

Program Summary

Introduction: The Center for Environmental and Rural Health (CERH) provides a forum for promotion of outstanding basic and applied science programs focusing on the impact of environmental factors on human health and disease in rural communities. Research efforts of CERH investigators are supported by centralized core facilities that advance the scientific discovery process, enhance the quality of research programs, and attract young faculty into the field of environmental health sciences.

Research Cores: The Biostatistics and Community Health core focuses on interrelated research projects to develop mathematical models to analyze complex data sets arising from environmental health studies, improve models for risk assessment, and investigate causes and methods of prevention of the most prevalent diseases in rural Texas. Investigators of the Chemical Biology core use chemical and biological approaches to address fundamental problems related to the pathogenesis of environmental diseases such as atherosclerosis, cancer, infections, neurologic disorders, and renal failure. The Nutrition core focuses on the study of nutritional and environmental interactions that influence atherosclerosis, cancer, and immune-mediated inflammatory diseases. The Reproductive and Developmental Biology core focuses on the adverse impacts of environmental agents on all aspects of reproduction and development including gametogenesis, conception, pregnancy and embryonic morphogenesis.

Facility Cores: Six facility cores support the research programs of members, their students, postdoctoral fellows, and staff. The Analytical Services core assists investigators with the development and implementation of environmental sampling strategies and detection of DNA adducts and drug metabolites. The Biostatistics and Computational Services core provides extensive network and software support, data management, and statistical support for research projects. The Genomics and Bioinformatics core provides support for analysis of global gene expression using DNA chip technology. The Image Analysis core provides a host of non-invasive imaging tools to probe the role of environmental factors on cellular homeostasis along with traditional digital microscopic imaging and camera-ready digital printing. The Protein Technologies core offers comprehensive approaches for analysis of peptides and proteins. The Transgenic core provides support in the generation and characterization of transgenic mice produced by pronuclear injection or homologous recombination.

Community Outreach and Education: Outreach activities focus on training and education in human health and the environment in rural Texas. In collaboration with community leaders, lay educators, and other NIEHS Core Centers, efforts are directed at the implementation of community-based educational programs in “colonias” along the Texas-Mexico border. Grades K-12 and professional education programs are also being developed in collaboration with multiple units. Services for the local community include broadcast of a monthly TV segment in the Bryan/College Station viewing area, production of articles focusing on environmental health issues for bilingual education, and collaborations with established local community outreach programs.

Pilot Projects: The CERH provided financial support for several research projects consistent with the major scientific themes of the Center. Six projects were funded in 2001 to support collaborative research and enhance the success rate of grant applications submitted to secure external funding.

Research Highlights – 2001

Title: Advances in Microarray Technology and Bioinformatics

Significance: Two key goals of molecular analysis of disease are to use gene-expression information to diagnose disease and to produce therapies based on the disruption or correction of the aberrant function of gene products whose activities are central to the disease pathology. Correction would be accomplished either by the use of drugs already known to act on these gene products or by developing new drugs targeting these gene products. The development of microarray technology makes possible the measurement of expressions for thousands of genes at once. Diagnosis requires the design of a classifier that takes gene-expression levels as inputs. For instance, classification can be between different kinds of cancer or different stages of tumor development. Three critical issues arise in classifier design: (1) designing a classifier from sample data that provides good classification over the general population; (2) estimating the error of a designed classifier when data is limited; (3) selecting from a large set of genes a small subset whose expression levels are to be used for classification. Work of Dr. Dougherty focuses on these issues, with particular attention to small-sample issues.

Publications:

- Dougherty, E. R. Small sample issues for microarray-based classification. *Comparative and Functional Genomics* 2, 28-34, 2001.
- Hedenfelk, I., Duggan, D., Chen, Y., Radmacher, M., Bittner, M., Simon, R., Meltzer, P., Gusterson, B., Esterller, M., Raffeld, Yakhini, Z., Ben-Dor, A., Dougherty, E., Kononen, J., Bubendorf, L., Fehrle, W., Pittaluga, S., Gruvverger, S., Loman, N., Johannsson, O., Olsson, H., Wifond, B., Sauter, G., Kallioniemi, O. P., Borg, A. and Trent, J. Gene expression profiles distinguish hereditary breast cancers. *New England Journal of Medicine* 34, No. 8, 2001.
- Theera-Umpon, N., Dougherty, E.R. and Gader, P. Non-homothetic granulometric mixing theory. *Pattern Recognition* 34, 2547-2560, 2001.

Title: Regulation of Vascular Smooth Muscle Phenotype by Localized Environmental Factors

Significance: Atherosclerosis and hypertension are chronic diseases that affect millions of Americans each year. These diseases are characterized by increased proliferation of vascular smooth muscle cells. Emily Wilson's laboratory is working to define the role of the localized environment in regulating changes in smooth muscle cells seen in hypertension and atherosclerosis. Specific interest is in how oxidative stress and changes in mechanical load affect gene expression and growth properties of smooth muscle and how these changes contribute to vascular disease. Several genes that are regulated by changes in mechanical strain have been identified and the mechanisms regulating these processes are being investigated. It has been shown that the extracellular matrix proteins that smooth muscle cells are associated with can modulate the response to mechanical strain and growth factors. Current studies are also focusing on how oxidative stress alters growth of smooth muscle cells with the hope of identifying key genes that are involved in promoting atherosclerosis. Through understanding the mechanisms regulating gene expression and cellular phenotype of smooth muscle cells, researchers will soon be able to understand the changes associated with vascular disease that affect many Americans.

Publications:

- Davis, J.J., Wu, X., Nurkiewicz, T.R., Kawasaki, J., Gui, P., Hill, M.A. and Wilson, E. Regulation of ion channels by protein tyrosine phosphorylation. *American Journal of Physiology: Heart and Circulation Physiology* 281, H1835-H1862, 2001.
- Wu, X., Davis, G.E., Meininger, G.A., Wilson, E. and Davis, M.J. Regulation of the L-type calcium channel by alpha-5 beta-1 integrin requires signaling between focal adhesion proteins. *Journal of Biological Chemistry* 276, 30285-30292, 2001.
- Wilson, E., Parrish, A.R., Williams, E.S., Bral, C.M., Mitchell, D.M. and Ramos, K.S. Collagen suppresses the proliferative phenotype of allylamine injured vascular smooth muscle cells. *Atherosclerosis* 162, 289-297, 2002.

Administrative Core

Description: The Administrative Core functions to facilitate research, service and outreach activities for CERH investigators and to ensure fiscal integrity of the Center. The core provides leadership in environmental health to the Texas A&M community and promotes expansion of outstanding environmental health research programs that address health concerns of citizens in rural communities of the State of Texas and beyond. The routine activities coordinated by the Administrative Core include: scheduling of Scientific Advisory Group and Facility Core meetings; coordination of biannual and annual meetings of the Internal and External Advisory Boards, respectively; coordination of the Visiting Speakers Program; development of the annual CERH thematic scientific conference; development of contacts and interactions with other EHS Centers and NIEHS staff; administrative and scientific support of collaborative CERH-sponsored grant proposals; coordination of pilot project program call for proposals, review of facility core operations, preparation of CERH annual report; and development of contacts with State and Federal elected officials and their staff to ensure that they are aware of the CERH and its potential services.

Members:

- Kenneth S. Ramos, Ph.D., Center Director, Professor, Departments of Veterinary Physiology and Pharmacology, Medical Physiology and Environmental and Occupational Health
- Stephen H. Safe, D.Sc., Deputy Director, Distinguished Professor, Departments of Veterinary Physiology and Pharmacology, Biochemistry and Biophysics and Environmental and Occupational Health
- Robert C. Burghardt, Ph.D., Associate Director, Professor, Department of Veterinary Anatomy and Public Health
- Jeannie Bowman, A.S., Office Associate, Department of Veterinary Physiology and Pharmacology
- Yvonne Kovar, Business Administrator, Department of Veterinary Physiology and Pharmacology

Internal Advisory Committee: The Internal Advisory committee consists of Deans from Colleges whose faculty are CERH investigators and the Vice-President for Research and Associate Provost for Graduate Studies. This Committee meets biannually to provide advice and guidance on scientific and administrative concerns and also serves as an advocate for the CERH within the Texas A&M University System.

- Richard Ewing, Ph.D., Chair, Vice-President for Research and Associate Provost for Graduate Studies

- H. Richard Adams, D.V.M., Ph.D., Dean, College of Veterinary Medicine
- Roderick McCallum, Ph.D., Interim Dean, College of Medicine
- Edward Hiler, Ph.D., Vice Chancellor and Dean, College of Agricultural & Life Sciences
- Joseph Newton, Ph.D., Interim Dean, College of Science
- Ciro V. Sumaya, M.D., M.P.H.T.M., Dean, School of Rural Public Health

External Advisory Committee: The External Advisory Committee provides critical input regarding CERH operations, thematic development, and evolution. The committee meets once a year usually during early spring and when possible, participates in the activities of the annual CERH meeting.

- Daniel Acosta, Ph.D., Chair, Dean, College of Pharmacy, University of Cincinnati
- Dennis M. Bier, M.D., Professor and Director, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine
- Steven D. Clarke, Ph.D., M.M. Love Chair of Nutritional, Cellular and Molecular Sciences, Department of Human Ecology, The University of Texas at Austin
- Edward R. McCabe, M.D., Executive Chair, Department of Pediatrics, UCLA – School of Medicine
- Roger O. McClellan, Ph.D., Scientific Advisor in Toxicology and Human Health Risk Assessment, President Emeritus, Chemical Industry Institute of Toxicology, Albuquerque, NM 87123

Institutional Commitment: The Life Sciences Task Force at Texas A&M University has made commitments in excess of \$2,000,000 to support training efforts in toxicology and reproductive biology, as well as infrastructure development in the areas of genomics and bioinformatics, imaging, proteomics, and transgenics.

Research Cores

Biostatistics and Community Health Research Core

The goal of the Biostatistics and Community Health Research Core is to develop new biostatistical methods related to environmental health and to perform community-based research studies to examine the relationship between risk factors and disease. The specific objectives of the members of the research core are:

- To develop biostatistical methods to help understand the origin of colon cancer, and how factors, such as apoptosis, are affected by chemicals and diet.
- To develop biostatistical methods to analyze correlated longitudinal and spatial data using subsampling methods.
- To link spatial and measurement error techniques to improve assessment of environmental exposures. Also, more generally, to develop new biostatistical methods for problems having missing and incorrectly measured data, including population-based pharmacokinetic modeling.
- To develop biostatistical methods for apportionment of the sources of environmental air pollutants and toxicants.
- To investigate the causes, mechanisms, and methods of prevention of the most prevalent diseases in rural Texas and other rural areas.
- To study the delivery of health services to people with chronic diseases and chronic physical disabilities in rural communities, particularly as it relates to conditions involving a significant environmental component.
- To analyze data derived from complex multi-stage samples to evaluate health outcomes and health services used in rural communities.

Members:

- Raymond J. Carroll, Ph.D., Director, Distinguished Professor, Department of Statistics
- Robert J. Buchanan, Ph.D., Professor, Department of Health Policy and Management
- James A. Calvin, Ph.D., Professor and Head, Department of Statistics
- Kirby C. Donnelly, Ph.D., Professor, Departments of Environmental and Occupational Health and Veterinary Anatomy and Public Health
- Catherine Hawes, Ph.D., Professor, Department of Health Policy and Management
- Bani Mallick, Ph.D., Associate Professor, Department of Statistics

- Marlynn L. May, Ph.D., Distinguished Lecturer, Center for Housing and Urban Development
- Charles D. Phillips, Ph.D., Professor, Department of Health Policy and Management
- Michael Sherman, Ph.D., Associate Professor, Department of Statistics
- Naisyin Wang, Ph.D., Professor, Department of Statistics
- Soujin Wang, Ph.D., Professor, Department of Statistics

Key Words:

- Chemometrics
- Genetic Epidemiology
- Longitudinal Data Analysis
- Measurement Error
- Missing Data
- Mixed Linear Models
- Non-linear Models
- Receptor Modeling
- Rural Health Research
- Spatial Modeling

Progress Report: The analysis of hierarchical functional data arising in nutrition experiments, especially DNA adducts and repair enzymes measured at the colonic crypt level is a major emphasis of research for core members Drs. Carroll, Mallick, N. Wang, and S. Wang. This project is multi-disciplinary with Nutrition Research Core members Drs. Chapkin, Lupton, and Turner. In addition to multiple papers, two Ph.D.'s in Statistics and one in Nutrition have resulted from this collaboration. Two Biostatistics students are funded to assist in the analysis of data arising from ongoing nutrition and cancer experiments.

Four NCI grants (CA57030, CA59034, CA61750, CA82907) have become multidisciplinary and were awarded continued funding after competitive review as a result of Core-sponsored activities. Drs. Carroll (Principal Investigator), Lupton, Mallick and S. Wang are funded for development of new statistical methodology focusing on the hierarchical structure of the Chapkin-Lupton-Turner experiments (CA57030). Drs. Chapkin and Lupton (Principal Investigators) have funding for experiments designed to elucidate the role of specific PKC isozymes in colon tumor development by using a targeted pharmacological inhibitor in vivo in combination with overexpression and antisense strategies in vitro (CA59034). Drs. Carroll, Chapkin, Lupton (Principal Investigators), Turner and Wu are also funded for comparison of small and large intestinal epithelial cells to determine causes for the development of colon cancer (CA61750), and Drs. Carroll, Chapkin, Lupton and Turner have funding for research to understand why n-3 fatty acids establish permissive conditions for butyrate-induced apoptosis (CA82907).

Drs. Carroll and N. Wang collaborate with Drs. Chapkin, Lupton, and Turner (Nutrition) to administer a training grant (R25CA90301) with the goal of training statistically oriented individuals to function as independent researchers in a multidisciplinary environment. To achieve this goal, a team specializing in Biostatistics, Statistics, Bioinformatics/Biomedical Imaging and the biology of nutrition and cancer has been assembled. Through a combination of didactic coursework, seminars, and research experiences, trainees, functioning as true collaborators in teams of biologists, make important contributions in the development of statistical methods targeted to experiments in nutrition and cancer.

Drs. Buchanan and S. Wang received funding from the National Multiple Sclerosis Society to analyze the nursing home care provided to people with multiple sclerosis and/or depression. This team is also profiling nursing home residents with chronic obstructive pulmonary disease, funded by a grant from the Alpha One Foundation. Their research utilizes the Minimum Data Set for Nursing Home Resident Assessment and Care Screening.

Drs. Calvin, Donnelly, and Mallick are members of the NIEHS-funded Superfund Basic Research Program grant “Procedures to Assess the Hazards of a Superfund Site” (P42ES04917) and are collaborating to construct spatially smoothed dose-response models (Bayesian hierarchical models) to study the effects of complex mixtures on Superfund sites. Dr. Safe, of the Chemical Biology Core, is Principal Investigator of the Superfund program. Drs. Autenrieth and Donnelly are conducting field experiments in Texas and Azerbaijan that are being used to develop models will recognize both the spatial and measurement errors incumbent in such problems and will also be used to aid the design of site selection in Azerbaijan. Drs. Calvin and Donnelly are developing a model to assess early childhood exposure to pesticides from daily activities on the U.S. Mexico border. The Superfund Basic Research Program is a highly interdisciplinary venture involving multiple CERH members.

Drs. Hawes (Principal Investigator), Buchanan, Carozza, May, and Phillips collaborate through The Southwest Rural Health Research Center to conduct basic health services research focusing on reducing health disparities and building the capacity of rural health systems to address special health needs of vulnerable, minority, and rural populations, particularly those with chronic diseases and disabilities. This project is funded by the Federal Office of Rural Health Policy and Health Resources and Services Administration.

