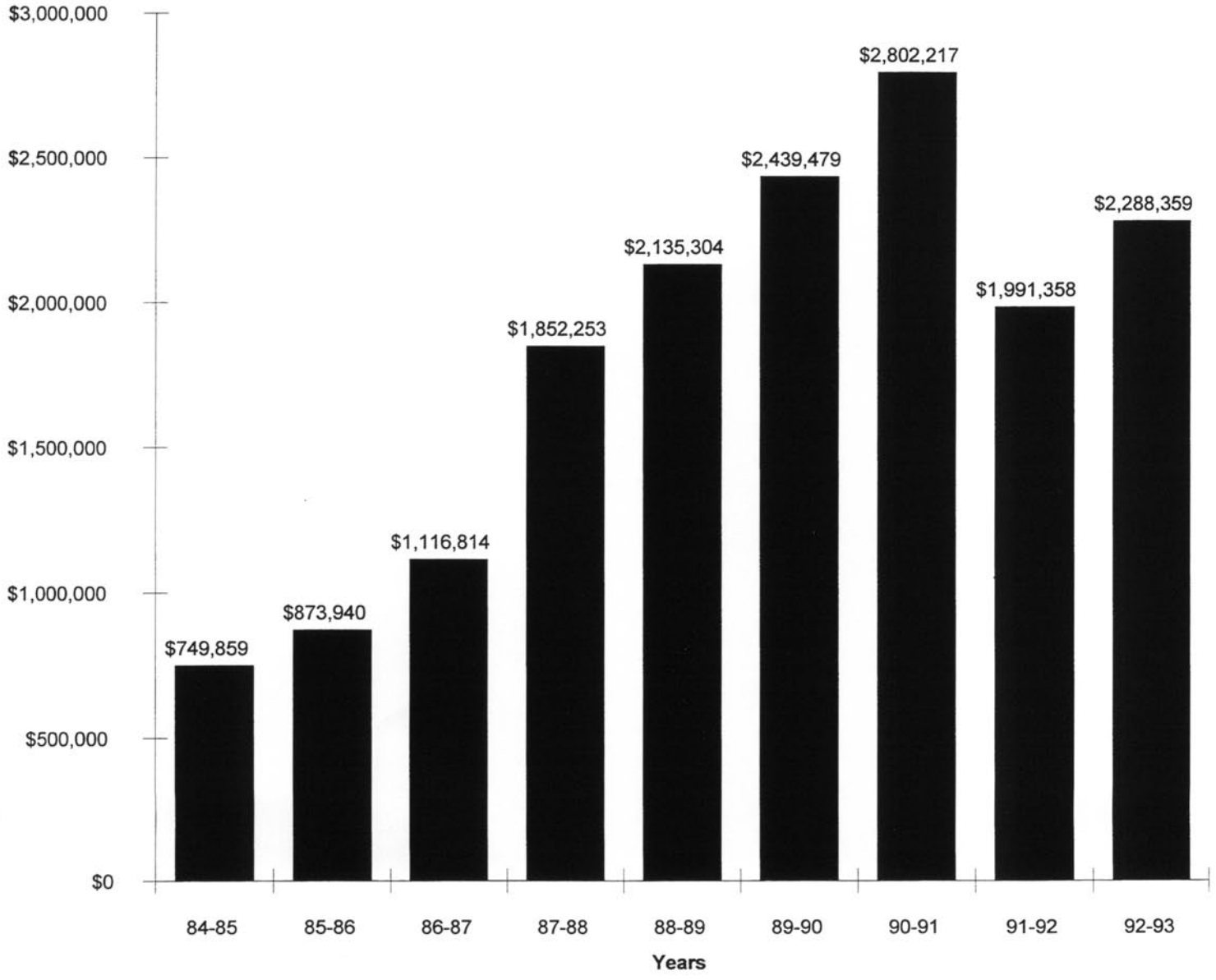
The effects of sex- and strain-related differences in megakaryocytopoiesis and platelet production in C3H and BALB/C mice were studied during the year by **DR. T. MCDONALD.** The studies were carried out on castrated male and ovariectomized female C3H and BALB/C mice, along with suitable intact controls. The results support their previous work, showing that intact male BALB/C mice had higher platelet counts and $\%^{35}S$ incorporation into platelets than did intact female BALB/C mice. Further, both intact BALB/C and C3H male mice had higher platelet counts than their castrated counterparts. Castration caused increased numbers and decreased sizes of megakaryocytes in both strains of mice. Although a difference in megakaryocyte ploidy was not detected between intact male and intact female C3H mice, BALB/C female mice had lower ploidy megakaryocytes than did BALB/C male mice. Castrated BALB/C mice had lower ploidy megakaryocytes than did intact BALB/C male mice. They found a shift to a lower ploidy class with the removal of gonads in male mice; therefore, they believe that this finding is consistent with the hypothesis that *male sex hormones cause an increase in polyploidy and size of megakaryocytes.* In order to understand thrombocytopoiesis during pregnancy, megakaryocytes and platelets were examined during gestation and the early postpartum period, using the rat as a model. Mean platelet volume and platelet volume distributions were not significantly altered in late gestation of rats. Also, they showed that platelet survival in pregnant rats was not significantly different from that of non-pregnant females. In contrast, megakaryocyte concentration was significantly increased during late gestation and early postpartum. Their *data indicate that thrombocytopoiesis is substantially increased during late pregnancy, and that this increase is accompanied through an increase in megakaryocyte DNA content and size, as well as, megakaryocyte number.* The *data also revealed that pregnancy-associated hormonal changes which produce an increase in megakaryocyte DNA content and size differ from those which cause an increase in megakaryocyte number.*

