ARIZONA DISEASE CONTROL RESEARCH COMMISSION

1995-96 Annual Report

Arizona Disease Control Research Commission

ANNUAL REPORT
1995-96

Fife Symington, Governor

Jack Dillenberg D.D.S., M.P.H., Chairman

COMMISSION MEMBERS

General Public
Lois Emden
Frank Hidalgo
Orme Lewis, Jr.

Medical Community
Patricia Moore, Dr.P.H.
John Oakley, M.D.
Eladio Pereira, M.D.

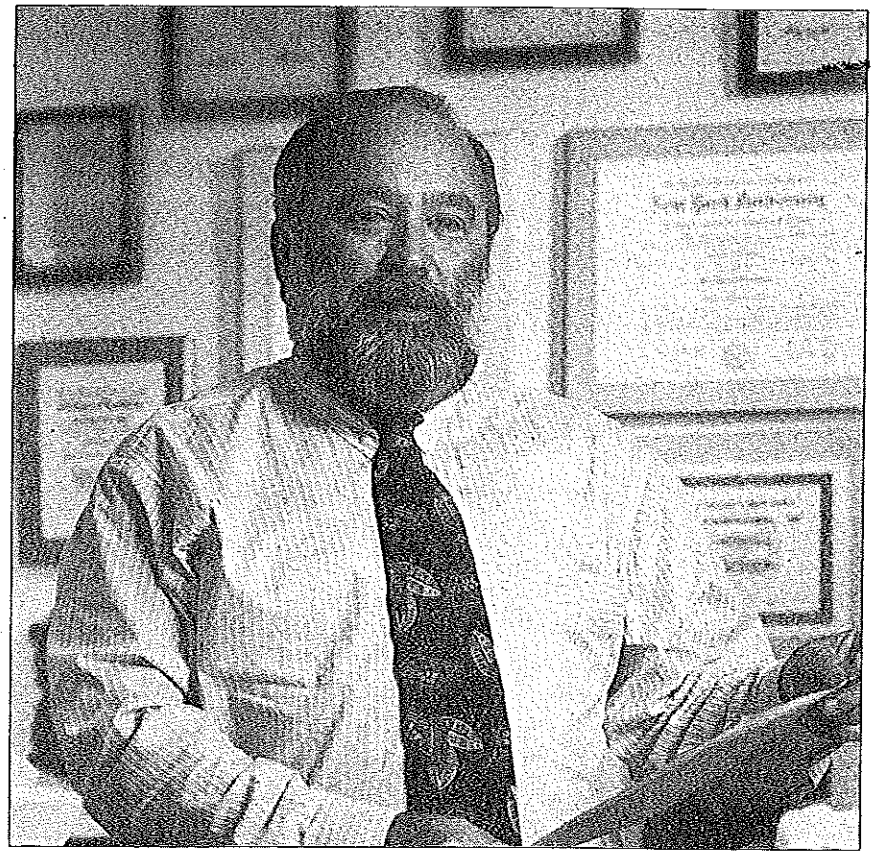
Scientific Research Community
Stan Lindstedt, Ph.D.
Henry Reeves, Ph.D.
Walter Williams, Ph.D., M.D.

Staff
Executive Director: Dawn Schroeder, D.D.S., M.A.
Administrative Services Coordinator: Damika Brock
Staff Assistant: Dan Powell
Accounting Specialist II: Bryon Winston

1740 West Adams Street, Room 102
Phoenix, Arizona 85007
Telephone: (602) 542-1028
Fax: (602) 542-6380

January 1997

TABLE OF CONTENTS



Message from the Chairman

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's report on its contributions to improving the health of Arizonans through funding essential scientific research. Established by the Arizona Legislature in 1984 to support research on the cause, epidemiology, diagnosis, treatment and prevention of diseases affecting Arizonans, the ADCRC is empowered to direct public funds to basic research on a wide range of medical and public health concerns. Funding comes through the legislative appropriations process and the Tobacco Tax Initiative, passed by the voters in November, 1994. The ADCRC receives five percent of the revenues collected from the tax to fund tobacco related medical research. The first recipients of these funds were selected in June of 1995. I am hopeful that the recent passage of Proposition 203, the "Healthy Arizona Initiative," will provide further revenues for ADCRC to continue its exemplary record in support of non-tobacco related essential scientific research.