Drs. Mallick and Carroll are doing statistical research with Drs. Lupton and Turner (Nutrition) on radiation exposure data. The overall goal of this project is to design diets that protect against radiation-induced carcinogenesis, reduce subsequent carcinogen-induced carcinogenesis, and improve immune function. Several experiments are being done to determine if an intervention diet reduces the potential danger to astronauts of radiation- and chemically-induced cancer of the colon and/or improves immune function.

Dr. May, distinguished lecturer with the Center for Housing and Urban Development (CHUD), which was awarded the prestigious Hammer Award from the National

Partnership for Reinventing Government, and the Secretary's Honor Award for outstanding performance as part of the Partnership for Change Team in improving health, nutrition, and housing conditions for residents of impoverished Texas colonias, continues to collaborate extensively with Dr. I. Ramos, Director of the Community Outreach and Education Program. During 2001, eight promotoras (community educators) were given environmental health education that they then delivered to 1500 households in the Cameron Park community. The strong interactions between the Center for Housing and Urban Development, the Community Outreach and Education Program, and CERH scientists have greatly enhanced the breadth of environmental health research and education efforts on the border.

The Biostatistics and Community Health Research Core encourages interaction within its membership through joint seminars between Statistics and the School of Rural Public Health. Members also attend a weekly statistics laboratory meeting and occasional meetings of the Core members. There is a weekly Statistics and Nutrition research meeting for members working actively in joint grants.

Publications:

Bordelon, N.R., Donnelly, K.C. and George, S.E. Pentachlorophenol potentiates benzo[a]pyrene DNA adduct formation in adult but not infant B6C3F1 male mice. *Environmental and Molecular Mutagenesis* 37, 164-172, 2001.

Buchanan, R. and Smith, S.R. HIV care consortia and coverage of medications. *AIDS and Public Policy Journal* 16, No. 7, 2001.

Buchanan, R.J., Wang, S.J., Huang, C.F. and Graber, D. Profiles of nursing home residents with multiple sclerosis using the minimum data set. *Multiple Sclerosis* 7, No. 3, 2001.

Buchanan, R.J., Wang, S.J. and Huang, C.F. Nursing home residents with HIV and anemia. *Aids Patient Care and Standards* 17, No. 7, 2001.

Buchanan, R., Chakravorty, B. and Smith, S. Eligibility policies for the state AIDS drug assistance programs. *Social Work in Health Care* 32, No. 3, 2001.

Buchanan, R.J., Wang, S.J. and Huang, C.F. Analyses of nursing home residents with HIV and dementia using the minimum data set. *Journal of Acquired Immune Deficiency Syndrome* 26, No. 3, 2001.

Cheng, S.C. and Wang, N. Linear transformation models for failure time data with covariate measurement error. *Journal of the American Statistical Association* 96, 706-717, 2001.

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at various intervals after intravenous administration of furosemide in horses. *American Journal of Veterinary Research* 62, 1349-1352, 2001.

Chu, K.K., Wang, N., Stanley, S. and Cohen, N.D. Statistical evaluation of the regulatory guidelines for use of furosemide in race horses. *Biometrics* 57, 294-302, 2001.

Falahatpisheh, M.M., Donnelly, K.C. and Ramos, K.S. Antagonistic interactions among nephrotoxic polycyclic aromatic hydrocarbons. *Journal of Toxicology and Environmental Health* 62, 101-118, 2001.

Garcia, S.S., Ake, C., Clement, B., Huebner, H.J., Donnelly, K.C. and Shalat, S.L. Initial results of environmental monitoring in the Texas Rio Grande Valley. *Environment International* 26, 465-474, 2001.

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Lemke, S.L., Mayura, K., Wang, N., Fickey, C. and Phillips, T.D. Evaluation of organophilic montmorillonite clay inclusion in zearalenone-contaminated diets using the mouse uterine weight bioassay. *Journal of Toxicology and Environmental Health* 62, 101-116, 2001.

Lin, X. and Carroll, R.J. Discussion of the paper by Lin and Ying. *Journal of the American Statistical Association* 96, 114-116, 2001.

Lin, X. and Carroll, R.J. Semiparametric regression for clustered data using generalized estimating equations. *Journal of the American Statistical Association* 96, 1045-1056, 2001.

McDougal, A., Wormke, M., Calvin, J. and Safe, S. Tamoxifen-induced antitumorigenic/antiestrogenic action synergized by a selective Aryl hydrocarbon receptor modulator. *Cancer Research* 61, 3902-3907, 2001.

McShane, L., Midthune, D.N., Dorgan, J.F., Freedman, L.S. and Carroll, R.J. Covariate measurement error adjustment for matched case-control studies. *Biometrics* 57, 62-73, 2001.

Morris, J.S., Wang, N., Lupton, J.R., Chapkin, R.S., Turner, N.D., Hong, M.Y. and Carroll, R.J. Parametric and nonparametric methods for understanding the relationship between carcinogen-induced DNA adduct levels in distal and proximal regions of the colon. *Journal of the American Statistical Association* 96, 816-826, 2001.

Ramos, I., May, M. and Ramos, K.S. Environmental Health Training of Promotoras along the Texas-Mexico Border. *American Journal of Public Health* 91, 568-570, 2001.

Reese, C.S., Calvin, J.A., George, C. and Tarpley, R.J. Estimation of fetal growth and gestation in Bowhead whales. *Journal of the American Statistical Association* 96, 915-938, 2001.

Smith, S. and Buchanan, R. The AIDS drug assistance programs and coverage of HIV-related medications. *Annals of Pharmacotherapy* 35, No. 2, 2001.

Strauss, W.J., Carroll, R.J., Bortnick, S.M., Menkedick, J.R. and Schulz, B.D. Combining datasets to predict the effects of regulation of environmental lead exposure in housing stock. *Biometrics* 57, 203-210, 2001.

Washburn, K.S., Donnelly, K.C., Huebner, J.J., Burghardt, R.C., Sewall, T.C. and Claxton, L.D. A study of 2,4,6-trinitrotoluene inhibition of benzo[a]pyrene uptake and activation in a microbial mutagenicity assay. *Chemosphere* 44, 1703-1709, 2001.

Chemical Biology Research Core

The goal of the Chemical Biology Research Core is to understand signaling pathways important in cellular homeostasis and disease, and to determine the modulatory effects of environmental toxicants and dietary factors on these processes. The core also focuses on molecular aspects of toxicology and environmental biology, as it relates to the development of novel molecular detection systems, remediation and detoxification, protein characterization, and protein engineering. At the beginning of 2001 Dr. Hagan Bayley stepped down as director of the Chemical Bio logy Research Core because the growth and development of the Health Science Center presented increased administrative demands on his time. Dr. Stephen Safe assumed leadership and continues as its director.

The specific objectives of the research core are:

- To determine molecular mechanisms associated with tissue-specific oxidative stress injury and altered gene expression associated with these processes.
- To investigate modulation of kinase dependent pathways and downstream effects including their role in tumor development.
- To determine molecular mechanisms of ligand activated receptors and their differential interactions with endogenous compounds and environmental contaminants.
- To investigate tissue specific modulation of gene expression by environmental contaminants and dietary factors.
- To define molecular mechanisms of action of several environmental chemicals including aromatic hydrocarbons, pesticides, environmental estrogens/antiestrogens, lead, and mercury.
- To develop biologically based molecular detection systems for analyses of interest in environmental biology.
- To develop molecular technologies for environmental decontamination and protection.

Members:

- Stephen H. Safe, D.Sc., Director, Distinguished Professor, Departments of Veterinary Physiology and Pharmacology, Biochemistry and Biophysics and Environmental and Occupational Health
- Hagan Bayley, Ph.D., Professor and Head, Department of Medical Biochemistry and Genetics
- Lori Bernstein, Ph.D., Assistant Professor, Department of Medical Pathology

- David Busbee, Ph.D., Professor, Department of Veterinary Anatomy and Public Health
- Edward Dougherty, Jr., Professor, Electrical Engineering Department
- Arthur Johnson, Ph.D., Professor, Department of Medical Biochemistry and Genetics
- Ann Kier, D.V.M., Professor and Head, Department of Veterinary Pathobiology
- Timothy Phillips, Ph.D., Professor, Department of Veterinary Anatomy and Public Health
- Kenneth S. Ramos, Ph.D., Professor, Departments of Veterinary Physiology and Pharmacology, Medical Physiology and Environmental and Occupational Health
- James Sacchettini, Ph.D., Professor, Department of Biochemistry and Biophysics
- Terry Thomas, Ph.D., Professor and Head, Department of Biology
- Evelyn Tiffany-Castiglioni, Ph.D., Professor and Head, Department of Veterinary Anatomy and Public Health
- James Wild, Ph.D., Professor, Department of Biochemistry and Biophysics
- Emily Wilson, Ph.D., Assistant Professor, Department of Medical Physiology

Key Words:

- Aging
- Aryl Hydrocarbon Receptor
- Biosensors
- Dioxin
- Endocrine Disruption
- Gene Regulation
- Molecular Detoxification
- Nitroaromatics
- Polycyclic Aromatic Hydrocarbons
- Pesticides

Progress Report: Dr. Safe's research activities are focused on the molecular biology of estrogen-regulated gene expression through estrogen receptor (ER)/Sp1 interactions in breast cancer cells. In addition, non-genomic pathways of estrogen action are also being investigated. The mechanisms of inhibitory Ah receptor-ER crosstalk are also being studied along with development of selective Ah receptor modulators (SAhRMs) for treatment of breast cancer. In collaboration with Dr. James Calvin (Biostatistics and Community Health), it was recently shown that SAhRMs synergistically interact with the antiestrogen tamoxifen to inhibit growth of mammary tumors in rodents. Research on the anticarcinogenic properties and mechanisms of action of diindolylmethane (DIM) analogs is also underway. In collaboration with Dr. T. Phillips of this core, a new class of DIM-derived peroxisome proliferator-activated receptor agonists have been discovered and their clinical potential as chemotherapeutic agents for treatment of multiple cancers is now being investigated.

In Dr. Bayley's laboratory, sensor elements are being made by engineering transmembrane protein pores. Analyte molecules modulate the ionic current driven through the engineered pores by a transmembrane potential. Stochastic sensing, which uses currents from single pores, yields both the concentration and identify (from its distinctive current signature) of an analyte. Several analytes can be detected simultaneously with a single sensor element. In one example, the bacterial pore-forming protein staphylococcal alpha-hemolysin, has been altered to allow detection of divalent metal cations by using mutagenesis to place a cation-binding site within the conductive pathway. Dr. Bayley is also doing research on the design and synthesis of biomolecular materials in which the properties of molecules found in nature are mimicked or extended to produce materials with unusual properties. One goal of this research is to discover low energy, environmentally benign methods for manufacture and disposal of materials.

Dr. Bernstein, in collaboration with Dr. Chapkin (Nutrition Research Core), has been working on identification of multiple adhesion molecules, MMPs, metalloproteinase inhibitors and interferon activatable proteins by cDNA microarray analyses and real time Polymerase Chain Reaction (PCR) whose expression is differentially modulated by TPA in tumor promotion susceptible and resistant cells. Dr. Bernstein's lab is also working on the cloning of partial and full-length cDNAs for p97, a new AP-1 DNA binding protein, and the identification of chromosomal translocations, chromosomal loss and widespread chromosomal instability in tumor promotion susceptible and resistant cells. She also has been collaborating with Dr. Ramos of this core in the identification of novel sensors of oxidative stress in vascular cells.

Dr. Dougherty's research is focused in two areas. One is the analysis of the granulometric properties of particulate images. The second is the analysis of data from cDNA microarrays in which his research encompasses processing raw image data; ratio analysis; simulation models for cDNA micorarray images; analyzing the inference capability of sample-based clustering; development of classification methods dependent on small gene sets for cancer classification; estimating and analyzing the coefficient of determination in expression data; and modeling genomic network behavior. Much of the research is in collaboration with the National Human Genome Research Institute, M.D. Anderson Cancer Center, and the University of Sao Paulo in Brazil. Dr. Dougherty is collaborating with fellow Center investigators (Carroll, Chapkin, Lupton, Ramos and Thomas) on a project involving clustering expression data with respect to the effects of antioxidants on reversing signal changes caused by tumor inducing agents, and another involving classification and prediction of expression changes related to cancer in the context of dietary changes.

Dr. Johnson's laboratory is working on projects related to apolipoprotein B processing, GPI-anchor attachment to proteins to cholesterol dependence of protein sorting at the translocon to mechanisms of hole formation in mammalian membranes by bacterial toxins. Perhaps most notable is our discovery that BiP, a protein chaperone found in the lumen of the endoplasmic reticulum (ER), is responsible for sealing the aqueous pore through the mammalian translocon during membrane protein integration. Thus, BiP

maintains the permeability barrier of the ER membrane during integration, a new functional role for BiP that is extremely important.

Dr. Kier's laboratory, in collaboration with Dr. Schroeder (Nutrition Research Core) has created several lines of transgenic mice overexpressing sterol carrier protein-2 (SCP-2) or acyl CoA binding protein (ACBP). They have produced gene-targeted mice in which the entire sterol carrier protein-2/sterol carrier protein-x (SCP-2/SCP-x) gene has been ablated, as well as mice in which only the SCP-x product has been targeted. Significant progress has been made inbreeding these mice to the C57B1 strain. Research is ongoing with nutrition and metabolism studies in these mice, as well as using overexpression LM cell lines to study immunocolocalization of nuclear transcription factors associated with this family of fatty acid carrier proteins. Dr. Kier is collaborating with Drs. Piedrahita and Schroeder on projects targeting an ablation of the SCP-2 protein product independent of the SCP-x protein product and intercrossing LFABP gene ablated mice with the SCP-2/SCP-x knockout. Dr. Piedrahita has collaborated in design of the SCP-2 gene targeting construct to be used for making the gene targeted SCP-2 mice.

Dr. T. Phillips' laboratory is using molecular modeling techniques to design various phyllosilicate clays for the detection and detoxification of environmental chemicals. This research will be beneficial to rural communities, where incidence of exposure to aflatoxins, mycotoxins, and halogenated aromatic hydrocarbons are often elevated. Dr. T. Phillips and his staff are working to understand surface chemistry and mechanisms involved in the interactions of these toxins with phyllosilicate clay minerals. Research to form symmetrical, resonance-stabilized chelate by the dicarbonyl system of aflatoxins with certain metal ions in the clay is also being done.

Dr. Ramos' research focuses on genetic and molecular mechanisms of environmental stress and cell injury. A major thrust in his laboratory is the study of redox signaling pathways involved in transcriptional and post-transcriptional control of *Ha-ras*, *L1Md* retrotransposon, osteopontin, *grp78* and Wilms' tumor suppressor gene. The study of functional interactions between basic helix-loop-helix, leucine zipper, and zinc finger transcription factors with accessory factors involved in enhanceosome complex assembly, and their contribution to human disorders of growth, differentiation and development is emphasized. Drs. Ramos and Tiffany-Castiglioni collaborate in a project to study regulation of glucose-regulated protein 78 (*grp78*) in renal and brain cells.

Dr. Sacchettini's laboratory studies interactions between proteins and their ligands or substrates. Several techniques are used for examination of the molecular details of these types of interactions including x-ray crystallography, microcalorimetry and molecular biology. Human amyloid disorders, including familial amyloid polyneuropathy, familial amyloid cardiomyopathy and senile systemic amyloidosis are linked to deposition of insoluble TTR in peripheral nerves and heart tissues. Hydroxy-PCBs bind TTR with high affinity. In collaboration with Dr. Safe, new TTR ligands based on a hydroxybipheny backbone are currently being investigated and x-ray crystallographic analysis and binding assays show that some congeners exhibit high affinity for TTR.