During the past year, COE provided support for a post-doctoral fellow to work with **DRS. D. FRAZIER** and **DR. M. BREIDER.** The project entitled "Enhancement of photodynamic tumor therapy with angiogenic cytokines" was supported by the Thompson Cancer Survival Center. This project *examined the effect of cytokines on*

cytoxicity induced by photsensitizing drugs in tumor cells and endothelial cells. Preliminary drug transport studies were conducted. The proposal to the Beckman Laser Institute *will examine transport of these photosensitizing agents across the blood-brian barrier and cytotoxicity to neuroblastoma cells, brian capillary endothelial cells and astrocytes*. COE has also provided support for two doctoral students. The two projects (1) use of photodynamic therapy for autologous bone marrow transplantation and (2) synergistic activity of cisplatin and radiation, will be completed this year.

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 *(Final Year of Initial Commitment)* 1988-89			**Year 6** *(Year 01 as Accomplished Center)* 1989-90		**Year 7** *(Year 02 as Accomplished Center)* 1990-91		**Year 8** *(Year 03 as Accomplished Center)* 1991-92		**Year 9** *(Year 04 as Accomplished Center)* 1992-93	
	Target	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)
2. Books or book chapters published		7	(0.30)	17	(0.65)	14	(0.54)	6	(0.32)	7	(0.35)
3. Published proceedings		21	(0.91)	37	(1.42)	42	(1.62)	24	(1.26)	17	(0.85)
Total Publications:	2.82	102	(4.43)	122	(4.69)	153	(5.89)	113	(5.95)	102	(5.10)
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)
2. National Meetings	1.25	55	(2.39)	28	(1.08)	44	(1.69)	36	(1.89)	47	(2.35)
C. Abstracts	0.30	33	(1.43)	66	(2.54)	48	(1.85)	47	(2.47)	53	(2.65)
Number of faculty included in Center		23	.	26	.	26	.	19	.	20	.
Number of Visitors	11	10	.	17	.	17	.	12	.	12	.

TABLE 2.