The Annual Report is prepared and submitted in January of each year to the Governor, the President of the Senate and the Speaker of the House of Representatives. We appreciate their continued support.

The Commission Members

Nine Commissioners guide the work of the Arizona Disease Control Research Commission. They are appointed by the Governor and confirmed by the Senate. Three members represent the General Public, three the Medical Community and three the Scientific Research Community. Commissioners are appointed for three year terms, and may be reappointed. The terms of three members expire each year. The Director of the Department of Health Services serves as Chairman of the Commission and *ex-officio* member. The Chairman and Commissioners who served during 1995-96 are presented below.

Jack Dillenberg, D.D.S., M.P.H.
Director
Arizona Department of Health Services

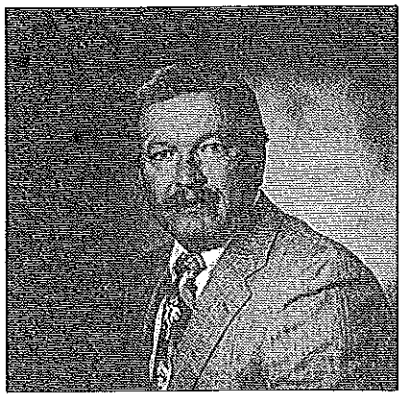
Chairman Dillenberg was appointed Director of the Arizona Department of Health Services by Governor Fife Symington in late August 1993. Dr. Dillenberg began with the ADHS in 1986 when he became Chief of the Office of Dental Health. In 1991, he became Assistant Director for Community and Family Health Services, where he served until appointed ADHS Acting Director on March 12, 1993. Chairman Dillenberg is a graduate of Tulane University and earned his D.D.S. degree at New York University and his Masters in Public Health from the Harvard School of Public Health, where he was honored as recipient of the 1993 Alumni Award of Merit in recognition of his major contributions to public health.

General Public

Lois Emden, M.S.
Nutritional Counselor
Paradise Valley

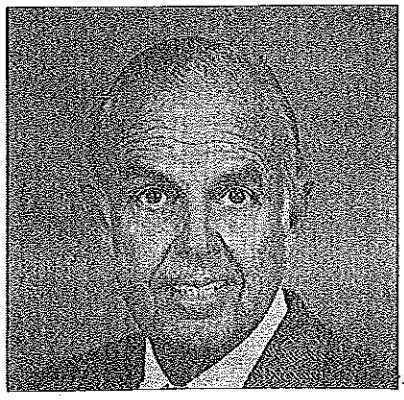
Commissioner Emden received her B.S. in 1963 and M.S. in Education from Hofstra University in 1967. She is an active participant in the Cancer Awareness Programs sponsored by the Arizona Cancer Center. Commissioner Emden served as consumer representative for Scientific Peer Review with the 1995 Department of Defense Breast Cancer Research Program. She is a Phoenix Art Museum Docent and was active in the Kiva School Art Masterpiece Program. Commissioner Emden was appointed by Governor Symington in 1994 and her term will expire in May of 1997.

Frank Hidalgo
Assistant Vice President, Institutional Advancement
Arizona State University



Commissioner Hidalgo has been Assistant Vice President for University Relations at Arizona State University since 1986. He served as Executive Director of the Governor's Council on Employment and Training in 1983. Commissioner Hidalgo received his B.A. in Education in 1964 from Arizona State University. He has served as a board member for numerous organizations, including Chicanos por la Causa and the Hispanic Women's Foundation. Commissioner Hidalgo was originally appointed to the Commission in 1991 and reappointed in 1993 by Governor Symington. His term expires in May of 1997.