Dr. Wild's research group focuses on the molecular characterization and manipulation of specific enzymes involved in detoxification of pesticides and organophosphate nerve gases. Studies on the mechanism of detoxification of organophosphate neurotoxins primarily involve the genetic and biochemical analysis of the plasmid-borne organophosphorus-degrading gene of *Pseudomonas diminuta* and its unique broad-spectrum organophosphate hydrolase activity. The organophosphorus degrading genes (opd) from two different plasmids in the soil bacteria *P. diminuta* and *Flavobacterium* have been sequenced and their structural organizations are being characterized. Applications of this system have led to development of an enzyme-based biosensor that is capable of the direct and discriminatory detection of various OP neurotoxins. In addition, the gene has been productively inserted into plants and soil fungi for developing an *in situ* decontamination system for environmental bioremediation.

A toxicogenomics initiative lead by Drs. Ramos and Thomas is being developed through interactions between CERH and several system-wide components. As part of institutional initiatives and a program project grant application currently in preparation by several members of this core (Dougherty, Ramos, Safe and Thomas), CERH is developing computational infrastructure necessary for DNA microarray analysis. Dr. Dougherty has facilitated application of the multiple data analysis strategies developed at the National Human Genome Research Institute to microarray data generated at Texas A&M University.

Members of the Chemical Biology Research Core promote scientific collaborations within this group, with investigators in other CERH Research Cores, and with other collaborators on campus and in other university/research laboratories through weekly seminar programs and participation in several collaborative research activities, as well as informal meeting settings. Joint efforts to promote new technologies in genomics and transgenics for CERH investigators have also led to new collaborations.

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Nutrition Research Core

The primary goal of the Nutrition Research Core is to promote active collaboration among members of the Nutrition core and the other Center cores, primarily focusing on gene-environment interactions. The individual scientists in the Nutrition Research Core serve as the interface between nutritional problems observed in the field and the molecular mechanisms behind those specific problems. Of particular interest is the role of dietary factors in outcomes associated with environmental exposures to toxic chemicals, especially as it relates to colon cancer and cardiovascular disease.

The specific research aims of the core include:

- Identify diet components that reduce or delay onset of chemically induced and “spontaneous” colon carcinogenesis, and thus determine potential diet interventions that ameliorate the impacts of environmentally derived insults.
- Evaluate non-invasive biomarkers of colon cancer as a means of monitoring food born anticarcinogens.
- Determine how over- and under-consumption of dietary minerals (e.g. copper and iron) may exacerbate the response to environmental toxins.
- Evaluate how previous nutritional status affects the response to environmental challenges, and if the response can be modulated by changes in diet.
- Elucidate the cellular and molecular mechanisms by which diet modulates immune-mediated inflammatory disease, and alters host resistance to infectious pathogens.
- Identify diet components that reduce atherosclerotic cardiovascular disease.
- Determine dietary influences on fetal small intestinal development and enzyme expression.

Members:

- Joanne R. Lupton, Ph.D., Director, Regent’s Professor, Department of Animal Science
- Robert S. Chapkin, Ph.D., Professor, Department of Animal Science
- Edward D. Harris, Ph.D., Professor, Department of Biochemistry and Biophysics
- David N. McMurray, Ph.D., Professor, Department of Medical Microbiology and Immunology
- Friedhelm Schroeder, Ph.D., Professor, Department of Veterinary Physiology and Pharmacology
- Guoyao Wu, Professor, Department of Animal Science

Key Words:

- Atherosclerosis
- Biomarkers
- Cardiovascular disease

- Colon cancer
- Environmental carcinogens or mutagens
- Immune responsiveness
- Minerals
- Phytochemicals

Progress Report: Members of the Nutrition Core and members of the Biostatistics and Community Health Research Core are collaborating on several research projects that have resulted in key publications in the area of colon cancer and statistical theory. New and renewed projects involving collaborations between Drs. Lupton, Chapkin, Turner, Walzem and Wu (Nutrition) and Drs. Carroll, Mallick, and Wang (Biostatistics and Community Health) include Lupton and Chapkin's research to determine the effects of dietary fiber and fat on colon cancer incidence (CA82907), Carroll and Lupton's work to develop new statistical methods for problems involving nutrition and cancer (CA57030) and a National Space Biomedical Research Institute grant for studies to design diets that protect against radiation-induced carcinogenesis and improve immune function (NPFR00202, Lupton, Principal Investigator). An NIH training grant (R25CA90301), one of the first of its kind in the nation, is a collaboration between members from these two Research Cores, along with Dr. Dougherty (Chemical Biology Research Core) to train biostatisticians about carcinogenesis and the relevance of diet in disease occurrence and prevention.

Members of Dr. Lupton's research group together with Drs. Chapkin and Turner are making progress in the area of understanding the cumulative effects of oxidative DNA damage resulting from metabolism of dietary components resulting from carcinogens that produce methylation adducts in DNA. Recent studies have documented that oxidative DNA damage resulting from radiation exposure amplifies the response to a chemical carcinogen by increasing in the size and severity of these lesions. Further work has indicated that dietary fatty acid composition (n-3 fatty acids) affects mitochondrial function, which establishes a permissive environment whereby the short chain fatty acid butyrate is able to initiate apoptosis in colonocytes. Together this work links colon cancer development to various aspects of oxidative stress generated by exogenous radiate or dietary sources of pro-oxidant molecules, which have an independent effect on colon cancer development, but that can combine to increase susceptibility to induction of the disease by chemical carcinogens that cause methylation damage to DNA.

Dr. Chapkin has work in progress to determine the ability of specific nutrients to influence cellular "signaling cascades" in the colon. These cascades are required to transmit signals from outside the cell into interpretable signals inside the cell. The ability of diet to regulate the transmission of information can alter physiological responses such as cell growth, differentiation or death, ultimately determining the risk for developing cancer. These studies will help determine whether diet can be used as a legitimate treatment to reduce the likelihood of developing colon cancer. Ongoing studies focus on the effect of dietary fat and fiber on plasma and mitochondrial membrane function in relation to oncogene function and colonocyte cytokinetics. Since chronic inflammation of the colon enhances colon cancer risk, experiments to elucidate the effects of diet on

colonic and T-cell receptor mediated activation are planned in collaboration with Dr. McMurray. These studies will be complemented by micorarray analyses to find and quantify subtle regulatory relationships among genes that regulate colon cancer and chronic inflammation.

Dr. Harris' research has focused on the control of a gene that codes for a copper-transporting ATPase in humans and higher animals. Studies have revealed active elements in the distal promoter region of the gene that may play a role in developmental expression. Reporter gene constructs have shown that the gene is refractory to elevated copper, but responds strongly to low environmental copper. Active elements in the proximal region coordinate with newly discovered upstream elements in controlling expression. This research helps to understand how cells maintain homeostatic levels of a toxic, yet nutritionally essential heavy metal.

Dr. McMurray, in addition to his collaborations with Dr. Chapkin on the activation of the T-cell and its modulation by diet, is also studying pulmonary tuberculosis using a guinea pig model. His research has demonstrated that chronic, moderate protein deficiency is associated with profound impairment of T lymphocyte functions that render malnourished animals less responsive to vaccination and less able to control pulmonary infection with virulent tubercle bacilli. These studies have great significance for the role of dietary protein in enhancing vaccine efficacy and reducing the incidence of tuberculosis in malnourished human populations, such as those along the Texas-Mexico border.

Dr. Schroeder's laboratory has successfully transfected transformed fibroblast cells with the cDNA encoding the various lipid transfer proteins to obtain overexpression of fatty acid, fatty acyl CoA, and sterol carrier proteins. Immunocolocalization and confocal microscopy identified the intracellular organelles where these proteins are targeted. In collaboration with Dr. A. Kier (Chemical Biology Core), he continues to overexpress sterol carrier proteins in transgenic mice. By applying confocal and multiphoton imaging technologies, images of a naturally occurring fluorescent sterol in living cells have been obtained. Furthermore, sterol carrier protein expression dramatically inhibited HDL mediated reverse cholesterol transport in living cells. The transfected cells are being utilized to examine the effects of environmental toxicants that might interact with these proteins and thereby alter the normal lipid metabolism and/or expression of toxicity.

Members of the Nutrition Research Core get together each week at the Faculty of Nutrition seminar series. There are also combined weekly lab meetings for the Lupton, Chapkin and Turner laboratories in addition to a weekly meeting of Statistics and Nutrition and bi-monthly meetings of the Nutrition Research Core.

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Reproductive and Developmental Biology Research Core

The goals of the Reproductive and Developmental Biology Research Core is to understand how estrogen-like molecules and other steroids present in the environment, known commonly as endocrine disruptors, influence tissues that express high levels of estrogen receptor during fetal and neonatal periods, or disrupt reproductive and nervous system functions in adults. Core investigators also study how steroid hormone agonists impact all aspects of reproduction and development, including gametogenesis, conception, establishment and maintenance of pregnancy, and normal development of the conceptus (embryo/fetus and its membranes) in animals and humans.

The specific research aims of the Reproductive and Developmental Biology Research Core are to:

- Understand the impact of environmental toxicants on reproduction and development.
- Identify and characterize genes conferring susceptibility to environmentally induced congenital malformations.
- Develop methods for the genetic modification of small and large animals.
- Define molecular regulation of steroid hormone receptors by environmental effectors.
- Determine the action of environmental toxicants on various aspects of cellular signal transduction.
- Determine how environmental factors affect brain development.
- Identify genes that are transcribed and translated during normal pre-implantation and peri-implantation development.

Members:

- Fuller W. Bazer, Ph.D., Director, Professor, Department of Animal Science, Veterinary Anatomy and Public Health and Institute of Biosciences and Technology
- Louise Abbott, D.V.M., Ph.D., Associate Professor, Department of Veterinary Anatomy and Public Health
- Robert Burghardt, Ph.D., Professor, Department of Veterinary Anatomy and Public Health
- Les Dees, Ph.D., Professor, Department of Veterinary Anatomy and Public Health
- Larry Johnson, Ph.D., Professor, Department of Veterinary Anatomy and Public Health
- James Martin, M.D., Assistant Professor, Institute of Biosciences and Technology
- Wallace L. McKeehan, Professor, Institute of Biosciences and Technology
- Rajesh Miranda, Ph.D., Assistant Professor, Department of Medical Anatomy and Neurobiology
- Jorge Piedrahita, Ph.D., Associate Professor, Department of Veterinary Anatomy and Public Health

- Thomas E. Spencer, Ph.D., Assistant Professor, Institute of Biosciences and Technology
- James West, Ph.D., Professor, Department of Medical and Neurobiology
- Mark Westhusin, Ph.D., Associate Professor, Department of Veterinary Physiology and Pharmacology

Key Words:

- Cloning
- Genetic Sensitivity
- Neuronal Differentiation
- Nuclear Transplantation
- Pre-implantation
- Signal Transduction
- Steroid Receptors
- Teratogens
- Transgenics
- Uterine Environment

Progress Report: Drs. Abbott and West continue to collaborate on a project to explore the underlying mechanisms responsible for alcohol-induced detrimental effects on brain deficits, neuronal loss to be specific, during the brain growth spurt period. The hypothesis being tested are: that the mode of cell death induced by alcohol treatment are different (moderate doses of alcohol lead to apoptosis, high alcohol doses lead to necrosis; that there are changes in the ratios of key apoptotic regulator proteins (Bcl-2 family) and in the involvement of neurotrophic factors (BDNF & GDNF) in alcohol-induced Purkinje cell loss; and that alcohol-induced cell death is attenuated by supplementing with neurotrophic factors in an organotypic explant culture system. Dr. Abbott is also collaborating with Dr. Ramos (Chemical Biology) on the role of apoptosis genes in hydrocarbon-induced deficits in nephrogenesis.

Drs. Bazer and Spencer carry out reproductive biology research with an emphasis on hormonal, cellular and molecular mechanisms regulating uterine development, pregnancy recognition, uterine function, and fetal-placental development. The potential therapeutic value of prolactin and placental lactogen is an area of research with both sheep and pigs as model systems. They collaborate with Drs. Burghardt, Piedrahita, Jaeger, and Wu to determine the role of placental nitric oxide and polyamine synthesis in the ovine uterus, fetus and placenta during pregnancy. Dr. Bazer's laboratory is also studying the roles of Fibroblast Growth Factor-7 (Keratinocyte Growth Factor) in uterine biology and pregnancy in pigs, as well as researching ovine interferon tau, the maternal recognition of pregnancy signal in ruminants.

Dr. Burghardt, in collaboration with members of the Reproductive and Developmental Biology Research Core including Drs. Bazer, Jaeger, Spencer and Ing, continues to investigate conceptus – maternal interactions associated with implantation and placentation in domestic species. Particular emphasis is directed at the analysis of

integrin mediated attachment, adhesion and signal transduction. Dr. Burghardt is also collaborating with members of the Nutrition Research Core (Drs. Lupton, Chapkin, Harris and Turner) and Chemical Biology Research Cores (Ramos, Safe, Phillips, Tiffany-Castiglioni, Donnelly, Parrish, and Busbee) involving the development and adaptation of modern imaging tools to mechanistic analysis of cellular physiology and pathophysiology. Strategies to analyze frequency encoded calcium signaling are being used to characterize uterotonin-induced calcium oscillations that result in muscle contraction, and to identify intracellular and extracellular pools of calcium responsible. This technology is being applied to the analysis of altered intracellular calcium homeostasis by environmental toxicants including studies of uptake, subcellular partitioning, and metabolism of aryl hydrocarbon receptor-binding ligands (using benzo[a]pyrene as a model compound), and their effects on cellular homeostasis.

Dr. McKeehan is using genetic engineering to develop mouse models that more closely mimic human disease symptoms, especially prostate cancer. Models will be used for pre-clinical trials on prevention and treatment.

Dr. Dees continues to study alcohol interactions with puberty related hormones and patterns of menstrual development in rhesus monkeys. He has shown that alcohol impacts intraovarian hormonal systems and modulates steroidogenic acute regulatory protein in the prepubertal rat ovary. Other studies show that lipopolysaccharide-induced leptin release is neurally mediated, and that Lamprey gonadotropin-releasing hormone-III selectively releases follicle stimulating hormone in the bovine.

Dr. L. Johnson determined that the level of spermatogenesis in North American men has declined in recent years, and established outreach education programs for enhancing environmental and health science education in grades 6-8 in rural public schools. He collaborates with Drs. I. Ramos (COEP) and Donnelly (Biostatistics and Community Health) in community outreach efforts.

Dr. Martin's research is on molecular mechanisms controlling cell growth and differentiation in the context of vertebrate embryogenesis and causes of birth defects. He studies the roles of homeobox genes in cell growth and differentiation within the craniofacial skeleton using targeted gene mutations through "knockout" technology and related transgenic techniques to express genes of interest in mice. Another interest is to understand how environmental factors, such as teratogens, interact with the genome to generate congenital defects. He recently has initiated collaborations with Dr. Richard Finnell, the newly appointed Director of the Institute of Biosciences and Technology in Houston.

Dr. Piedrahita has continued efforts to develop transgenic pig chimeras using primordial germ cell-derived cell lines and elucidate apoptotic pathways affecting in vitro survival of porcine primordial germ cells in culture.

Dr. Spencer has reported that endometrial glands are required for the ovine uterus to support the estrous cycle and establishment of pregnancy by using the novel Uterine

Gland Knock Out (UGKO) ewe model and that endometrial glands in adult uterus express novel and known mRNAs that provide a basis for a genomics approach to enhancing uterine capacity and embryotrophic potential, as well as potential tools for gene targeting. He has also shown that endometrial gland morphogenesis and function during pregnancy requires down-regulation of the progesterone receptor by progesterone itself and sequential actions of conceptus hormones including interferon tau, placental lactogen and placental growth hormone. Dr. Spencer has begun collaborating with members of the genomics program project initiative led by Drs. Ramos and Thomas.