RESEARCH PROJECTS FUNDED EXTERNALLY
REPORT PERIOD 1992-93

PROJECT DIRECTOR	SOURCE	TOTAL AMOUNT AWARDED	**ESTIMATED** EXPENDITURES 7-1-92/6-30-93
BOCHSLER, P. N. *Bovine Lipoplysaccharide Binding Protein and Mechanisms of Macrophage Activation*	USDA 9/1/91-8/31/93 (Extension to 8/31/94)	140,000	42,000
BOCHSLER, P. N. *Molecular Basis of Endothelial Cell Sensitivity to Lipopolysaccharide*	USDA 9/15/92-9/30/94	95,000	37,601
BOCHSLER, P. N. *The Bovine CD14 Receptor: A Link in Endotoxin-mediated Macrophage Activation*	USDA 9/15/92-9/30/94	150,000	59,375
BRIAN, D. A. *Coronavirus Structure and Replication*	NIH 9/1/89-8/31/94	552,187	140,716
BRIAN, D. A. *Mechanism(s) of Coronavirus RNA Replication and Packaging*	USDA 9/15/92-9/30/95	200,000	52,782
DAWSON, D. A. **SCHULTZ, T. W.**, Co-investigator *Mixture Teratogenesis: Relation to Mechanisms and QSARs*	NIEHS/NIH 4/1/91-3/31/93	138,043	51,768
HAHN, K. **SCHULLER, H. M.**, Co-investigator Contract--*B859-035 Treatment of Dogs with Osteosarcoma*	BYK Gulden Pharmaceuticals 1/1/93-12/31/95	63,890	21,120
MCDONALD, T. P. Contract--*Development of Assays for Thrombopoietin*	Genentech 3/1/88-2/28/93 (Extension to 2/28/95)	175,832	29,304
Performance of Assays for Thrombopoietin	3/1/88-7/31/93 (Extension to 2/28/95)	112,700	18,780
MCDONALD, T. P. *Characterization of Thrombopoietin*	AmGen 10/1/92-12/31/95	73,671	17,001
MCDONALD, T. P. *Thrombopoietin: Immuno-assay & Characterization*	NIH 12/1/88-11/30/93	548,681	109,740

PROJECT DIRECTOR	SOURCE	TOTAL AMOUNT AWARDED	**ESTIMATED** EXPENDITURES 7-1-92/6-30-93
McDonald, T. P. Purchase of: *Matrix 96 Direct Beta Counter*	NHLBI Small Instrumentation Program 9/1/92-8/31/93	17,225	17,225
Munson, L. *The Pathological Effects of Melengestrol Acetate in Captive Wild Felids*	AAZPA Conservation Center 10/1/92-9/30/93	37,807	28,359
Oliver, S. P. *Mastitis Research*	Alcide Corporation 12/90-12/94	57,752	14,438
Oliver, S. P. *Mastitis Research*	Monsanto Agri Co 11/90-12/92	25,576	12,788
Oliver, S. P. *Factors Influencing Bovine Mononuclear Cell Function during the Nonlactating Period*	USDA-SEA 9/90-9/92	98,388	12,300
Oliver, S. P. *Mastitis Research*	H. B. Fuller Co. 4/91-12/94	31,778	8,664
Effectiveness of chlorhexidine as a premilking teat disinfectant for the prevention of bovine mastitis	H. B. Fuller Co. 1992-1993	24,052	12,026
Chlorhexidine Milk Residues Associated with Premilking and Postmilking Teat Disinfection	H. B. Fuller Co. 1992-1993	3,732	3,732
Oliver, S. P. *Mastitis Research*	Upjohn Company 10/91-12/94	6,293	1,992
Immunization of dairy cows against Streptococcus uberis: Efficacy of an Experimental Vaccine during the Nonlactating Period	Upjohn Company 1991-1993	54,225	27,112
Oliver, S. P. *Selenium and Vitamin E in Disease Resistance*	BASF 1992-1993	17,000	10,000
Oliver, S. P. *Use of Genetic Markers as Indicators of Mastitis Resistance and Milk Production in Jersey Cattle*	American Jersey Cattle Club 1993	6,000	3,000