Orme Lewis, Jr.
Managing Director, Select Investments, L.L.C.
Phoenix



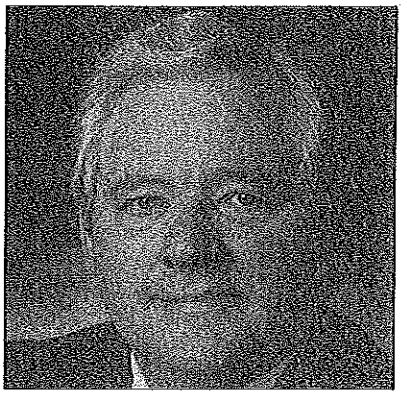
Commissioner Lewis oversees commercial real estate interests and participates in environmentally sensitive businesses. He currently serves on the governing boards of the Arizona Historical Foundation, Arizona State University Foundation, Phoenix Children's Hospital, Polycystic Kidney Research Foundation and is a former member of the Governor's Regulatory Review Council and the U.S. Advisory Committee on Mining and Mineral Research. Commissioner Lewis was elected to the 23rd and 24th Arizona State Legislature. He received his B.S. in Economics in 1958 from the University of Arizona. Commissioner Lewis was appointed by Governor Symington to the Commission in 1995 and his term will expire in May, 1998.

Medical Community

Patricia D. Moore, Dr.P.H., R.N.
Chair, Division of Community Health Nursing
Arizona State University

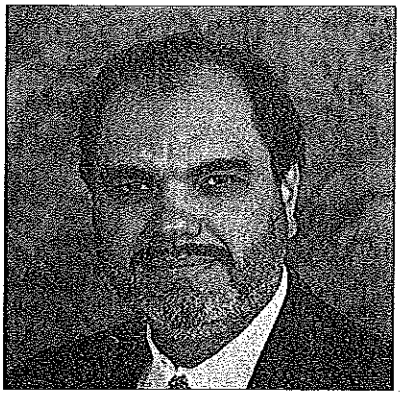
Commissioner Moore received her Doctorate and Master's degrees in Public Health from the Johns Hopkins University School of Hygiene and Public Health and a Master's degree in Nursing from the Catholic University of America. Commissioner Moore is an Associate Professor and Chair of the Division of Community Health Nursing, Arizona State University. She is a Fellow of the American Academy of Nursing. Appointed to the Commission by Governor Symington in 1993, Commissioner Moore was reassigned to the Medical Community in May of 1994. She was appointed to a second term in 1996 and her term expires in 1999.

John E. Oakley, M.D.
Family Practice
Prescott



Commissioner Oakley received his A.B. from Washington University, St. Louis, and an M.D. from the University of Missouri, College of Medicine. He completed a rotating internship and four year residency in General Surgery at St. Louis County Hospital. A member of the Arizona Medical Association for 33 years, Commissioner Oakley has also served as President and Vice President. For 15 years he was a preceptor professor for the University of Arizona, College of Medicine. Commissioner Oakley has practiced general medicine and surgery in Prescott since 1962. Commissioner Oakley was appointed by Governor Symington in 1994 and his term expires in May 1997.

Eladio Pereira, M.D., F.A.C.P.
Chief, Internal Medicine
Mariposa Community Health Center
Nogales



Commissioner Pereira received his B.S. in Chemistry from Georgia Institute of Technology in 1979. He graduated *Magna Cum Laude* from Emory University School of Medicine in 1983. He completed his internal medicine residency in 1986 at the Emory University Affiliated Hospitals and joined the staff at the Mariposa Community Health Center that year. He returned to Emory University where he was an Assistant Professor of Medicine and Director of the Intensive Care Unit at Grady Memorial Hospital from 1990 to 1992. He has been a Fellow of the American College of Physicians since 1993. In 1994 he initiated and continues to oversee, as program planner, annual Southern Arizona Conferences for primary care physicians. He was appointed to the Commission by Governor Symington in 1995 to complete the term of Commissioner Carlos Gonzales. His term expires in May 1996.