The Reproductive and Developmental Biology Research Core promotes interaction among its members through the "ReproForum," a weekly seminar series organized by Core members. Collaboration and interactions are also facilitated as Core members participate in the Texas Forum on Female Reproduction held each year in the Texas Medical Center in Houston and co-sponsored by the CERH.

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Analytical Services Facility Core

Description: Research investigating the impact of environmental factors on rural public health requires collaborations between multiple disciplines. For example, interpretation of community health studies is facilitated by collaborative efforts between community-based researchers, epidemiologists and toxicologists to evaluate disease frequency and estimates of exposures. The interpretation of *in vitro* and *in vivo* studies to investigate mechanisms through which chemicals produce their toxic effect is enhanced by the availability of data to identify toxic metabolites. Finally, organized production and evaluation of data from these studies require establishment of a detailed Quality Assurance/Quality Control (QA/QC) program and the implementation of Standard Operating Procedures (SOPs) for routine laboratory techniques to improve the precision, accuracy and reproducibility of data generated by Center investigators.

The specific aims of the Analytical Services Facility Core are to:

- Provide analytical support including sample preparation, extraction and standard analytical measurements to Center investigators.
- Provide assistance to Center investigators in the development and implementation of a sampling strategy for field investigations to estimate exposures in support of community-based research studies.
- Assist in the development and implementation of SOPs and/or Quality Assurance Project Plans for Center researchers.

The Facility Core supports investigators in the Chemical Biology, Reproductive and Developmental Biology, and Biostatistics and community Health Research Cores of the CERH. The majority of these projects have involved analytical services or assistance in the collection of field samples. The core also collaborates with the NIEHS Center at Rutgers University to support several jointly funded EPA initiatives.

Members:

- K.C. Donnelly, Ph.D., Director, Associate Professor, Departments of Veterinary Anatomy and Public Health and Environmental and Occupational Health
- A. Bokelman, QA/QC Officer, Department of Veterinary Anatomy and Public Health
- L.Y. He, Ph.D., Research Chemist, Department of Veterinary Anatomy and Public Health
- T.J. McDonald, Ph.D., Associate Research Scientist, Department of Civil Engineering
- G.D. Zhou, M.D., Associate Research Scientist, Department of Veterinary Anatomy and Public Health

Equipment and Facilities:

- Field Sampling and Safety Equipment – includes full-face respirators, boots, coveralls, and various disposable equipment. Trowels for surface soil sampling,

an extension for surface water sampling, and a HEPA vacuum cleaner for sampling indoor house dust.

- HPLC with PhotoDiode Array Detector – High Pressure Liquid Chromatography equipped with a PhotoDiode Array detector for trace analysis of polycyclic aromatic hydrocarbons (PAHs) and their metabolites. The Core maintains several HPLC columns for both trace chemical analysis and preparative scale separation of complex mixtures.
- Two Gas Chromatographs with EC, FID and NP detectors – these Gas Chromatographs are used for routine chemical analysis. The detectors are analyte specific, with the NP detector primarily used for detection of organophosphate insecticides, the FID for analysis of simple PAH mixtures, and the EC detector for chlorophenols and other halogenated hydrocarbons.
- Zymark Turbovap Concentrator – used for reducing solvent volume of sample extracts. This unit will be combined with an Accelerated Solvent Extractor for efficient sample preparation.
- Tecator Soxhlet Extractor – used for extraction of soils, sediments and solid waste. This equipment reduces time and solvent required for standard Soxhlet extraction.
- Greenhouse Space – approximately 600 square feet of space is available for sample preparation in bioremediation studies. The greenhouse is temperature controlled and includes a rainfall simulator for collection of runoff water from contaminated soils.
- Gas Chromatograph-Mass Spectrometer (GC/MS) and Liquid Chromatograph-Mass Spectrometer (LC/MS) – The GC-MS is available for quantitative analysis of environmental samples, while the LC/MS-MS is used for trace analysis and analysis of unknown samples such as PAH degradation products produced from treatment with ozone.
- Tracor 540 GC – with a flame ionization detector and an electron capture detector, another Tracor 540 GC with a nitrogen-phosphorous detector and a Hewlett-Packard 5890 GC with a flame ionization detector.

Usage and Benefits: A new technology available through the Analytical Services Facility Core is DNA postlabeling. ASC staff members have completed the initial analyses of a series of tissues from Drs. Lupton, Chapkin and Turner (with the Nutrition Research Core). Previous studies have shown that Type 1 I-compounds are present in reduced levels in tumor cells when compared to adjacent normal cells. In addition, treatment of cells with 2,3,7,8-tetrachlorodibenzo-p-dioxin (a suspected tumor promoter) has been found to reduce the frequency of Type 1 I-compounds in tissues. Thus, research suggests that a reduction in Type 1 I-compounds in tissues is associated with an increased risk of cancer. The current study shows that relative adduct levels for Type 1 compounds are higher in colonic DNA from animals with fish oil in their diet than from animals with corn oil in their diet. This corresponds with findings that corn oil animals also have higher levels of colon tumors in long-term studies. In addition, administration of the tumor carcinogen azoxymethane also appears to have produced a reduction in the relative levels of Type 1 I-compounds in colon cells. ASC staff are presently isolating DNA from small intestine to complete the analysis of these tissues for Type 1 I-compounds. A

significant portion of effort over the past 9 months has been the development of a protocol for analysis of Type I and Type II adducts from colon tissue. Colon tissue historically has been difficult to analyze due to interferences. Analytical Services staff have revised existing protocols and have been able to obtain reproducible TLC plates with increased sensitivity. This collaboration would not have occurred without the support of the CERH.

The Analytical Services Core continues to collaborate with Dr. J. Calvin of the Biostatistics and Community Health Research Core to provide support for the Rio Bravo Health Child Project. This study is designed to provide a more accurate model for estimating intake and absorption of pesticides by small children (aged 6 months to 4 years). Although most exposure models treat children as small adults, numerous studies have demonstrated that the rates of absorption, distribution and metabolism in children are very different from adults. The Rio Bravo (Rio Bravo is a small colonia south of Laredo, Texas) study is designed to measure not only intake and excretion of pesticides in children, but also behavioral activities that may influence intake. The Core developed procedures, trained field staff (Promotoras), and assisted in sample collection and processing. The initial stages of the project, a series of SOPs were developed (in English and Spanish) for the collection of house dust, hand wipe and urine samples, as well as procedures for conducting the interview and videotaping of the children. Core personnel also conducted training on sampling in the field. The Analytical Services Facility staff also receives and processes samples for analysis. Dr. T. McDonald is analyzing hand wipe and house dust samples, and the urine samples are being processed at the Centers for Disease Control by Dr. L. Needham.

The Rio Bravo study involves a total of four sampling periods (spring and fall for two years). Samples collected during the spring and fall of 2000 were analyzed for more than 20 organochlorine and organophosphate insecticides. The data indicate that DDT was the most common chemical detected. DDT and DDT metabolites were detected in 20 of 20 house dust samples and 35 of 37 dermal rinse samples. Although the organophosphate (OPs) pesticides were detected less frequently, concentrations of OPs were generally 10 to 100-fold higher than were observed for the organochlorines. Results from the third and fourth sampling conducted in the spring and fall of 2001 are being processed. When combined with data from the analysis of urine and videotapes (to enumerate hand-to-mouth activities), these studies will provide valuable information to characterize the rate and extent of pesticide absorption and excretion in small children.

Center members receive priority service from the Analytical Services Facility Core. The Core has been crucial in providing usage of the Liquid Chromatograph-Mass Spectrometer and the DNA postlabeling. Services such as Quality Assurance and Quality Control are provided to CERH members at no cost, and other services are available at a reduced charge.

Biostatistics and Computational Services Facility Core

Description: The goal of this core is to support four different activities of CERH investigators in all Research Cores.

- Help Desk – The first line of support for short-term statistical design and analysis questions comes from the Help Desk maintained in the CERH main offices. Services are provided at no charge on a no-appointment basis. One research assistant staffs the desk ten hours per week. The core also supports long-term collaborative research by funding 25% effort of a research assistant for those research teams whose work involves more extensive statistical modeling and analysis.
- Statistical Consulting – Services are provided for long-term statistical design and analysis and to instruct researchers on new statistical methods applicable to their research activities.
- Computational Services – A web site (<http://cerh.tamu.edu>) highlighting the Center's outreach and education efforts has been developed. A three-minute streaming video features the focus and goals of the CERH and a 12-month interactive calendar advises members of upcoming seminars, meetings and conferences. An electronic mail system has also been implemented which integrates members regular mail system allowing for a wider communication base.
- Request for Services System – An on-line service request system for all facility cores has been developed. Facility Cores, as well as the Administrative Core, are able to track services rendered and schedule efficiently.

Members:

- James A. Calvin, Ph.D., Director, Professor and Head, Department of Statistics
- Michael T. Longnecker, Ph.D., Associate Director, Professor, Department of Statistics
- James Snell, D.V.M., Ph.D., System Administrator, Department of Veterinary Anatomy and Public Health
- Linda Perry, Staff Assistant, Department of Statistics
- Ilsung Chang, Research Assistant, Department of Statistics
- Scott Holan, Research Assistant, Department of Statistics
- Kyeong Eun Lee, Research Assistant, Department of Statistics
- Yolanda Munoz, Research Assistant, Department of Statistics
- Christie Spinka, Research Assistant, Department of Statistics

Equipment and Facilities:

- Compaq PC Server that supports the CERH mail system and web site
- PC in Help Desk office to support statistical consulting activities
- PC used for web site development
- HP LaserJet printer in Help Desk office to support statistical consulting activities

Usage and Benefits: Statistical support of the Center investigators is the primary mission of this facility. Through core activities, research teams are able to perform complete analyses and design cost effective experiments. Texas A&M University does not provide a statistical consulting service and, thus, without the CERH, Center investigators would not have access to statistical support without creating personal contacts and paying for any and all contacts, regardless of the request. As the Center continues to grow this service is becoming a dominant activity of the facility.

This facility core has also developed a CERH electronic mail system that is integrated with each member's own regular mail system. Group aliases for research and facility cores, as well as other recognized groups have been established. This structure facilitates communication spread throughout the Texas A&M campus and sites in Houston.

Dr. Calvin and Dr. Donnelly (Biostatistics and Community Health) are actively involved in a project to evaluate border health issues with respect to the exposure of children to pesticides.

Dr. Calvin continues to collaborate with Dr. Safe of the Chemical Biology Research Core. They have published the results of their studies of the combined antitumorigenic activity of tamoxifen and 6-methyl-1, 3, 8-trichlorodibenzofuran (MCDF) in a rat mammary tumor model in *Cancer Research*.

Dr. Calvin is working with Dr. Donnelly (Biostatistics and Community Health) on experiments studying the effectiveness of microorganisms in bioremediation.

Dr. Calvin and Dr. R. Finnell reinitiated collaborations on genetic factors of neural tube defects with the help of CERH graduate research assistant, Ilsung Chang.

Drs. Carroll and N. Wang (Biostatistics and Community Health) collaborated with Drs. Lupton and Chapkin (Nutrition), in their research to understand the responses of regions of the small and large intestines and the mediating effects of diet in experimentally induced tumorigenesis. CERH graduate assistant Christie Spinka has assisted them.

Drs. Sherman, C. Phillips, and Hawes (Biostatistics and Community Health) along with CERH graduate assistants Scott Holan and Yolanda Munoz are actively involved in a review of assisted living facilities and conditions. The main goal of the research is to determine the major factors within the assisted living facility that contribute to successful outcomes for the elderly residents of the facility.

Dr. S. Wang collaborated with Dr. Buchanan on the analyses of nursing home residents with HIV and dementia.

Dr. Longnecker and CERH graduate research assistant Kyeong Eun Lee have provided experimental design and statistical analysis assistance to numerous members of the CERH throughout the past year through the Help Desk program.

Genomics and Bioinformatics Facility Core

Description: The goal of the Genomics and Bioinformatics Facility Core is to support the global gene expression research efforts of CERH investigators. During the second year, the Facility Core was expanded to include microarray technology as part of the services offered by the core. Due to the need for functional genomics capabilities, Dr. Terry Thomas, Head, Department of Biology and Director, Laboratory for Functional Genomics, was recruited to guide these efforts. Subsequently, in consultation with CERH members and the Internal/External Advisory Boards, it was decided that services previously provided by the DNA Facility Core could be obtained at competitive prices elsewhere, and that the primary needs of CERH investigators were in global gene expression analysis. Hence, in 2000, the core was renamed the Genomics and Bioinformatics Facility Core and Dr. Thomas was named director. The goal of the Genomics and Bioinformatics Facility Core is to provide all CERH investigators access to state-of-the-art functional genomics and bioinformatics resources.

The specific aims of the core are to:

- Facilitate CERH investigator efforts to obtain or develop unigene sets for construction of relevant DNA microarrays.
- Construct DNA microarrays that will facilitate CERH Research Core objectives.
- Coordinate gene expression experiments of CERH Core users.
- Compile and display expression data.
- Acquire or develop new technologies to advance global gene expression analysis capabilities. This includes developing and comparing alternative and new transcriptional profiling platforms.

Members:

- Terry Thomas, Ph.D., Director, Professor and Head, Department of Biology
- Edward Dougherty, Ph.D., Director of Computer Assisted Medical Diagnostics Imaging Laboratory (CAMDI), Professor, Department of Electrical Engineering
- Phillip Beremand, Ph.D., Associate Research Scientist, Department of Biology
- Laurie Davidson, Ph.D., Research Associate, Department of Animal Science
- Jamie Schroeder, B.S., Research Scientist, Department of Biology

Equipment and Facilities: Genomics Infrastructure has been enhanced through upgrades to GeneMachines Omnigrad robot, acquisition of Affymetrix 428 fluorescence scanner, and implementation of Real Time PCR and Affymetrix Gene Chip Technology platform. The following resources are available to CERH investigators:

- cDNA library construction, arraying and curation – Directionally cloned representative cDNA libraries are constructed and robotically arrayed at a density of 50,000 – 100,000 clones in 384 well microtiter plates using a Qbot (Genetix, Inc.). High-density nylon filters are made using the Qbot or the BioMek (Beckman Coulter) robots for screening by virtual subtraction. The Genomics and Bioinformatics Facility Core serves as a technical resource for library construction and screens using limiting amounts of material (a few hundred cells).

Using the Qbot, libraries can also be rearranged following virtual subtraction screens. These services are provided on a fee-for-service basis.