PROJECT DIRECTOR	SOURCE	TOTAL AMOUNT AWARDED	**ESTIMATED** EXPENDITURES 7-1-92/6-30-93
POTGIETER, L. *Development of Technology for use in the Discovery and Production of a Commercial BVD Vaccine and Diagnostic*	Upjohn Company 2/1/90-12/31/92	75,000	25,000
POTGIETER, L. *Cloning and Sequencing of BRSV G Glycoprotein and Detection of Strain Divergence*	USDA Special Grant 8/1/90-7/31/93	149,974	49,991
ROUSE, B. T. *Immunity Mechanisms in Herpesvirus Infections*	NIH 5/1/89-4/30/94	1,130,370	234,132
ROUSE, B. T. *Keratitis Treatment with Drug-containing Immunoliposomes*	National Eye Institute (NIH) 9/30/87-9/29/92	718,935	38,907
Mechanisms in Herpetic Stromal Keratitis	National Eye Institute (NIH) 9/30/92-9/29/97	774,745	152,586
ROUSE, B. T. *Liposome Microencapsulation of Vaccine Antigens*	NIAID 6/1/90-5/31/95	828,289	162,807
ROUSE, B. T. *Herpes Zosterification*	Smithkline Biologicals 12/15/89-12/31/94	124,746	25,000
ROWLES T. K. *Characterization Isolated Rodent Microvessels/ Astrocytes Co-cultures*	Environmental Protection Agency (EPA) 4/7/93-4/6/95	9,484	789
SCHULLER, H. M. *Mechanisms of Neuroendocrine Lung Carcinogenesis by Nitrosamines*	Shannon Award Institute (NIH) 9/1/91-8/31/93	100,000	48,504
SCHULLER, H. M. *Transplacental Carcinogenicity of NNK*	NIH 9/1/89-11/30/92 (Extension to 11/30/93)	491,219	93,137
SCHULLER, H. M. *Characterization of Induced Neuroendocrine Lung Cancer*	National Cancer Institute (NIH) 2/7/92-1/31/95	464,975	176,642
SCHULLER, H. M. Contract--*Testing of Anti-carcinogenic Effects of Niguldipine*	Byk Gulden Pharmaceuticals 3/1/88-12/31/94	382,925	52,497

PROJECT DIRECTOR	SOURCE	TOTAL AMOUNT AWARDED	**ESTIMATED** EXPENDITURES 7-1-92/6-30-93
SCHULTZ, T. W. *Chaos in Microbial Systems*	NSF 12/1/89-11/30/92	111,615	15,500
SHULL, R. *Molecular Study of MPS I: Gene Therapy in a Canine Model*	NIH 8/1/92-7/31/96	1,001,653	114,944
SLAUSON, D. O. *Leukocyte Function and Host Defense in Developing Calves*	USDA Competitive 8/15/90-9/30/93 (Extension to 9/30/93)	120,000	39,000
SLAUSON, D., Advisor for D. Dean *Signalling Pathways in LPS-Stimulated Lung Macrophages*	NIH Training Grant 11/30/91-11/30/94	97,500	26,540
WILKINSON, J. E. & GODKIN, J., Co-investigator *Transforming Growth Factors in Early Pregnancy in the Cow*	USDA Special 8/1/89-6/30/92 (Extension to 6/30/93)	149,653	35,488
WILKINSON, J. E. *Molecular Genetics of PKD in the Transgenic TG737 Mouse*	NIH 9/30/92-9/29/95	406,639	121,608
WILKINSON, J. E. *Immunobiology of the Scurfy Mouse*	NIH 9/30/92-5/31/95	579,594	143,464
Total			$2,288,359

Plans For Next Year

III. PLANS FOR NEXT YEAR

CARCINOGENESIS AND DEVELOPMENTAL THERAPEUTICS GROUP

Dr. H. Schuller will continue to pursue research goals and devote considerable efforts to the integration of other faculty into the interdisciplinary research programs under the umbrella of the "Carcinogenesis and Developmental Therapeutics Program. Dr. Schuller has served on four NIH site visit teams for the review of Cancer Center grants and program project grants, and she was an ad hoc member of two NIH study sections. She has agreed to become a regular member of the NIH chemical pathology study section in January 1994. Dr. Schuller has also been appointed as associate editor of the international journal *Carcinogenesis*.