Scientific Research Community

Stan Lindstedt, Ph.D.
Professor of Biology
Northern Arizona University

Commissioner Lindstedt received his B.S. in Biology in 1970 from the University of Southern California and his Ph.D. in Zoology from the University of Arizona in 1977. He completed a National Science Foundation Fellowship in the Department of Anatomy, University of Berne, Switzerland in 1981. Commissioner Lindstedt is Treasurer and Steering Committee member of the American Physiological Society. He is the author of numerous articles and serves as a referee for a number of scientific publications including *Science*, *Nature* and the *Journal of Applied Physiology*. Commissioner Lindstedt was appointed to the Commission by Governor Symington in 1995 and his term expires in May, 1998.

Henry Reeves, Ph.D.
Professor Emeritus
Arizona State University

Commissioner Reeves was a Professor of Microbiology at Arizona State University from 1969 to 1993. During that time he also served as Chair of the Department of Microbiology from 1970 to 1973 and as Vice President for Research from 1985 to 1991. On leave from ASU, he served as Director of the National Science Foundation. Commissioner Reeves received his B.S. from Franklin and Marshall College and his M.A. and Ph.D. from Vanderbilt University. He was appointed to the Commission by Governor Symington to complete the term of Commissioner James Bloedel in 1995. His term expires in May, 1996.

Walter Williams, Ph.D., M.D.
Associate Professor, Department of Radiology
University of Arizona

Commissioner Williams received his Ph.D. in Physical Chemistry from Purdue University in 1968 and his M.D. from Yale University in 1980. Commissioner Williams was a member of the Science Team for the Voyager Spacecraft Missions to Jupiter and Saturn and was a Senior Scientist at the Jet Propulsion Laboratory, California Institute of Technology prior to returning to school to complete his medical studies. Commissioner Williams is an experienced teacher and researcher. He has authored numerous publications in the areas of physics and medicine. Commissioner Williams was appointed by Governor Symington in 1994 and his term expires in May, 1997.

Summary of 1995-96 Commission Activities

The Commission had 68 contracts with medical and health researchers in Arizona as of July, 1995. Contract summaries are contained in Sections A-C. The section headings list the source of funding (unrestricted medical research, appropriated funds and tobacco related research, tobacco tax funds) and the year the contracts are in, first second or third. Seven contracts received funding from both appropriated and tobacco tax revenues. These are marked with an asterisk, [*] and funds received from tobacco tax are enclosed in parentheses.

The appearance of the Annual Report has changed significantly this year. Abstracts for each project outlining the progress made are usually published separately late in the spring. They have been included here as have scientific publications and presentation or published abstracts. Presentation abstracts list the scientific meeting where they were delivered. Lay summaries for new awards have been added to Section E to provide an overview of the new research. These changes should provide a more complete description of the work of the Commission and the impact this program has on both the scientific community and the state of Arizona.

Approximately 1030 Requests for Proposals (RFPs) for 1995-96 awards were mailed to potential applicants in August 1995. The amount available for new tobacco related research contract awards was approximately $2,000,000. In response to the RFP, the Commission received 72 proposals in October, 1995. Section D lists the research proposals received.

In late November and early December the proposals were sent to a panel of national scientific and medical experts for peer review and evaluation. In January and February, the Commission received approximately 240 proposal evaluations prepared by more than 100 out-of-state peer reviewers. Three reviews were sought for each proposal. The proposals and evaluations were distributed to the Commissioners who were formed into three, three-person subcommittees to facilitate the final review process. In April the Commissioners selected 36 award-winning research projects from among the 72 applications submitted. The New Awards are listed in Section D. During 1996-97, the ADCRC will be managing 81 contracts.