- High throughput (HT) EST analysis – HT methods are available for template preparation (BioMek 2000), reaction assembly (Robbins Hydra 96), HT PCR and HT sequencing. Two ABI 377 (Perkins Elmer) sequencers are available; thus >70,000 ESTs can be generated per year. Additional sequencing capacity (>20,000 reactions) is available on a ABI 3700 96 channel capillary electrophoresis sequencer housed in the Borlaug Southern Crop Improvement Center. One-pass sequences are determined from each cDNA; the estimated sequence length per EST is 400-500 nucleotides.
- Transcriptional profiling – The facility has assembled a toolbox with enabling technologies for the global analysis of gene expression programs. The two-color fluorescent hybridization microarray system developed by Brown and colleagues at Stanford is used because it is one of the most flexible and robust transcriptional profiling technologies available in an academic setting. A gridding robot from Genemachines is in operation. The upgraded Genemachines OmniGrid gridding robot uses up to 48 “pins” to develop sub-nanoliter volumes of liquid in approximately 100 micrometer diameter features on as many as 100 glass slides (1” x 3”) at 100 micrometer spacing. This process allows fabrication of DNA microarrays with up to 40,000 gene sequences per slide. Hybridization is detected using cDNA probes synthesized with one of two color fluorescent deoxyribonucleotide precursors (Cy3 and Cy5). A confocal laser scanning device (ScanArray® 3000; Genreal Scanning Lunonics, Inc.) is used to detect and quantify hybridization signals. The Scan Array® 3000 is specifically designed to scan DNA microarrays fabricated on glass slides using the Cy3 and Cy5 fluorescent labels. Scan rates of 4 min per 20 x 20 um arrays allow rapid data acquisition at 10um resolution.
- Real Time PCR – Once cDNAs of interest are identified, experiments to confirm that a particular clone shows the expression pattern indicated by the array are required. Quantitative PCR using an internal standard to monitor each reaction and allow comparisons between different reactions will thus be completed.
- Informatics – The Facility has its own informatics capability for EST analysis and transcriptional profiling. Databases are searchable with standard search algorithms, including several versions of BLAST. The internal databases available use two software packages for analysis of the microarray data. One is Visage HDG Analyzer from Genomic Solutions that runs on a Sun Microsystems ULTRA 10 workstation. The second is ArraySuite, an application developed at the National Human Genome Research Institute (NHGRI) for the IPLab software that runs on Rower MacG4s. Dr. Dougherty recently joined the CERH to provide leadership in bioinformatics and to apply the multiple data analysis strategies developed and used at NHGRI. Several other commercial software packages will be acquired with grant funds over the next five year period to enhance and expand downstream analysis capabilities, including GeneSpring (Silicon Genetics, Inc.) and Spotfire (Spotfire, Inc.). Under the leadership of Dr. Dougherty, a robust bioinformatics cluster is being developed for use by CERH investigators for

- microarray analysis. His efforts are closely aligned with the Biostatistics and Computational Services Facility Core to synergize the overall effort.
- Genemachines Omnigrad robot (upgraded 9/16/00) for preparing DNA microarrays
 - ScanArray 3000 fluorescence scanner (GSI Lumonics) for scanning DNA microarrays
 - Affymetix 428 fluorescence scanner
 - Three (3) ABI 377 (Perkin Elmer) automated sequencers (96 well format)
 - BioMek 2000 (Beckman Coulter) robot for plate replication, filter preparation and repetitive pipetting
 - Qbot (Genetic, Inc.) for picking and arraying (rearranging) bacterial colonies and HD filter preparation (This is a shared instrument located elsewhere on campus)
 - Multiple 96 well PCR machines
 - Robbins Hydra 96 liquid handling robot for reaction assembly, etc.
 - One (1) Sun ULTRA 10, three (3) Pentium II PCs, one (1) Pentium III dual processor PC and one (1) Mac G4

Computer Assisted Medical Diagnostics Imaging Laboratory (CAMDI)

- Six (6) networked Dell workstations
- Two (2) Unix workstations
- Three (3) Mac Power PCs

Usage and Benefits: The Genomics and Bioinformatics Facility Core, in conjunction with the Laboratory for Computational Functional Genomics hosted three training seminars to implement the GeneSpring (Silicon Genetics) array analysis software. There are also two new workstations for array analysis using GeneX, an XML-based gene expression database. Ongoing research projects are briefly described below.

Noninvasive Detection of Colon Cancer Diagnostic Markers using DNA Microarrays *PIs: R. Chapkin and J. Lupton*

As part of this initiative, studies are planned to evaluate gene expression profiling over time to predict experimentally verifiable phenotypic characteristics (i.e. DNA damage, aberrant crypt foci and tumors) that are relevant to colon tumor initiation, promotion, and progression. Rats are being exposed to an environmental carcinogen and dietary modifiers of tumorigenesis and examined over time (0 hour, 12 hour, 10 week, and 36 week post carcinogen exposure). RNA from the diet and carcinogen treatment groups will be compared with a single standard to find and quantify subtle regulatory relationships among genes.

Molecular Mechanisms Regulating Function of the Endometrium During Pregnancy *PIs: T. Spencer and F. Bazer*

The endometrial glands of the pregnant ovine uterus express genes, such as growth factors and cytokines, that encode secretory proteins. These gland-derived proteins directly regulate conceptus survival and growth. A uterine gland knockout (UGKO) sheep model has been developed by exposing neonatal ewes to progesterin from birth to eight weeks. Inappropriate exposure to progesterone blocks endometrial gland

development (or adenogenesis). Uteri of adult UGKO sheep lack endometrial glandular epithelium but not luminal epithelium. UGKO uteri cannot support establishment of pregnancy. PCR-based suppression subtraction hybridization, and differential display PCR analysis determined that endometrial glands express specific genes, which are not found in other uterine, cell types. DNA microarray based transcriptional profiles are being developed using RNA from endometrium from Day 14 pregnant ewes, Day 14 Cyclic normal ewes, and Day 14 bred UGKO ewes. Target genes on the microarrays are comprised of a human unigene set obtained by the CERH/GBFC. For future experiments, relevant clones will be obtained and used to create a unique cDNA microarray for analysis of the endometrium of prolific and non-prolific breeds of sheep. Furthermore, given that pregnancy loss in the UGKO model parallels conditions in humans, endometrial samples of women who exhibit early embryo loss infertility will be compared to normal fertile women using the unique cDNA microarray.

Effect of AhR Agonists on the Transcriptome of Breast Cancer Cells

PI: S. Safe

Aryl hydrocarbon receptor (AhR) agonists inhibit 17-beta estradiol induced cellular and molecular responses, including protein and gene expression, in rodent uterus and mammary cells and in human breast cancer cells. For example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a prototypical agonist; it also inhibits development and growth of mammary tumors in rodent models. Selective AhR modulators (SahRMs) are being developed for treatment of breast cancer in women. The importance of specific genes in the initiation and growth of mammary tumors has not been well defined; therefore transcriptional profiling and associated genome wide gene discovery strategies are potentially powerful tools in the identification of novel genes involved in this important disease process. DNA microarray based gene expression studies utilizing human, mouse and rat unigene sets are being conducted to identify genes whose expression is modulated by AhRs. In addition, suppression subtractive hybridization (SSH) coupled with DNA microarray analysis is being used to identify new genes whose expression is modulated by AhRs. A pilot study using SSH has already identified 33 known and 2 unknown E2-responsive genes in MCF-7 cells that are inhibited by AhR agonists (Chen et al., 2001), thus validating the approach.

Xenobiotic-Activated Gene Expression in Atherogenesis

PI: K. Ramos

Environmental hydrocarbons have been implicated as significant risk factors in human atherosclerotic vascular disease. However, to date little is known about molecular mechanisms of vascular gene deregulation by environmental injury. Using benzo(a)pyrene (BaP) as a model compound, this laboratory has shown that interference with gene transcription is a critical event for the induction of proliferative (i.e. atherogenic) phenotypes in rat, mouse, quail, and human vascular smooth muscle cells (vSMCs) (Ramos, *Ann. Rev. Pharmacol. Toxicol.*, 29, 243-265, 1999). Challenge of vSMCs with BaP and related aromatic hydrocarbons triggers a complex cellular response that culminates in simultaneous activation of overlapping signal transduction cascades involving oxidative stress and aryl hydrocarbon receptor (Chen and Ramos, *J. Biol. Chem.*, 275, 27366-27376, 2000). Based on these findings it is hypothesized that

epigenetic mechanisms involving redox and aryl hydrocarbon receptor signaling coordinately regulate gene sets that control genomic integrity and induce atherogenic phenotypes. Comprehensive analysis of the atherogenic response triggered by BaP requires examination of complex patterns of global gene expression during the course of the atherogenic response. The most effective manner in which this analysis can be completed involves use of DNA microarray technology. Therefore, experiments are in progress to: 1) Evaluate coordinate patterns of gene expression during the course of the BaP atherogenic response *in vivo*; 2) Identify novel genes targeted by BaP in vSMCs and; 3) Identify gene clusters predictive of atherogenic outcomes *in vitro* and *in vivo*.

The Avian Bursa of Fabricius: A Novel Toxicogenomics

PI: L. Berghman

The main objective of this project is to develop cDNA microarrays for the expression profiling of the chicken bursa of Fabricius to explore the value of the bursa of Fabricius as a biological sensor system for immunotoxicological substances, either under lab conditions or in resident birds from a biotope under study. This project comprises of isolation of intact mRNA from chickens at different stages of development, EST library construction, EST sequence analysis, microarray construction and finally testing in model situations in the lab (on neonatal chickens) and in the field (on related species).

Requests for Genomic and Bioinformatics Facility Core services are taken on a first-come, first-serve basis, and consideration is given to the nature of the project in relation to the goals of the CERH. Genomics and Bioinformatics Facility Core service fees are discounted for CERH investigators and access to technical support, robotic equipment and scanners limited to CERH members.

Image Analysis Facility Core

Description: The Image Analysis Core provides CERH investigators with access to state-of-the-art microscopy and image analysis services. The specific objectives are to provide instrumentation and services for:

- All aspects of specimen preparation for ultrastructural analysis and immunocytochemistry.
- Digital imaging, image processing and analysis.
- Quantitative single and multiparameter steady-state analysis of vital fluorescence endpoints within living and/or stabilized cells and tissues.
- Quantitative single and multiparameter kinetic analysis of endpoints of cellular homeostasis mechanisms.

The mission of the Image Analysis Core is fully integrated with the activities of the CERH. Image Analysis staff are working with CERH investigators in all cores to continue development and application of new methods to enhance mechanistic assessment of cellular physiology and pathophysiology. Improvements in analytical microscopy resources remain a priority. Applications using the newly installed multiphoton microscope have been integrated into routine support services for CERH investigations. Technology development continues to be a priority. During the past year a proposal was submitted to develop and validate a Real Time Imaging Signal Analyzer instrument capable of performing not only high speed image acquisition, but also automated statistical data analysis and presentation in real time to study biological systems in vivo and in vitro.

Members:

- Robert C. Burghardt, Ph.D., Director, Professor, Departments of Veterinary Anatomy and Public Health and Medical Physiology
- Rola Barhoumi, Ph.D., Associate Director, Research Scientist, Department of Veterinary Anatomy and Public Health
- Helga Sittertz-Bhatkar, Ph.D., Research Scientist, Department of Veterinary Anatomy and Public Health

Equipment and Facilities:

Electron microscopy:

- *Zeiss 10C high resolution transmission electron microscope* with goniometer and cryo-stage, micro-dose focusing control.
- *Bio-Rad Radiance2000MP multiphoton microscope* equipped with a tunable (700 – 1050 nm) Spectra Physics Ti:Sapphire femtosecond pulsed laser with Millennia XsP Argon ion pump laser, Krypton/Argon ion and Red Diode lasers interfaced with a fully equipped Nikon T300 inverted microscope.
- *Arcturus PixCell II Laser Capture Microdissection System*, a slide based cell capture system which utilizes a microscope fitted with an infrared laser to attach cells of interest onto a film which can be transferred to a tube for extraction of DNA, RNA or protein and subsequent molecular analysis.

- *Meridian Ultima Confocal microscope* equipped with a multi-line, UV/Visible EnterpriseTM argon ion laser, 3 high quantum efficiency photomultiplier tubes for detection, and an array of detection filter sets to provide fluorochrome versatility.
- *Meridian InSIGHT Point laser scanning confocal microscope* equipped with 100 mW argon ion and 75 mW Krypton ion lasers, capable of direct ocular viewing in real time and real color. An array of detection filter sets on a filter wheel is computer-controlled and integrated with a cooled intensified CCD camera.
- *Scanalytics CELLscanTM fluorescence deconvolution workstation* supported by a Zeiss Axioplan inverted fluorescence microscope with 100 W mercury source, a Quantix high resolution digital camera, widefield image capture, and image deblurring software consisting of a constrained iterative fluorescence deconvolution algorithm.
- *Digital Imaging and Image Analysis Workstation* optical microscopy workstation consisting of a Zeiss Axioplan 2 Research Microscope interfaced with a Hamamatsu 3 chip color camera supported by a Power Macintosh G3 Computer with several image capture and analysis software packages, a Kodak XLS 8650 PS Color/B&W Digital Printer, and an Epson, Expression 636 scanner.
- *Zeiss PMIII Light Microscope* interfaced with a Nikon DXM-1200 color digital camera and equipped for brightfield, phase contrast, fluorescence and Nomarski differential interference contrast microscopy along with video recording capabilities.
- *Bio-Tek FL600FA Fluorescence/Absorbance Reader*, supporting flexible kinetic assays with top and bottom probes, a fluorescence excitation and emission range of 300-635 nm and 350-700+ nm respectively and probe diameters of 5, 3, 1.5 and 1.0 mm with full plate reading (6 to 384 well) in less than 30 sec, temperature control from ambient +6 to 50°C.

A full range of support equipment including specimen preparation equipment, cryostats, microtomes, Balzers freeze-fracture and freeze-etch instrumentation, micromanipulation/microinjection equipment, electroporation and electroinsertion equipment, complete tissue culture facilities, -80C freezers, evaporation equipment, darkroom facilities, and custom-made real time signal analysis hardware and software are also available in the Image Analysis Laboratory.

Usage and Benefits: CERH investigators who utilized services of the Image Analysis Facility Core during 2001 are:

- Biostatistics and Community Health Research Core: Drs. Calvin and Donnelly.
- Chemical Biology Research Core: Drs. Busbee, Parrish, T. Phillips, Ramos, Safe, Tiffany-Castiglioni, Tjalkens and Wilson.
- Nutrition Research Core: Drs. Chapkin, Harris, Lupton and Turner.
- Reproductive and Developmental Biology Research Core: Drs. Abbott, Barhoumi, Bazer, Burghardt, Dees, Ing, Jaeger, L. Johnson, Piedrahita, Spencer, West and Westhusin.

During the past year, the monthly allocation of instrument time has been consumed in the generation of data for projects covered by the mission of the CERH. Examples include:

- Analysis of natural and chemically modified sorbent materials used for remediation of toxicant- and bacteria-contaminated water and soil (Dr. T. Phillips; Chemical Biology Research Core).
- Laser capture microdissection of brain, testis, and uterine tissues for harvesting specific cell types for RNA and protein analysis (Dr. Abbott, L. Johnson, West, Spencer; Reproductive and Developmental Biology Research Core)
- Analysis of germ cell apoptosis in male reproductive tract (L. Johnson; Reproductive and Developmental Biology Research Core)
- Analysis of cerebellar neuronal innervation, glial cell function, calcium channels, and toxicant-induced neuronal cell apoptosis (electron microscopy, brightfield/darkfield in situ hybridization; immunocytochemistry, digital imaging and image analysis) (Drs. Abbott, Tiffany-Castiglioni, Tjalkens; Chemical Biology and Reproductive and Developmental Biology Research Cores).
- Analysis of docosahexaenoic acid (DHA), the major n-3 polyunsaturated fatty acid (PUFA) in fish oil, to modulate intracellular Ras trafficking in colonic epithelial cells using (GFP)-Ras fusion constructs (multiphoton and confocal) (Drs. Chapkin, Turner, Lupton, Barhoumi; Nutrition and Reproductive and Developmental Biology Research Cores)
- Analysis of ozonated metabolites of benzo(a)pyrene (confocal microscopy and multiparameter kinetic analysis of vital endpoints) (Drs. T. Phillips, Donnelly; Chemical Biology and Biostatistics and Community Health Research Cores).
- Analysis of alcohol toxicity on onset of puberty; neuronal innervation of ovary (digital imaging) (Dr. Dees, Reproductive and Developmental Biology Research Core).
- Study of uterine biology during the peri-implantation period as targets for endocrine disruptors. Endpoints include uterine secretion of osteopontin, interferon-inducible ubiquitin cross-reactive protein, uterine milk protein, 2',5'-oligoadenylate synthetase, receptors including, integrin, prolactin, EGF, FGF-10, and HDG, and glycoconjugate markers of uterine receptivity (brightfield/darkfield in situ hybridization, immunocytochemistry, vital imaging, digital imaging and image analysis) (Drs. Bazer, Burghardt, Jaeger, Spencer, Safe, Wu; Chemical Biology, Nutrition and Reproductive and Developmental Biology Research Cores).
- Functional analysis of frequency encoded intracellular calcium signaling. Identification of codes in the frequency domain signaling (FFT and wavelet transform) and the action of environmental toxicants and endocrine disruptors on these coded signals in liver and uterine smooth muscle cells (Drs. Burghardt, Safe, T. Phillips, Ramos, Donnelly; Biostatistics and Community Health, Chemical Biology and Reproductive and Developmental Biology Research Cores).
- Dietary factor modulation of colonocyte proliferation and apoptosis, and action of dietary lipids on Ha-*ras* expression (fluorescence detection of cell cycle and apoptosis markers, detection of reactive oxygen species and mitochondrial damage leading to apoptosis, measurement of intracellular GSH and pH) (Drs.