Fetal tissues are more sensitive to the effects of chemical and physical carcinogens than are adult tissues. This suggests that the embryos and fetuses of pregnant women are at a greater risk of developing cancer from environmental exposures than is the adult population. It is thus important to gain further understanding of the mechanism(s) that help modulate the fetal organism's response to environmental toxicants, and to determine how the genetic background of the individual fetus and its mother can modulate its response to envrionmental carcinogens. **Dr. M. Miller**'s goal for the coming year is to determine the molecular mechanisms governing the pathogenesis of lung tumors in mice differing in their susceptibility to polycylic hydrocarbon-mediated tumor formation. The data will show how the genetic make-up of the individual and the modulating influences of maternal phenotype can influence susceptibility to cancer as a result of early in utero exposure to environmental chemicals.

Dr. K. Hahn's long term goal for the Cytogenetics Laboratory is to determine the in vitro and/or in vivo efficacy of novel cancer treatment strategies using proven methods in genetic toxicology. With recent support from Bristol Laboratories and the National Cancer Institute of donated drugs, he will seek to determine quantitatively if SR-2508 (etanidazole), an oxygen-mimetic nitroimidazole, potentiates sublethal DNA damage and cytotoxicity in EMT-6 tumor cells and/or normal peripheral blood lymphocytes when concurrently exposed to subtherapeutic doses of either radiation or chemotherapy in vitro.

GROWTH FACTORS GROUP

Dr. R. Shull will continue to investigate methods of either stem cell enrichment or elimination of differentiated cells from populations to be subjected to gene transfer. Even with relatively high titer vectors, working with unenriched marrow mononuclear cells requires very large volumes to be effective. Monoclonal antibodies to c-kit, the target for stem cell factor have been obtained, and they will test these by FACS in the coming year. Another antibody (DH59B) reacts with an epitope on differentiated granulocytes and

For **Dr. T. Rowles**, next year will involve further development of protocols for the in vitro assessment of neurotoxicity. These protocols include transport studies, transepithelial electrical resistance and biochemical assays. Such protocols will be used to assess the integrity of an in vitro model of the blood brain barrier. *A valid model of the blood brain barrier will allow drug and toxicant testing with a reduction in the number of animals used and an increase in the numbers of chemicals tested.* Additionally, this model will provide a way to study mechanisms of neurotoxicity.

Dr. D. Frazier is working with **Drs. T. Rowles** and **T. Schultz** to develop an in vitro model of the blood-brain barrier for evaluation of transport and cytotoxicity of environmental toxicants and cancer chemotherapeutics. Preliminary studies of Dr. Frazier and others suggested that cytokines are important mediators of transport of cytotoxic drugs across peripheral capillary endothelial cells and into tumor cells. Angiogenic cytokines may be even more important as mediators of transport across brain capillary endothelial cells (blood-brain barrier).

INFECTIOUS DISEASES AND POPULATION MEDICINE GROUP

Dr. D. Brian's research is a systematic analysis of the molecular structure, replicational strategy, and medical significance of coronaviruses, one family of RNA viruses that cause primarily gastroenteric and respiratory diseases in animals and humans. Their major effort focuses on two gastroenteric coronaviruses, the porcine, transmissible, gastroenteritis, coronavirus (TGEV) and the bovine enteric coronavirus (BCV). Both are significant veterinary pathogens and each serves as a model for one of two major antigenic subgroups of mammalian centeric coronaviruses. They also conduct some studies on the mouse hepatitis coronavirus and human coronaviruses.