SECTION A

CONTINUING CONTRACTS

UNRESTRICTED MEDICAL RESEARCH

YEAR THREE

Flink, Ph.D., Irwin

University of Arizona

Award Amount FY 1996: **$21,250**

Characterization of Cellular Nucleic Acid Binding Protein

The β-myosin heavy chain (MHC) gene encodes a protein of about 200 kDa.. The βMHC is one member of about thirty two different contractile proteins which function together during each contraction of the heart. Expression of the βMHC gene is regulated by thyroid hormone and also in a developmental and muscle-specific manner. Extrinsic factors, such as hypertension, control expression of the BMHC gene. Additionally, various cardiomyopathies are associated with BMHC gene defects which lead to myofibrillar disarray, altered heart performance, and early death. In Arizona and throughout the U. S., mutations within the βMHC coding region result in an autosomal disorder where approximately one-half of the affected individuals suffer from early mortality. An understanding of the factors which regulate transcription of the βMHC gene is essential for developing therapeutic interventions to improve heart performance during diseased states. In this study we have characterized a human protein, previously called Cellular Nucleic Acid Binding Protein (CNBP), which regulates βMHC gene expression by interacting with the βMHC gene promoter. Two isoforms of CNBP, α and β, have been characterized and the complete genomic sequence of CNBP has been determined. Additionally, the cDNA encoding CNBP has been identified in *Xenopus* (frog), in order to learn more about the role of CNBP during development. Preliminary results indicate that CNBP is expressed in the mature unfertilized oocyte and also during early embryonic development in ectodermal and mesodermal tissues.

Publications:

Flink I, Morkin E. Alternative Processed Isoforms of Cellular Nucleic Acid Binding Protein Interact with a Suppressor region of the Human *β*-Myosin Heavy Chain Gene. J. Biol. Chem. 270: 6959-6965; 1995.

Flink I, Morkin E. Organization of the gene Encoding Cellular Nucleic Acid Binding Protein. Gene 163: 279-282; 1995.

Abstract:
Flink I, Morkin E. Genomic Organization and Mutational Analysis of the Cellular Nucleic Acid Binding Protein (CNBP) Gene. Presented at the International Society for Heart Research; 1995.

Ulreich, Ph.D., Judith B.

University of Arizona

Award Amount FY 1996: **$44,947**

Immunotoxicology of Common Groundwater Contaminants in Arizona

Research was conducted to determine whether the groundwater contaminants trichloroethylene (TOE). dichlorothylene (DOE) and perchloroethylene (POE), which have been found to contaminate wells in Arizona and nationwide have an adverse effect on the immune system. In prior years, we demonstrated in a mouse model, that these agents caused alterations in the cells that control the immune system. This year, long term studies were conducted where animals were exposed to the water contaminants for up to several weeks and then challenged by injection of tumor cells. Greatly increased numbers of tumors were observed in treated animals, even when treatment had been discontinued prior to challenge with the tumor cells. The immune systems of the mice were, therefore, compromised since resistance to tumors decreased even after exposure to the toxicant ceased. These findings emphasize the importance of closing contaminated wells, testing nearby wells over time and following the health of affected populations.

Publications:

Ulreich J, Harris D, Luedke D. Differential Effects of Trichloroethylene and Dichloroethylene on the Murine Immune System. The Toxicologist 14:29; 1994.

Ulreich J, Harris D, Spofford C. Halogenated Hydrocarbons Inhibit Natural Killer Activity in Spleen Cells from Exposed Mice. The Toxicologist 15:226; 1995.

Balcazar, Ph.D., Hector

Arizona State University

Award Amount FY 1996: **$16,130**

The Effect of Acculturation on Birth and Postnatal Outcomes Among Mexican American Women in Arizona

The overall objective of this cohort study "The Arizona Perinatal Acculturation Project" (APAP) was to examine the effects of Americanization (acculturation) on birth outcomes among Mexican American women. In phase one of the study, a detailed prenatal survey was administered to 500 pregnant women enrolled in the project. Each woman provided data for the following factors: sociodemographic and economic profiles, acculturation, family support, coping strategies, family cohesiveness, previous pregnant risk factors, pregnancy risk-taking behaviors, and diet. Phase two consisted of a postnatal survey administered at least three months after the infant's birth date. A total of 269 women provided data for this second phase. Subjects were recruited from two health care agencies, the Tidwell Service Area or Clinica Adelante and a WIC site located in the Phoenix metropolitan area.

Findings from the prenatal component of APAP (phase 1) revealed that acculturation as well as protective family factors (as indicators of adherence to traditional Mexican cultural values) were important determinants of health-risk behaviors among Mexican American women. Furthermore, combined measures of cultural protection (represented by classification schema based on acculturation and family cohesion scales) provided further evidence that cultural factors are strongly associated with health-related risk behaviors (including diet) among Mexican American women.