Chapkin, Lupton, Turner, Burghardt; Nutrition and Reproductive and Developmental Biology Research Cores).

- Analysis of cadherin-catenin complexes in mercury exposed kidney and phenotypic profiles of cultured adult and embryonic renal cells following repeated cycles of hydrocarbon injury (electron microscopy; brightfield imaging and image analysis). (Drs. Parrish, Burghardt; Chemical Biology and Reproductive and Developmental Biology Research Cores and Pilot Project).
- Analysis of daily sperm production and abnormal spermatozoa as an endpoint for the action of endocrine disruptors (light microscopy, morphometric analysis, electron microscopy) (L. Johnson, Ing; Reproductive and Developmental Biology Research Core).
- Analysis of estrogen, progesterone, and growth factor receptor expression in conceptus tissues as targets for endocrine disruptors. Evaluation of environmental estrogens on conceptus trophoblast cells (digital imaging and image analysis, brightfield and darkfield in situ hybridization; immunocytochemistry, multiparameter kinetic analysis of vital fluorescence endpoints) (Drs. Ing, Jaeger, Bazer, Safe; Chemical Biology and Reproductive and Developmental Biology Research Cores).
- Nuclear translocation of transcription factors and transcriptional activation of gene expression, e.g. interferon-tau induced translocation of STAT proteins, NF-kappa B, estradiol activation of DNA polymerase (immunocytochemistry fluorescence deconvolution and confocal microscopy) (Drs. Bazer, Spencer, Ramos, Burghardt, Safe; Chemical Biology and Reproductive and Developmental Biology Research Cores).

The majority of these investigations address fundamental questions related to the mechanisms by which environmental toxicants cause cellular injury and mechanisms to prevent or reverse these effects. CERH investigators benefit from the rapid turn-around time that is facilitated by the CERH-supported technical staff position. Image Analysis Facility Core services are provided at no charge to CERH investigators up to \$16,000.00 per year for analytical microscopy instrumentation time and \$6,000.00 for supplies. This includes chamber slides, fluorescence probes and camera-ready digital photographs.

Protein Technologies Facility Core

Description: The goals of the Protein Technologies Facility Core have been expanded to facilitate basic and applied research by providing state-of-the-art analytical and preparative protein chemical and consulting services, including automated N-terminal sequencing, amino acid analysis and electrophoretic and chromatographic protein separations of proteins and peptides. The laboratory also offers a wide range of ancillary techniques for protein/peptide identification and micro-characterization including protein fragmentation and reversed phase HPLC fingerprinting. The core is also actively involved in developing new approaches and protocols for advanced technology in protein characterization to further the Center's research goals. The Laboratory for Biological Mass Spectrometry complements the protein chemistry services offered to CERH members. The laboratory provides modern mass spectrometry capabilities to CERH researchers, including several high resolution time-of-flight (TOF) mass spectrometry instruments.

The specific aims of the Protein Technologies Facility core are to provide services for:

- Primary structure elucidation for protein identification using high resolution mass spectrometry and automated Edman sequencing techniques.
- Micro-characterization of protein post-translational modifications using mass spectrometry and amino acid analysis and automated Edman sequencing techniques.
- Development and execution of novel protein and peptide separations using electrophoretic and chromatographic techniques.
- Education and training of Center investigators on modern techniques of protein purification and analysis.

Members:

- Lawrence Dangott, Ph.D., Director, Research Scientist, Department of Chemistry
- Shane Tichy, Ph.D., Department of Chemistry
- Virginia Johnson, M.S., Protein Specialist, Department of Chemistry
- To be named, Protein Specialist, Department of Biochemistry and Biophysics

Equipment and Facilities:

- Time-of-flight mass spectrometers: PerSeptive Biosystems, Inc. Voyager Elite XL high resolution of greater than 10,000 can be achieved up to m/z ratios of 10,000 with mass measurement accuracy of less than 10 ppm. PerSeptive Biosystems, Inc. Voyager STR high resolution matrix-assisted laser desorption ionization (MALDI).
- ThermoFinnegan LCQ DecaXP Electrospray ionization (ESI) ion trap mass spectrometer: This is an instrument for direct analysis of solutions containing biological samples. The instrument is especially well suited for studies of protein de novo sequencing and protein identification.
- High-resolution tandem TOF instrument equipped with photodissociation: This instrument is used for developing peptide sequencing using mass spectrometry. Ionization is achieved by using either ESI or MALDI and the fragment ion mass

spectrum (used to extract the amino acid sequence) can be obtained using metastable ion, collision-induced dissociation, and/or photodissociation.

- Ion mobility-TOF instruments: There are two prototype instruments designed and built in our laboratory that can be used for studies of the size or conformation of proteins and peptides. These instruments are well suited for analyzing complex mixtures of proteins and protein digests.
- Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry: Two FTICR instruments are used for collaborations and applications research. FTICR provide unique capabilities for analysis of large biomolecules. One FTICR is equipped with capabilities for ion mobility measurements, determination of volumes of gas-phase ions similar to gel-electrophoresis.
- Hewlett Packard G1005A Protein Sequencer
- Hewlett Packard AminoQuant Amino Acid Analyzer
- Hewlett Packard 1100 Liquid Chromatographer system
 - Model G1315A Diode Array Detector
 - Model G1312A Binary Pump
 - Model G1313A Autosampler
 - Model G1316A Thermostated Column Heater
- Hewlett Packard Model 1046A Fluorescence Detector
- Hewlett Packard Model Protein Chemistry Workstation
- Pharmacia Explorer 10 Liquid Chromatograph
- Pharmacia IPGPhor Isoelectric Chromatography System
 - Model 626 Pump
 - Model 486 Tunable Absorbance Detector
 - Model 600S Controller
- Waters Model 600 Liquid Chromatography System
- LC Packings Probot
- Savant Model AES10 10-speed Vac Concentrator
- Gibson Model FC204 Fraction Collector

Usage and Benefits: Over the last two years the Protein Technologies Facility Core has interacted with over 16 CERH scientists and 30 students and postdoctoral fellows on a variety of projects ranging from amino acid analysis to proteomic-style protein identification. A number of these projects have resulted in publications. Several of the projects are funded by CERH pilot grants. Included here is a short list of specific projects done in collaboration with the Protein Technologies Facility Core staff.

- 2-D Gel analysis of proteins found in diseased leaner mouse brain and normal mouse brain for the purpose of identifying abnormal proteins. Target proteins will be proteolytically digested, the peptide fragments analyzed mass spectrometry (MS), and the protein identified using database searching (Dr. L. Abbott; Reproductive and Developmental Biology Core).
- Accurate mass determination of proteins using high-resolution matrix assisted laser desorption/ionization mass spectrometry (HR-MALDI-MS) (Dr. H. Bayley; Chemical Biology Research Core).

- 2-D gel analysis and N-terminal sequencing of p97 for purposes of protein identification and molecular cloning (Dr. L. Bernstein; Chemical Biology Research Core).
- Assisted in purification of Ha-*ras*. Mass mapping of Ha-*ras* for identification of post-translational modifications of Ha-*ras* proteins using ESI-MS (Dr. R. Chapkin; Nutrition Research Core).
- Western Blot analysis of equine Sertoli and Leydig cell antigens for purposes of protein localization (Dr. L. Johnson; Reproductive and Developmental Biology Research Core).
- N-terminal protein sequence analysis of various proteins (H3, DT3, DT1, F10, PG) for purposes of cloning (Dr. W. McKeehan; Reproductive and Developmental Biology Research Core).
- Accurate mass determinations of proteins using HR-MALDI-MS (Dr. T. Phillips; Chemical Biology Research Core).
- Gel isolated GST-ARE DNA-binding proteins were proteolytically digested and their fragments analyzed using N-terminal sequencing and MS for identification (Dr. K. Ramos; Chemical Biology Research Core).
- Accurate mass determination of proteins using HR-MALDI-MS (Dr. J. Sacchettini; Chemical Biology Research Core).
- Amino Acid analysis and accurate mass determination of sterol carrier proteins. Proteins were mass mapped in order to determine point mutations. Mass Mapping of acyl-CoA binding proteins (Dr. F. Schroeder; Nutrition Research Core).
- 2D gel separations and protein identification from knockout ovine uterine washes (Dr. T. Spencer; Reproductive and Developmental Biology Research Core).
- Quantitative amino acid analysis of ovine fallopian tube and oviductal fluids for purposes of preparing a synthetic oocyte culture medium (Dr. Westhusin; Reproductive and Developmental Biology Research Core).
- N-terminal sequence analysis from PVDF of TNT de-nitrifying protein for purposes of cloning. Identification of unknown TNT remediating proteins using proteolytic digestion and HR-MALDI-MS (Dr. J. Wild; Chemical Biology Research Core).

CERH members have the availability of CERH funded personnel dedicated to CERH projects. Each CERH project begins with a planning meeting with the PI, Core Director and appropriately trained personnel. The technology is subsidized by the CERH with preference given to first time Protein Technologies Facility Core CERH investigators.

Transgenics Facility Core

Description: The goal of the Transgenics Facility Core is to support Center investigators in the generation and characterization of transgenic mice produced by pronuclear injection and homologous recombination in a cohesive and cost-efficient manner. The specific objectives of the Transgenics Facility Core are to:

- Assist with the design and fabrication of DNA constructs for pronuclear injection and homologous recombination.
- Provide services for the inactivation of specific genes by homologous recombination in embryonic stem cells.
- Provide the ability to generate transgenic mice by pronuclear injection.
- Generate germ line chimeras from selected transgenic ES cell lines via blastocyst injection and breeding of chimeric mice.
- Provide assistance with screening and maintenance of transgenic mouse lines and create segregating or non-segregating congenic inbred strains.
- Assist with the morphological and pathological analysis of transgenic lines.
- Provide computer-assisted image acquisition, image editing, and data collection and analysis.

Members:

- Ann Kier, Ph.D., Co-Director, Professor and Head, Department of Pathobiology
- Jorge Piedrahita, Ph.D., Co-Director, Associate Professor, Department of Veterinary Anatomy and Public Health
- Bert Binas, Ph.D., Associate Professor, Department of Pathobiology
- Patrick Dunne, Ph.D., Assistant Professor, Department of Veterinary Anatomy and Public Health
- Christie Fickey, Mouse Breeding Laboratory Coordinator, Department of Pathobiology
- Danilo Landrock, Microinjection Laboratory Coordinator, Department of Pathobiology
- John Roths, B.S., Associate Research Scientist, Department of Pathobiology

Equipment and Facilities:

- Molecular Biology Division: Tissue culture hoods and incubators, Recombinant DNA-associated equipment for electrophoresis, PCR, sequencing, and Southern analysis. Additionally, facilities are available for micro-injection of embryos for both pronuclear injection and chimera generation. Equipment includes two tissue culture hoods, four CO₂ incubators, a Zeiss Axiovert inverted microscope equipped with brightfield, phase contrast, differential interference contrast (Nomarski) and epi-fluorescence, a Sutter horizontal pipetter puller, a deFonbrune microforge, a set of Leitz micromanipulators, an Eppendorf microinjector 5242, and a Micro-g vibration-isolation table.
- Microinjection Division: State-of-the-art microinjection equipment includes a Nikon Diaphot inverted microscope system equipped with brightfield, phase contrast, differential interference contrast (Nomarski) and epi-fluorescence, laser

scanning confocal microscopy using NIH image and Metamorph software, two Narshige micromanipulators, a Sutter horizontal pipette puller, a deFonbrune microforge, a gimbal gas piston vibration-isolation table, a computer controlled, robotic, capillary-gap immuno and *in situ* hybridization staining apparatus (Tekmate, Biotek Solutions Corp.), a fully equipped microscope imaging facility featuring Olympus Vanox Light Microscope for light, Nomarski, epifluorescence and polarized light microscopy, both Sony and Optronics high resolution video cameras with output to video printer or to gray-scale or 24-bit RGB frame grabbers in a Power Macintosh G4 computer, software for complete image densitometry including color segmentation and morphometry (Ultimate Optilab, Labview and Labview Concept Vi, Virtual instruments, imaging modules from Engineering Technology Concept, Groton, CT, Fuji phosphoimager, and Alpha Innotech Chemiluminescence gel documentation imager.

- Morphology/pathology Division: State-of-the-art morphometric analysis with light, fluorescence, laser scanning confocal microscopy using NIH Image and Metamorph software, video digital capture and electronically linked black and white and color glossy printers.

The facility maintains its mice in a small specific pathogen free (SPF) barrier designed for transgenic animal production at the Laboratory Animal Resource Facility (LARR). Dr. A. Kier maintains the core colonies needed for pronuclear and gene targeted mice (FVB, C57BL, and other strains for embryos, feeder cells, vasectmized males, and recipient female mice). The Kier laboratory collaborated with LARR for the pathology in the pathogen surveillance monitoring conducted by the LARR, and generation of the 25 embryo-derived mouse lines, to attain LARR SPF status in 1966.

Usage and Benefits: The Transgenics Facility Core continues to work on creating transgenic and gene ablated mice with Dr. Schroeder. To date they have created several lines of transgenic mice overexpressing sterol carrier protein-2 (SCP-2) or acyl CoA binding protein (ACBP) for Drs. Schroeder and Atshaves. The group has produced gene-targeted mice wherein the entire sterol carrier protein-2/sterol carrier protein-x (SCP-2/SCP-x) gene has been ablated and mice wherein only the SCP-x product has been targeted and is in the process of inbreeding these mice to the C57B1 strain. They are working on targeting an ablation of the SCP-2 protein product independent of the SCP-x protein product, and also intercrossing LFABP gene ablated mice with the SCP-2/SCP-x knockout.

Two members of Dr. Kier's staff and several students affiliated with CERH faculty completed Dr. Piedrahita's constructs course. Dr. Piedrahita has been assisting in the design and problem solving aspects of the SCP-2 gene targeting construct. This construct is now complete and Dr. Kier is electroporating the construct into ES cells, PCR screening, and will be microinjecting blastocysts for making the gene targeted SCP-2 mice.