Additional structural and nonstructural protein-encoding genes of ORSV have been cloned in **Dr. L. Potgieter**'s laboratory. In the next year, these ORSV recombinant clones will be sequenced and analyzed, as has been done for the G and 1C genes. They also plan to subclone the ORSV genes into the baculovirus expression system for production and analysis of ORSV proteins. Eventually, they hope to evaluate the immune response to, and correlates of protection for ruminant RSV. Furthermore, since it is not known if ovine RSV strain is circulating in cattle, his laboratory plans to assess the presence of antibodies to their ovine RSV G glycoprotein serum samples from cattle in different geographical locations using a sensitive immunoassay such as ELISA. Such information is important to determine strain variability among cattle and to identify the strain necessary for inclusion in any multivalent vaccine. They also plan to continue investigation of 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.

Dr. J. Oliver will participate in endophyte-free and endophyte-positive tall fescue pasture studies. Studies will include collection of both forage from pastures and serum

from cattle throughout the grazing season to determine alkaloid content by HPLC and GC methods. These profiles will be correlated with other physiological response factors, including animal growth, hair quality scores, serum prolactin, serum endothelin and serum cortisol levels.

Dr. S. Oliver will be working on a new project entitled "DNA probes for typing and subtyping bacterial pathogens isolated from dairy cattle." Application of DNA probes for identification and subtyping of pathogenic bacteria could allow diagnostic laboratories to examine a wider variety of organisms, enable identification of bacterial pathogens that cannot be cultured easily in vitro, allow rapid screening and subtyping of a large number of organisms which would be very useful during an outbreak of an animal disease, enhance development of strategies for monitoring disease control programs and reduce costs of handling, processing, identification and subtyping of bacterial pathogens. The objective for the first year of this study is to develop a model system for identification of pathogenic bacteria using specific oligonucleotide primers targeted at 16 S rRNA sequences.

INFLAMMATION AND HOST DEFENSE GROUP

During the next academic year **Dr. B. Rouse**'s efforts will be directed at finding explanations for the preferential accumulation of CD4+ T cells in ocular tissues identifying possible target antigens which they recognize and evaluating the role of nitric oxide radicals as responsible for tissue damage.

Dr. P. Bochsler will expect to explore new research possibilities as they arise by incorporating new techniques, and by pursuing collaborative studies when bovine LBP is isolated and purified in sufficient quantity. Should opportunity arise, to continue PMN-related research, he would like to continue this as time and personnel allow. A specific goal is to have at least three research manuscripts published in refereed journals.

The specific goals for **Dr. D. Slauson** for the coming year are to continue to dissect the signalling 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 data his laboratory has been able to obtain to date support their contention that the pathways used by LPS are at least partially G-protein-linked and PKC-dependent. A second objective will be to determine the role of Ca^{2+} in these signalling pathways.

Budget Forms

REPORT F

SCHEDULE 1
TENNESSEE HIGHER EDUCATION COMMISSION
CENTERS OF EXCELLENCE
1992-93 BUDGET AND PROPOSED 1993-94 BUDGET

	1992-93 Actual Expenditures			1993-94 Proposed Budget		
	Matching	Appropriations	Total	Matching	Appropriations	Total
Revenue						
New State Appropriation		506,500	506,500		506,000	506,000
Carryover from Previous Appropriation					15,068	15,068*
New Matching Funds	253,250		253,250	253,000		253,000
Carryover from Previous Matching				7,579		7,579
Total	253,250	506,500	759,750	260,579	521,068	781,647
Expenditures						
Salaries						
a. Faculty	39,250	78,620	117,870	40,932	81,988	122,920
b. Other Professional	54,020	108,203	162,223	51,904	103,963	155,867
c. Clerical/Supporting	18,240	36,539	54,779	17,773	35,600	53,373
d. Assistantships	5,070	10.155	15,225	8,762	17,550	26,312
e. Students	7,566	15,149	22,715	8,325	17,675	25,000
Total Salaries	124,146	248,666	372,812	127,696	255,776	383,472
Fringe Benefits	23,045	46,158	69,203	26,867	53,816	80,683
Total Personnel	147,191	294,824	442,015	154,563	309,591	464,155
Travel	416	835	1,251	1,665	3,335	5,000
Software						
Books and Journals						
Other Supplies	33,874	67,851	101,725	47,974	95,219	143,192
Equipment	58,277	116,075	174,262	49,950	100,050	150,000
Maintenance	4,525	9,063	13,588	4,995	10,005	15,000
Student Fees	1,389	2,783	4,172	1,433	2,868	4,300
Scholarships						
Consultants						
Renovation						
Other (Specify)						
Total Non-personnel	98,481	195,607	294,998	106,016	211,477	317,492
GRAND TOTAL	245,671	491,432	737,013	260,579	521,068	781,647

*There was $6790 obligated in equipment that did not get paid in 92-93.