The postnatal component of APAP (phase 2) investigated the determinants of birthweight (as a continuous variable) and low birthweight (expressed as less or equal to 2900 grams). Acculturation (as measured by a 5-item scale) was not associated with these outcomes. For birthweight, factors found to be negatively associated in the multivariate model were: medical problems during pregnancy, history of a previous premature infant and living with a large number of relatives and other adults. Positive predictors of birthweight were: increased saturated fat and thiamin consumption, maternal height, male infant, longer gestation, and having received a great number of types of prenatal advice. The multiple logistic model revealed a decreased probability of low birthweight associated with increased: length of gestation, weight gain during pregnancy, maternal height, number of children living in the household, number of types of prenatal advice, and Americanized food preference. Increased risk of weight <2900 gm was associated with: increased number of relatives and other adults living in the household, history of a previous premature infant, and medical problems during pregnancy.

Even though the acculturation hypothesis was not confirmed relative to birth outcomes in this sample, the relationships found of acculturation with other family characteristics and health-related behaviors strongly suggest the need to continue exploring cultural factors in clinical settings so that the Arizona health care system responds appropriately to meet the needs of subgroups of Mexican American women.

Leischow, Ph.D., Scott J.

University of Arizona
Award Amount FY 1996: **$21,250**

The Safety and Efficacy of Nicotine Patch for Smoking Cessation in a Self-Help Program

Currently, the nicotine patch is only available in a prescription setting through a physician. All drugs that are to be switched from prescription classification to over-the-counter need to be tested in an over-the-counter setting to insure that the drug will not only be used safely, but that it will also be effective in such a setting. This self-help study is tracking how people use the Nicotrol™ patch on their own without physician consultation. The study is also evaluating whether or not the patch is more, less or equally effective in an over-the-counter setting as compared to a prescription setting. Finally, this study will look at how much people are willing to pay for the patch (which is a factor in determining the success of the switch to over-the-counter status). In this self-help study, 300 people had the opportunity to purchase patches to try to quit smoking.

Shand, Ph.D., Richard F.

Northern Arizona University
Award Amount FY 1996: **$19,476**

Isolation and Characterization of Halocin Genes from Extremely Halophilic Archaebacteria

Antibiotics are chemicals that are produced by microorganisms. For example, penicillin is an antibiotic that is made by the mold *Penicillium* and is effective against many types of bacteria that cause disease. The vast majority of antibiotics in use today are not made of protein; that is, they are not compromised of amino acids linked together in a chain. We are studying "protein antibiotics" called "halocins" that are made by bacteria that grow in extremely salty (NaCl) environments like the Dead Sea. We have successfully purified two other halocins, halocin Hal R1 and halocin S8. Comparing the gene sequence and protein structures of these halocins will be the first step in understanding how these protein antibiotics work, which is important for the citizens of Arizona if we are to design future protein antibiotics to fight disease.

Dodson, Ph.D., Mark S.

University of Arizona

Award Amount FY 1996: **$21,247**

Biochemical Characterization of the Helicase-Primase of Herpes Simplex Virus Type 1

Herpes virus encodes an enzyme called helicase-primase that is required for its reproduction. Our goal is to characterize the biochemical properties of this enzyme. Knowledge of these properties may be used to design antiviral compounds that block the activity of the enzyme. We have characterized the interaction of this enzyme with DNA and with nucleotide fuel molecules such as ATP. We have found that the enzyme must first bind to single-stranded DNA of a certain length in order to burn the ATP fuel molecule. The helicase-primase is made up of three distinct proteins. We have also determined that a new technique for mapping the sites of interactions between proteins can be used to map the interactive sites on the proteins comprising the helicase-primase. This technique may enable us to identify sites that can be targeted for the development of drugs that block the assembly of the enzyme.

Grimes, Ph.D., William J.