The Transgenics Core has successfully produced over 25 mice for pronuclear injection/overexpression of a construct for Dr. Safe, which was microinjected into a

mouse strain deficient in a specific estrogen receptor. This was particularly challenging since the mice had to be heterozygous, because the homozygote deficient mice have severely decreased fertility. The mice have been transferred to Dr. Safe's laboratory for screening, and more potentially overexpressed mice will be produced for the Safe lab as needed. There is also a DNA construct design for overexpression under discussion with Dr. Ramos' laboratory, which will be produced for injection soon. The Transgenics Core has closely advised Dr. Chapkin and staff on the design of a construct for overexpression, and this preparation should also be ready to inject in January 2002.

The Facility Core completed several outstanding projects in November, crediting the increased efficiency and productivity of microinjectionist, Danilo Landrock. The transgenic mouse records have been updated into database form and embryo deriving several strains of mice into the Veterinary Animal Facility for founder colony safety. This required the development of several different hormone regimens for each strain of mouse. The core is developing the ability to perform speed congenics by PCR, which will allow inbreeding back from the hybrid 129/C57B1, produced by the nature of gene targeting more rapidly. This technique will be of interest to investigators with knock out mice, because it is highly desirable for reproducibility of phenotype and for publication to perform experiments on an inbred strain of mouse having congenicity at all loci.

Dr. Kier now serves as the resource for LARR infectious disease monitoring and pathology. This service is provided to assist the LARR in the prevention of infectious diseases in mice which may affect animal research, thus helping all CERH animal users.

Requests for molecular biology services by CERH investigators are taken on a first-come, first-serve basis, and consideration is given to the nature of the project in relation to the goals of the CERH. Fees cover up to \$1,200 in upfront costs for CERH investigators. For microinjection and pathology services CERH investigators receive first priority. The first microinjection series is free to CERH members and additional requests are discounted by 25%.

Community Outreach and Education Program

Description: The primary goal of the COEP is to educate rural communities in Texas on how to reduce potential environmental exposures associated with human illness and to provide target communities with scientifically sound information to deal with environmental issues. The specific objectives are:

- To train promotoras and colonia residents along the Texas-Mexico border on relevant environmental health issues.
- To help implement environmental health science education in grade levels 6-8 in rural settings.
- To use multiple media resources to educate target communities.
- To collaborate with other Texas COEPs in enhancing educational materials and training of promotoras in the colonias along the Texas-Mexico border, and in training high school science teachers and students on environmental health.
- To establish a Brazos Valley community outreach initiative to address environmentally related health issues in the Brazos Valley Region of Central Texas.

Members:

- Irma N. Ramos, M.D., COEP Director, Center for Environmental and Rural Health
- Louise C. Abbott, D.V.M., Ph.D., Instructor, College of Veterinary Medicine
- Gary Badger, D.D.S., M.S., CERH Member, Pediatric Dentist in Private Practice
- Trina Davis, M.E., PEER Director, College of Education
- John Denton, Ph.D., PEER Co-Director, College of Education
- K.C. Donnelly, Ph.D., Instructor, College of Veterinary Medicine
- Charles Farnsworth, Ph.D., Instructor, College of Veterinary Medicine
- Adriana Garza, Promotora, Center for Housing and Urban Development
- Larry Johnson, Ph.D., PEER Principal Investigator, College of Veterinary Medicine
- Marlynn May, Ph.D., Instructor, Center for Housing and Urban Development
- Kenneth S. Ramos, Ph.D., Instructor and CERH Director, College of Veterinary Medicine
- Maria G. Rebollan, Promotora, Center for Housing and Urban Development
- Stephen H. Safe, D.Sc., Instructor and CERH Deputy Director, College of Veterinary Medicine
- Josephine Saldana, Promotora, Center for Environmental and Rural Health
- Adelina Sanchez, Promotora, Center for Housing and Urban Development
- Belinda Sanchez, Promotora, Center for Environmental and Rural Health
- Teresa Serna, Promotora, Center for Housing and Urban Development
- Mark Sicilio, M.D., CERH Member, Pediatrician, Scott and White Health Clinic
- Norma Viega, M.S., Promotora Coordinator, Center for Housing and Urban Development
- Gregoria Villegas, Promotora, Center for Environmental and Rural Health

- Rosemary Walzem, Ph.D., Instructor, College of Agriculture and Life Sciences
- Graciela Zamorano, Promotora, Center for Environmental and Rural Health

Collaborating Organizations:

- Bush School of Government and Public Service
- Center for Housing and Urban Development
- Community Partnership Board
- KBTX TV-3, Bryan/College Station
- International Consortium for the Environment
- South Texas Promotora Association
- Texas A&M Agricultural Extension Service
- Texas A&M Engineering Extension Service
- Texas A&M University School of Rural Public Health
- Texas A&M University Baylor College of Dentistry
- Texas A&M University College of Medicine
- Texas A&M University College of Veterinary Medicine
- Texas Department of Health
- University of Texas Medical Branch, Galveston
- University of Texas M.D. Anderson Cancer Center

2001 Highlights:

- Environmental Health Science Education of Colonia Residents (Phase III)

The environmental health educational project in the colonias of the Lower Rio Grande Valley continues to expand. Through collaborations with the University of Texas Medical Branch, Galveston and the University of Texas M.D. Anderson Cancer Center, new modules on skin cancer and asthma have been added to the Train the Trainer curriculum. The COEP team analyzed data provided by the promotoras after completing their training. These data were used to update the environmental health education material for residents, and to develop flip charts to facilitate and increase understanding of material by the community residents. Promotoras helped refine the design and implementation of the training program to colonia residents.

Promotoras also completed computer training for community education and outreach. Once equipped with knowledge and appropriated materials about environmental health, Promotoras moved on to Phase Three (Community Residents Education) in which they conducted educational sessions with their neighbors throughout the community, providing culturally relevant environmental health education to colonia residents.

- Environmental Health Education in the Bryan/College Station Community

To help educate our immediate community, the COEP provides coordination and oversight of a monthly TV show featuring topics on human health, entitled “Brazos Valley This Morning – Segment “Fit for Life” in our local TV station viewing area (KBTX-TV3). Regular guests are two COEP members, Drs. M. Sicilio and Gary Badger. Other CERH scientists have participated in this program. Among the topics presented in this program are: *Children’s Dental Health, Allergic Rhinitis, Lead Poisoning and Tooth Development, Protect Your Child from Poison, Early Childhood Cares, Swimmer’s Ear, Children and Adolescent Nutrition, Sealants for Health Teeth, Halloween and Sweets, and How to Prevent Abscessed Teeth.*

- Collaborations with other NIEHS Center Outreach Programs

Funding was secured from NIEHS to collaborate with UT MD Anderson Cancer Center on the development of a module entitled A High School Education Module on the Ethical, Legal and Social Implications (ELSI) of Genomic Screening. This proposal will extend the work of the COEP in an important area for young people reaching adulthood and facing important decisions about health care and privacy.

Members of the CERH COEP collaborated with the Dr. Robin Fuch-Young, Outreach Director at the Center for Research on Environmental Disease (CRED), University of Texas Smithville, in a mutually beneficial exchange of talent and materials in which Dr. Fuch-Young gave a special class on skin cancer to the promotoras and provided the Environment and Cancer module for the CERH Environmental Health curriculum. In exchange, the CERH COEP’s high school science/health curriculum CD-ROM and participated in the CRED bench tutorials program.

In collaborations with Dr. Edward G. Brooks and the COEP at the University of Texas Medical Branch-Galveston, Dr. Brooks provided the asthma module for the CERH curriculum and included asthma booklets and learning materials for the promotoras. Dr. Brooks also gave an asthma lecture in Cameron Park and performed an evaluation of potentially hazardous conditions in the colonia. CERH speakers gave presentation for the Summer K-12 Bilingual Institute for Teacher Training sponsored by University of Texas Medical Branch-Galveston.

Materials and Publications:

Web Site Publications:

- Preventing Carbon Monoxide Poisoning
Como Prevenir Intoxicacion con Monoxido de Carbono
- Environmental Tobacco Smoke (ETS) and the Health of Children, Adolescents, and Adults

Contaminacion Ambiental Secundaria al Humo del Cigarrillo (ETS), Y la Salud de Ninos, Adolescentes y Adultos

- Sun-Safety Tips
El Sol y la Salud de su Piel
- Holiday Health Highlights
Consejos de Salud para Temporada de la Navidad
- COEP Newsletter

Brochures:

- Preventing Food Poisoning
Como Prevenir Intoxicacion Alimenticia
- Holiday Health Highlights
Consejos de Salud Durante la Navidad
- Environmental Tobacco Smoke and Children
Contaminacion Ambiental Secundaria al Humo de Cigarrillo y la Salud de su Nino
- Birth Defects: Environmental Factors and Folic Acid
Defectos Congenitos: Factores Ambientales y Acido Folico
- Preventing Carbon Monoxide Poisoning
Como Prevenir Intoxicacion con Monoxido de Carbono

Bilingual Articles Published in “*La Prensa*”, a local Newspaper:

- Environmental Tobacco Smoke and Children
Contaminacion Ambiental Secundaria al Humo del Cigarrillo y la Salud de su Ninos
- Poisonous Plants and Children: Tips for the Holidays
Plantas Venenosas y los Ninos: Consejos para la Epoca de Navidad

Articles Published:

- Ramos, I., May, M. and Ramos, K.S. Environmental Health Training of Promotoras along the Texas-Mexico Border. *American Journal of Public Health*, 91, 568-570, 2001.

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Pilot Projects Funded in 2001

Year of Funding: 2001

Title: Dissection of the Signaling Pathways of Rotavirus NSP4

Investigator(s): Judith M. Ball, Ph.D.

Funded Value: \$ 20,000

Description: Rotaviruses (RV) are important causes of life-threatening diarrheal disease in infants and young children. A new concept of viral pathogenesis was offered with the discovery of the first viral enterotoxin, RV nonstructural protein 4 (NSP4). NSP4 is a multi-functional, novel secretory agonist that induces [Ca²⁺] mobilization and ion flux in intestinal cells. Yet, the mechanistic details of the signaling pathway whereby NSP4 is enterotoxic and the role of caveolae in NSP4 function remain unknown. The working hypothesis is NSP4 binds a specific surface receptor that is localized to caveolae and activates a specific isoform of phospholipase C (PLC). The specific aims of this project are to: 1) determine if NSP4 activates the phosphoinositide (PI) signaling pathway by interacting with molecules localized to caveolae; 2) determine if NSP4 requires endocytosis to transduce a signal or remain extracellular; 3) determine the specific isoform of phospholipase C (PLC) that is activated by NSP4.

Positive Outcomes

Publications

- Guerrero RA, Ball JM, Krater SS, Pacheco SE, Clements JD, Estes MK. 2001. Recombinant Norwalk virus-like particles administered intranasally to mice induce systemic and mucosal (fecal and vaginal) immune responses. *J Virol* 75:9713-22.

Huang H, Schroeder F, Zeng C, Estes MK, Schoer JK, Ball JM. 2001. Membrane interactions of a novel viral enterotoxin: rotavirus nonstructural glycoprotein NSP4. *Biochemistry* 40:4169-80.

Year of Funding: 2001

Title: Development of a Non-invasive Point-of-Test Lung Cancer Diagnostic

Investigator(s): John W. Bevan, Ph.D.

Funded Value: \$ 20,000

Description: The objective of this research project is to develop low cost, reliable and widely available point-of-test technology for lung cancer screening of human patients. This technology will be non-invasive and based on state specific detection of molecular markers characteristic of lung cancer through monitoring of human breath. This project proposes to: 1) develop a prototypical instrument with the necessary ultra high sensitivity and specificity for detection and quantitation of molecular markers for lung cancer; 2) apply the prototypical instrumentation to the definitive detection and quantitation of the molecular markers in gaseous metabolic emissions from immortalized control and lung cancer cell lines; 3) carry out preliminary tests on collected human breath samples which will provide the ultimate criterion of the efficacy of the proposed technology.

Year of Funding: 2001

Title: Ethanol Neurotoxicity and Amygdala NMDA Receptors: An in vitro Model

Investigator(s): Brian A. McCool, Ph.D.

Funded Value: \$ 20,000

Description: Alcohol abuse in rural settings emphasizes the need for rational design of drug therapies. While numerous brain regions interact during fear/anxiety, the amygdala is a central component of the neural circuitry controlling such behaviors. Chronic ethanol administration in rats potentiates the functional expression of excitatory NMDA-type ionotropic glutamate receptors. Adaptive responses to chronic ethanol by NMDA receptors may be related to receptor inhibition by ethanol. Alternatively, the amygdala receives a rich variety of modulatory neuropeptides, growth factors, and catecholamine neurotransmitters from distant brain regions. This project will examine the role of receptor-dependent adaptations to chronic ethanol by culturing isolated adult rat amygdala neurons in vitro and by measuring the properties of NMDA receptors expressed in these cultures. Whole-cell patch clamp and single-cell RTPCR in cultured neurons prior to and following chronic ethanol exposure will test the hypothesis that NMDA receptor adaptation in vivo is a primary characteristic of the receptor.

Positive Outcomes

Publications

- McCool BA, Farroni JS. 2001. Subunit composition of strychnine-sensitive glycine receptors expressed by adult rat basolateral amygdala neurons. *Eur J Neurosci* 14:1082-90.
- McCool BA, Farroni JS. 2001. A1 adenosine receptors inhibit multiple voltage-gated Ca²⁺ channel subtypes in acutely isolated rat basolateral amygdala neurons. *Br J Pharmacol* 132:879-88.

Year of Funding: 2001

Title: Nitric Oxide and Astroglia in Manganese Neurotoxicity

Investigator(s): Ronald B. Tjalkens, Ph.D.

Funded Value: \$ 20,000

Description: Chronic exposure to manganese (Mn) results in neurological deficits strikingly similar to those observed in idiopathic Parkinson's Disease. The etiologic role of environmental factors such as Mn in the onset and progression of neurodegenerative disorders is the subject of much current scientific interest and debate. Manganese readily crosses the blood-brain barrier and accumulates in astroglial cells, resulting ultimately in neuronal death and active gliosis in affected regions of the basal ganglia. However, the mechanisms by which astrocytes participate in neuronal degeneration are unclear. Chronic oxidative stress and subsequent mitochondrial dysfunction are thought to underlie the neurotoxicity of Mn. It is hypothesized that Mn-induced increases in mitochondrial calcium activate nitric oxide production and predispose astrocytes to injury during periods of physiologic stress. These studies seek to uncover mechanisms by which Mn may perturb astrocyte function in a manner that could result in long-term disruption of normal neuro-glial interactions.

Positive Outcomes

Publications

- Brasuel M, Kopelman R, Miller TJ, Tjalkens R, Philbert MA. 2001. Fluorescent nanosensors for intracellular chemical analysis: decyl methacrylate liquid polymer matrix and ion-exchange-based potassium PEBBLE sensors with real-time application to viable rat C6 glioma cells. *Anal Chem* 73:2221-8.
- Taneja N, Tjalkens R, Philbert MA, Rehemtulla A. 2001. Irradiation of mitochondria initiates apoptosis in a cell free system. *Oncogene* 20:167-77.

Year of Funding: 2001

Title: Statistical analysis in complex disease gene mapping

Investigator(s): Ruzong Fan, Ph.D.

Funded Value: \$ 20,000

Description: This research focuses on devising methods of gene mapping for human diseases, applying them for real data arising from human genetic study and also simulated genetic data, and designing appropriate study experiments to carry out genetic research. We are interested in devising methods for understating the information in the rapidly growing databases for DNA sequences and proteins. We work on genomics emphasizing such questions on the boundary between biological and information sciences, i.e., bioinformatics. Our objectives are to create new models, algorithms, and statistical genetic softwares. In this proposal, we describe our research plan in the following areas: 1) linkage disequilibrium mapping of quantitative trait loci (QTL); 2) multi-locus transmission disequilibrium tests (TDT), linkage and association studies of QTL; 3) haplotype mapping methods.