Appendices

NAME: Kevin A. Hahn
TITLE: Assistant Professor
APPOINTMENT: Dept. of Environmental Practice

EDUCATIONAL BACKGROUND:

Degree	Year	University	Major
PhD	1992	Purdue	Cytogenetics/Oncology
DVM	1987	Purdue	Veterinary Medicine
BS	1983	Purdue	Animal Sciences

CAREER HISTORY:

Year(s)	Title	Employer
1991 - present	Assistant Professor of Oncology	Univ. of Tennessee
1987 - 1992	Cytogenetics Research Fellow	Purdue University
1988 - 1991	Clinical Oncology Resident	Purdue University
1987 - 1988	Clinical Oncology Intern	Purdue University

PROFESSIONAL SOCIETIES:

American Veterinary Medical Association, Veterinary Cancer Society, Veterinary Cooperative Oncology Group, American Association for Cancer Research, Association of Cytogenetic Technologists, International Society for the Study of Comparative Oncology, American Cancer Society, Knox County Unit.

SCIENTIFIC PUBLICATIONS
- Abstracts, Proceedings, & Non-peer reviewed, (total - 41), 1992 - July 1, 1993:

Hahn KA, Morrison WB, Chan TC. The concentration of doxorubicin and its associated metabolites in canine urine following a single administered dose. Tenth Annual Forum, American College of Veterinary Internal Medicine, San Diego, CA. May 28-31, 1992.

Hahn KA, Vonderhaar MA, Teclaw RF. An epidemiological evaluation of 1202 dogs with testicular neoplasia. Tenth Annual Forum, American College of Veterinary Internal Medicine, San Diego, CA. May 28-31, 1992.

Hahn KA, Richardson RC. The diagnostic and prognostic importance of cytogenetic aberrations identified in spontaneously occurring canine malignant lymphoma. Twelfth Annual Conference, Veterinary Cancer Society, Monterey, CA. October 18-21, 1992.

Bravo L, Hahn KA, Legendre AM, Daniel GB, Frazier DL, Rohrbach BW. Evaluation of pre- and post-cisplatin renal function in 23 tumor-bearing dogs. Twelfth Annual Conference, Veterinary Cancer Society, Monterey, CA. October 18-21, 1992.

Arrington KA, Frazier DL, Tabeling GS, Hahn KA, Legendre AM. The comparison of pharmacokinetics, hematologic parameters, and clinical signs of doxorubicin administration in small and large dogs. Twelfth Annual Conference, Veterinary Cancer Society, Monterey, CA. October 18-21, 1992.

Hahn KA, Richardson RC. The cytogenetic evaluation of 61 dogs with non-Hodgkin's lymphoma (NHL). Eighty-fourth Annual Meeting, American Association for Cancer Research, Orlando, FL. May 19-22, 1993.

Hahn KA. Prognostic Tumor Markers. Eleventh Annual Forum, American College of Veterinary Internal Medicine, Washington, DC. May 20-23, 1993.

- *Referreed Journals (total - 17), 1992 - July 1, 1993:*

Hahn KA, Richardson RC, MA Cline, Teclaw RF, Carlton WW, DeNicola DB, and PL Bonney. Is Maintenance Chemotherapy Appropriate for the Management of Canine Malignant Lymphoma? *J Vet Int Med* 1992;6:3-10.

Hahn KA, Widmer WR. What is Your Diagnosis. *J Amer Vet Med Assoc* 1992;200:221-222.