University of Arizona

Award FY 1996: **$26,013**

Peptides Bound to HLA and Human Disease

We have developed a new technology for determining how peptide antigens are bound and displayed by human histocompatibility antigens. These procedures first used biochemically purified class I proteins, and we have extended our methods to include synthesis of human HLA proteins in bacteria and more recently in insect cells. The latter produce about a hundred-fold increase in yields of products with higher solubility because of glycosylation. Our methods involve using beads containing random oligopeptides and incubated *in vitro* with HLA and β-2 microglobulin. Biologically relevant complexes are detected using an ELISA reaction allowing a determination of peptide structures which have a high potential for presentation by human HLA. Our methods have potential for the development of new approaches to the therapy of autoimmune diseases, AIDS, and cancer.

Publications:

Smith M, Lam K, Hersh E, Lebl M, Grimes W. Peptide Sequences Binding to MHC Class I Proteins. Molecular Immunology 31: 1431; 1994.

Floyd-Smith, Ph.D., Georgia

Arizona State University
Award Amount FY 1996: **$19,318**

Regulation of Cell Cycle Control Genes by Interferons

Interferons have been extensively used as therapeutic agents against cancer. Interferons act through other proteins that regulate cell proliferation by altering entry into and progression through the cell cycle. One of the interferons, interferon-β, shows promise as a potential treatment for brain cancer. Three types of interferon, interferon-α, interferon-β, and interferon-γ were tested for anti-proliferative activity in 5 brain cancer cell lines and interferon-β was found to have the greatest inhibitory effect on cell growth. Interferon-β inhibits growth of a rapidly growing cell line, AO2V4 which is resistant to other drugs used in treatment of this cancer. Growth of normal brain cells (astrocytes) was unaffected by interferon treatment. The observed decrease in cell numbers for cancer lines was not due to an increase in the rate of cell death, but rather to an apparent decrease in the rate of DNA synthesis in interferon-β treated cells. Several cell cycle control proteins were tested for their role in mediating interferon's anti-proliferative effects. One of these, protein kinase C, was found to be involved in the induction of an important interferon-inducible protein, 2-5A synthetase. Since protein kinase C is activated by growth factors are known tumor promoters, the interaction between cell signaling pathways activating protein kinase C and the interferon signaling pathway may explain some of the anti-proliferative actions of interferons.

Publications:

Shih N-Y, Floyd-Smith G. Protein Kinase C-δ mRNA Is Down-regulated Transcriptionally and Post-transcriptionally by 12-*O*-tetradecanoylphorbol-13-acetate. J Biol Chem 271: 16040-16046; 1996.

Garrison JI, Berens ME, Shapiro JR, Treasurywala S, Floyd-Smith G. Interferon-β Inhibits Proliferation and Progression through S phase of the Cell Cycle in Five Glioma Cell Lines. J Neuro-oncology, In Press.

Abstracts:
Floyd-Smith G, Wang Q, Yu F. Protein Kinase C id required for IFN-α Introduction of the p69/p71 Isoform of the 2-5A Synthetase. Growth Factor and Signal Transduction Symposium on Interferon Signaling. p. 22, 1996.

Hoffmann, Ph.D., Joseph J. **University of Arizona**

Award Amount FY 1996: **$21,250**

Potential Antitumor Agents from Southwestern Plants

During the life of this three-year seed project, we have tested 769 plants that generated over 2,000 extracts or fractions that were tested by Drs. Fred Valeriote and Tom Corbett of Wayne State University. Only those plants demonstrating the most selective solid tumor activity are investigated. This translates into success rate of 1.5% or about 12 plants in our case. This does not include the many moderately active leads that are worth studying. Last year's interesting active compound turned out to be too toxic for further development. However, the compounds responsible for the good selectivity were present in very small amounts in those plants. Therefore, in collaboration with desert legume project, we are growing related plants for harvest in order to preserve the few populations present in the deserts of the Southwest.