Positive Outcomes

Publications

- Fan RZ, Floros J, Xiong MM. 2001. Linkage transmission disequilibrium test of two unlinked disease loci; application to respiratory distress syndrome. *Advances and Applications in Statistics* 1:277-308.
- Fan, R.Z. and Ziong, M.M. Linkage and association studies of QTL for nuclear families by mixed models. Submitted, 2001.
- Fan, R.Z., Floros, J. and Xiong, M.M. Models and tests of linkage and association studies of QTL for multi-allele marker loci; submitted, 2001.
- Floros, J., Fan, R., Diangelo, S., Guo, X., Wert, J. and Luo, J. Surfactant protein (SP) B associations and interactions with SPOA in white and black subjects with respiratory distress syndrome. *Pediatric Institute* 43, 567-576, 2001.
- Floros, J., Fan, R., Matthews, A., DiAngelo, S., Luo, J., Nielsen, H., Dunn, M., Gewold, I.H., Koppe, J., van Sonderen, L., Farri-Kostaopoulos, L., Tzaki, M., Ramet, M. and Merrill, J. Family-based transmission disequilibrium test (TDT) and case-control association studies reveal surfactant protein A (SP-A) susceptibility alleles for respiratory distress syndrome (RDS) and possible race differences. *Clinical Genetics* 60, 178-187, 2001.

- Liu, G., Miller, D.P., Zhou, W., Thurston, S.W., Fan, R., Xu, L.L., Lynch, T.J., Wain, J.C., Su, L. and Christiani, D.C. Differential association of the codon 72 p53 and GSTM1 polymorphisms on histological subtype of no-small cell lung carcinoma. *Cancer Research* 61, 8718-8722, 2001.

Year of Funding: 2001

Title: The avian bursa of Fabricius: a novel toxicogenomic tool?

Investigator(s): Luc R. Berghman

Funded Value: \$ 10,000

Description: We are currently performing in vitro studies aimed at characterizing the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on avian immune cells using a recently developed chicken immune system microarray. The microarray analyses are performed in collaboration with Dr. J. Delrow (Fred Hutchinson Cancer Research Center, Seattle, WA). Avian B-cells (DT40) and macrophages (HD11) have been treated with 1 and 10 nM TCDD for 6 and 12 hours. Microarray analysis shows that B-cells are a target for TCDD. Significant shifts in the gene expression profile were observed for genes involved in apoptosis, in oxidative stress and in DNA repair. We are currently verifying these microarray-based observations by realtime PCR and by flow cytometric measurement of apoptosis (binding of annexin). Analysis of TCDD treated HD11 cells is under way. A purification strategy for avian T-cells is now being developed and TCDD-challenged T-cells will be analyzed essentially as indicated above.

Pilot Projects Funded Since Last Competing Renewal

Year of Funding: 2000

Title: Molecular Mechanisms of Methylmercury Toxicity

Investigator(s): Louise C. Abbott, D.V.M., Ph.D.

Funded Value: \$ 15,000

Description: We are using gene microarray technology to assess gene expression in neurons exposed to methylmercury. The major target of methylmercury, the organic form of mercury, is the nervous system, followed by the kidney. During chronic exposure, methylmercury accumulates primarily in the cerebellum and cerebellar granule cells are particularly sensitive to methylmercury. Apoptosis is the proposed mode of neuronal death occurring in cerebellar granule cells exposed to methylmercury. Apoptosis requires activation of gene transcription to proceed. We have completed a dose response study, exposing mice to different levels of methylmercury and examining their brains for evidence of neuronal cell death using Fluoro-Jade staining. After establishing an effective dose we collected brains from mice exposed to methylmercury and examined differential gene expression using cDNA microarrays for known cell death genes. Collaborations are being established between Dr. Abbott and other faculty including Drs. Ron Tjalkens, Evelyn Castiglioni, Jane Welsh, and James Wild.

Year of Funding: 2000

Title: Progestin Effects on Neonatal Ovine Brain and Reproductive Tract Development

Investigator(s): Thomas E. Spencer, Ph.D.

Funded Value: \$ 15,000

Description: Long-term research objectives are to define developmental mechanisms regulating development of the reproductive tract and brain in the neonatal ewe lamb. Results indicate that inappropriate exposure of the developing reproductive tract of the newborn ewe lamb to a potent 19-norprogesterin for only 8 weeks prevents normal development and differentiation of the endometrial glands in the uterus, thereby producing a Uterine Gland KnockOut (UGKO) phenotype. The adult UGKO ewes do not exhibit regular estrous cycles and are infertile. The phenotype of abnormal estrous cycle length could be attributed to effects of the progestin on postnatal brain development and altered hypothalamic or pituitary function. The fact that disruption of uterine development during critical organizational periods can alter the functional capacity and embryotrophic potential of the adult uterus reinforces the importance of understanding the developmental biology of uterine glands. Unexplained, high rates of peri implantation embryonic loss in humans and livestock may reflect defects in endometrial gland morphogenesis due to genetic errors, epigenetic influences of endocrine disruptors, and pathological lesions.

Positive Outcomes

Publications

- Asselin E, Johnson GA, Spencer TE, Bazer FW. 2001. Monocyte chemotactic protein-1 and -2 messenger ribonucleic acids in the ovine uterus: regulation by pregnancy, progesterone, and interferon-tau. *Biol Reprod* 64:992-1000.

- Choi Y, Johnson GA, Burghardt RC, Berghman LR, Joyce MM, Taylor KM, Stewart MD, Bazer FW, Spencer TE. 2001. Interferon regulatory factor-two restricts expression of interferon-stimulated genes to the endometrial stroma and glandular epithelium of the ovine uterus. *Biol Reprod* 65:1038-49.
- Fleming JA, Choi Y, Johnson GA, Spencer TE, Bazer FW. 2001. Cloning of the ovine estrogen receptor-alpha promoter and functional regulation by ovine interferon-tau. *Endocrinology* 142:2879-87.
- Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW, Spencer TE. 2001. Developmental biology of uterine glands. *Biol Reprod* 65:1311-23.
- Gray CA, Bazer FW, Spencer TE. 2001. Effects of neonatal progestin exposure on female reproductive tract structure and function in the adult ewe. *Biol Reprod* 64:797-804.
- Gray CA, Taylor KM, Ramsey WS, Hill JR, Bazer FW, Bartol FF, Spencer TE. 2001. Endometrial glands are required for preimplantation conceptus elongation and survival. *Biol Reprod* 64:1608-13.
- Johnson GA, Bazer FW, Jaeger LA, Ka H, Garlow JE, Pfarrer C, Spencer TE, Burghardt RC. 2001. Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. *Biol Reprod* 65:820-8.
- Johnson GA, Stewart MD, Gray CA, Choi Y, Burghardt RC, Yu-Lee LY, Bazer FW, Spencer TE. 2001. Effects of the estrous cycle, pregnancy, and interferon tau on 2',5'-oligoadenylate synthetase expression in the ovine uterus. *Biol Reprod* 64:1392-9.
- Johnson GA, Ying G-y, Spencer TE, Bazer FW. 2001. Isolation, immortalization and initial characterization of uterine cell lines: an in vitro model system for the porcine uterus In Vitro. *Cellular and Developmental Biology* 36:650-656.
- Ka H, Jaeger LA, Johnson GA, Spencer TE, Bazer FW. 2001. Keratinocyte growth factor is up-regulated by estrogen in the porcine uterine endometrium and functions in trophectoderm cell proliferation and differentiation. *Endocrinology* 142:2303-10.
- Palmarini M, Gray CA, Carpenter K, Fan H, Bazer FW, Spencer TE. 2001. Expression of endogenous betaretroviruses in the ovine uterus: effects of neonatal age, estrous cycle, pregnancy, and progesterone. *J Virol* 75:11319-27.
- Stewart DM, Johnson GA, Vyhldal CA, Burghardt RC, Safe SH, Yu-Lee LY, Bazer FW, Spencer TE. 2001. Interferon-tau activates multiple signal transducer and activator of transcription proteins and has complex effects on interferon-responsive gene transcription in ovine endometrial epithelial cells. *Endocrinology* 142:98-107.
- Stewart MD, Johnson GA, Bazer FW, Spencer TE. 2001. Interferon-tau (IFNtau) regulation of IFN-stimulated gene expression in cell lines lacking specific IFN-signaling components. *Endocrinology* 142:1786-94.
- Taylor KM, Chen C, Gray CA, Bazer FW, Spencer TE. 2001. Expression of messenger ribonucleic acids for fibroblast growth factors 7 and 10, hepatocyte growth factor, and insulin-like growth factors and their receptors in the neonatal ovine uterus. *Biol Reprod* 64:1236-46.
- Tenakoon D, Smith R, Stewart MD, Spencer TE, Nayak M, Welsh CJR. 2001. Ovine IFN tau modulates the expression of MHC antigens on murine

cerebrovascular endothelial cells and inhibits replication of Theiler's virus.
Journal of Interferon Cytokine Research 21:785-792.

Grants

- Mechanisms Regulating Uterine Morphogenesis. 1R01 HD38274. \$864,000. Funded by NIH. 4/1/01-3/31/05.
- Placental Lactogen Enhances Production Efficiency in Sheep. BARD US-3199-OICR. \$150,000. Funded by USDA. 9/1/01-8/31/04.
- Role of Endometrial Glands in Uterine Function. NRICGP 2001-35203-10700. \$260,000. Funded by USDA. 9/1/01-8/31/04.
- Placental nitric oxide and polyamine synthesis in pigs. NRICGP 2001-35203-02166. \$200,000. Funded by USDA. 9/1/01-8/31/04.
- Ovine Interferon-Tau Regulates Uterine Hormone Receptors. 2R01 HD32534. \$1,309,500. Funded by NIH-NICHD. 2/28/01-2/27/06.

Year of Funding: 2000

Title: Storage stability of selected polyaromatic hydrocarbons on solid-phase Extraction Disks

Investigator(s): Scott A. Senseman, Ph.D.

Funded Value: \$ 15,000

Description: Solid-phase extraction disks have been used effectively for the extraction of a wide variety of compounds from water samples including PAH's. Previous work has demonstrated that enhanced stability of various pesticides was achieved when solid-phase extraction disks were used as storage devices. The compounds studied demonstrated recovery better or at least as good as when compounds were stored in bottled water. If this technology could be developed further, it would be possible that shipping PAH analytes stored by solid-phase extraction disk would enhance cost-effectiveness, sample stability, sample integrity, and sample throughput. The aim of this research is to compare the stability of selected PAH's on solid-phase extraction disks and devise a reproducible method that will enable extraction of PAH's at the contaminated site onto solid-phase extraction disks, followed by shipping of disks to the analytical laboratory for chemical elution and analysis.

Positive Outcomes:

Publications

- Mueller, T.C., Senseman, S.A., Carson, K.H. and Sciumbato, A.S. Stability and recovery of triazine and chloroacetamide herbicides from pH adjusted water samples by using empore solid-phase extraction disks and gas chromatography with ion trap mass spectrometry. *Journal of AOAC International* 84, 1070-1073, 2001.

Year of Funding: 1999

Title: Detection of Environmental Estrogens by Stochastic Sensing

Investigator(s): Orit Braha, Ph.D.

Funded Value: \$ 15,000

Description: This project was designed to determine whether stochastic sensing with biosensors based on engineered ion channels could be used to detect presumed and accepted environmental estrogens. Stochastic sensing is based on the detection of individual binding events between analyte molecules and a single receptor, which acts as a biosensor element. In the present study, the receptor is the channel protein hemolysin (HL). The read-out is fluctuations in electrical current through the channel, which report binding events. The frequency of the events gives the concentration of the analyte. The nature of the binding events (e.g. magnitude and duration) provides a signature for identification of the analyte. The recent discovery that cyclodextrins can act as molecular adapters in the HL channel and mediate the detection of a variety of organic molecules has been employed. New results indicate that cyclodextrin allows for detection of endogenous estrogens (e.g. estriol and estrone).

Positive Outcomes

Publications

- Howorka, S., Movileanu, L., Braha, O. and Bayley, H. Kinetics of duplex formation for individual DNA strands within a single protein nanopore. *Proc. National Academy of Science in the U.S.A.* 98, 12996-13001, 2001.
- Miles, G., Cheley, S., Braha, O. and Bayley, H. The staphylococcal leukodocidin bicomponent toxin forms large ionic channels. *Biochemistry* 40, 8514-8522, 2001.
- Movileanu, L., Cheley, S., Howorka, S., Braha, O. and Bayley, H. Location of a constriction in the lumen of a transmembrane pore by targeted covalent attachment of polymer molecules. *Journal of General Physiology* 117, 239-252, 2001.
- Gu LQ, Dalla Serra M, Vincent JB, Vigh G, Cheley S, Braha O, Bayley H. Reversal of charge selectivity in transmembrane protein pores by using noncovalent molecular adapters. *Proc Natl Acad Sci USA* 97:3959-64, 2000.
- Movileanu L, Howorka S, Braha O, Bayley H. 2000. Detecting protein analytes that modulate transmembrane movement of a polymer chain within a single protein pore. *Nat Biotechnol* 18:1091-5.

Grants

- Stochastic Sensing of Medically-Relevant Organic Analytes.. \$159,076. Funded by Texas Advanced Technology Program. 2000-2001.

Year of Funding: 1999

Title: Oxidative Stress Disrupts Cadherin/Catenin Complexes

Investigator(s): Alan R. Parrish, Ph.D.

Funded Value: \$ 15,000

Description: Cell-cell adhesion is predominately mediated by the cadherin superfamily responsible for the regulation of calcium-dependent cell-cell adhesion in association with catenins. Despite the importance of the cadherin/catenin proteins, little attention has focused on the effect of environmental stress on this complex. Preliminary data suggests that oxidative stress disrupts normal protein interactions of the E-cadherin catenin

complex in liver slices. Interestingly, this effect was specific for certain populations of hepatocytes, with no effect on some parenchymal cells or on bile duct epithelium. As cadherin-dependent cell adhesion in the liver is also mediated by N-cadherin and catenin, it is hypothesized that oxidative stress selectively disrupts cadherin/catenin complexes. In this pilot project, precision-cut mouse liver slices were challenged with diamide or tert-butylhydroperoxide. The impact of chemically induced oxidative stress on protein interactions of each cadherin/catenin complex in the liver will be assessed by biochemical and histological techniques.

Year of Funding: 1998

Title: Dietary Fibers/Phytoestrogens and Colon Carcinogenesis

Investigator(s): Nancy D. Turner

Funded Value: \$ 15,000

Description: Published data suggest estrogen inhibits colon cancer and that estrogen receptor (ER) expression is reduced in colon tumors. This project determined if phytoestrogens maintain ER expression in colon samples (normal and tumor) from rats consuming wheat bran or oat bran and injected with saline or carcinogen (AOM). There were no diet differences in saline rats. Oat bran reduced ER levels by 19% in normal tissues from AOM-injected rats. Tumors had little ER protein, except in crypt remnants. Wheat bran tumors had more crypt remnants, and ER expression was maintained in those cells. Another research focus was the effect of diindolylmethane (DIM) on proliferation and apoptosis in the HT-29 colon cancer cell line. DIM and 4-CI-DIM decreased proliferation and increased apoptosis in this system.

Positive Outcomes

Publications

- Turner ND, Zhang J, Davidson LA, Chapkin RS, Safe S, Lupton JR. 1999. Diindolylmethane reduced HT-29 colon cancer cell number by decreasing proliferation and increasing apoptosis.

Grants

- Dietary estrogen and colon cancer. \$19,000. Funded by Houston Live Stock Show and Rodeo. 1/99-12/99.