Hahn KA, Knapp DW, Richardson RC, Matlock C. The Clinical Response of Nasal Adenocarcinoma to Cisplatin Chemotherapy in 11 Dogs. *J Amer Vet Med Assoc* 1992;200:355-357.

Hahn KA, Richardson RC, Knapp DW. Canine Malignant Mammary Neoplasia: Biological Behavior, Diagnosis, and Treatment Alternatives. *J Amer Anim Hosp Assoc* 1992;28:251-256.

Peter AT, Scheidt AB, Campbell JW, Hahn KA. Male pseudohermaphroditism of the testicular feminization type in a heifer. *Canadian Vet. Journal* 1993; in press.

Hahn KA, Chan TCK, Morrison WB, Hahn EA. High performance liquid chromatographic analysis of doxorubicin and its metabolites in canine urine. *J Amer Anim Hosp Assoc*, 1993; in press.

Hahn KA, Richardson RC, Hahn EA. Identification and quantification of serum alpha-fetoprotein in 50 dogs with spontaneously occurring tumors. *Res Vet Sci*, 1993; submitted.

Hahn KA, Richardson RC, Hahn EA, Chrisman CL. The diagnostic and prognostic importance of cytogenetic aberrations identified in spontaneously occurring canine malignant lymphoma. *Vet Path* 1993; submitted.

Bravo L, Hahn KA, Legendre AM, Daniel GB, Frazier DL, Rohrbach BW. Evaluation of renal function in 23 tumor bearing dogs treated with cisplatin. *J Vet Int Med* 1993; submitted.

Hahn KA, DeNicola DB, Richardson RC, Hahn EA. Canine oral malignant melanoma: The prognostic utility of an alternative staging scheme and a review of 41 cases. *J Small Anim Pract* 1993; submitted.

RESEARCH - *(Accepted proposals - 22; support - $281,133), 1992 - July 1, 1993:*

Hahn KA, Frazier DL, Breider MA, Panjehpour M. Quantification of Photodynamic and Hyperthermic Induced In vivo Sub-lethal DNA damage. Submitted to Center of Excellence, College of Veterinary Medicine, University of Tennessee. Accepted July 1, 1992, $15,000.

Hahn KA. Micronuclei Assay: A Predictive Indicator for Tumor Response to Treatment. Submitted to University of Tennessee College of Veterinary Medicine, Venture Grant Program. Accepted July 1, 1992, $3,762.

Bravo L, Legendre AM, Hahn KA, Lothrop CD. Serum cytokine concentrations in normal dogs and in dogs with lymphoma before and after chemotherapy. Venture Grant Program, University of Tennessee College of Veterinary Medicine. Accepted July 1, 1992, $2,000.

Hahn KA, Schuller HS, Legendre AM. B859-035 (Dexniguldipine) treatment of dogs with spontaneously occurring osteogenic sarcoma. Byk Gulden Pharmaceuticals, Hamburg, Germany. Accepted January 1, 1993, $63,890.

Hahn KA. Promoting the responsible handling of anticancer drugs by veterinary personnel. The Minkel Grant Program, University of Tennessee. Accepted January 28, 1993, $2,300.

Hahn KA. Use of SR-2508 in in vitro an in vivo clinical studies. National Cancer Institute. Accepted March 22, 1993. Drug support (Approximate value, $4,000).

Hahn KA. Efficacy of SR-2508 as a radiosensitizer at low and high radiation doses. University of Tennesssee College of Veterinary Medicine Center of Excellence. Accepted July 1, 1993. $17,300.

Hahn KA. Evaluation of cisplatin toxicity modulation by etanidazole. Venture Grant Program, University of Tennessee College of Veterinary Medicine. Accepted July 1, 1993. $1,500.

Frazier DL, Hahn KA. Cisplatin pharmacokinetics and clinical response in the dog. Supported, in part, by Bristol Laboratories (Approximate support, $35,000).

Frazier DL, Hahn KA. Adriamycin pharmacokinetics and clinical response in the cat. Supported, in part, by Adria Laboratories (Approximate support, $35,000).

Publication #R180101-09-001-94