As we ended the project this summer, our latest discovery turned out to be a very selective solid tumor plant extract with negligible toxicity towards normal cells. This implies that we can indeed develop non-toxic tumor selective drugs. To date the attempts to recover the active compounds have not resulted in any changes in this selective profile. Therefore, it is highly probable that new drugs will be developed through the results of this seed project. This can be stated with some confidence, because we are part of a cancer drug discovery group that has been funded for five years at $126,000 per year for the part conducted at the University of Arizona. This project is expected to start by September 30th of this year. Thus, this investment by the people of Arizona will bring close to $750,000 in new money to the state for research and development. But more importantly, the data indicate that we have a very good chance of developing a non-toxic drug to treat solid tumors, especially colon cancer, which has been on the increase nationally for the past several years. Since this type of cancer affects people over 50 more often, this program could result in helping to maintain a healthy retirement community in Arizona.

Moffet, Ph.D., John **St. Joseph's Hospital, Phoenix**

Award Amount FY 1996: **$20,679**

Molecular Mechanisms of Altered Basic FGF Function in Human Malignant Astrocytoma

In the development of glioblastoma multiforma (GBM), proteins which normally promote growth expressed continuously to give unregulated cell growth or cancer. We are studying one growth factor, basic fibroblastic growth factor (bFGF) or FGF-2, which has been found to be over-expressed in GBM tissue. Our studies have shown that bFGF and its receptor (FGFR1) are deregulated when compared to normal human brain astrocytes. We have elucidated the mechanisms which control the over expression of bFGF in human glioma cells and have shown they mimic bFGF regulation in normal astrocytes during the process of gliosis. We have found that FGFR1 is deregulated in glioma cells in a similar manner as bFGF. We are currently determining the molecular factors involved in the regulation of bFGF and its receptor, FGFR1.

Moffet J, Kratz E, Florkiewicz R, Stachowiak MK. Promoter Regions Involved in Density-Dependent Regulation of Basic Fibroblast Growth Factor Gene Expression in Human Astrocytic Cells. Proc Nat Acad of Sci 93:2470-2475; 1996.

Joy A, Moffet J, Neary K, Mordechai E, *et.al.* Nuclear Accumulation of FGF-2 is Associated with Proliferation of Human Astrocytes and Glioma Cells. Oncogene, In Press.

Parker, Ph.D., Roy **University of Arizona**

Award Amount FY 1996: **$21,248**

Identification of Genes Regulating Eucaryotic Gene Expression by mRNA Deadenylation

The proper expression of genes is required for essentially all biological processes. For example, cancer cells grow abnormally because they no longer properly regulate the expression of their genes. One important, yet poorly understood, step in gene expression is the control of mRNA degradation. Individual mRNAs can be degraded at very different rates and mutations that alter the normal decay of certain mRNAs can have dramatic consequences to the cell. We have utilized our knowledge of how mRNAs are degraded in yeast cells to identify mutations in genes that may encode the cellular machinery that degrades mRNAs. We have shown that these mutations affect the degradation of several mRNAs and have determined that one of these mutations is in the gene for the critical decapping enzyme. Given the similarities of biological processes in yeast and humans, it is anticipated that this work will allow insight into how mRNA turnover is controlled in humans.

Publications:

Hatfield L, Beelman CA, Stevens A, Parker R. Trans-acting Mutations that Inhibit mRNA Decapping in Yeast. Molecular and Cellular Biology, In Press.

Beelman CA, Stevens A, Caponigro G, LaGrandeur. E, *et. al.* An Essential Component of the Decapping Enzyme Required for Normal Rates of mRNA Decay in Yeast. Nature, In Press.

Kling, M.D., Pamela Jean

University of Arizona

Award Amount FY 1996: **$21,150**

Growth and Development Regulation of Erythropoietin Production

Anemia is an extremely common finding among premature infants. Premature infants less than 1500 grams birthweight (approximately 700 infants per year in Arizona) receive 8 transfusions for anemia before discharge to home. With transfusion, premature infants are susceptible to viral infections, transfusion reactions, and metabolic disturbances. Premature infants have anemia secondary to deficiency of a hormone, erythropoietin (in Greek, "to make red blood cells"). Our hypothesis was that the fetus and premature newborn infant have defective regulation of erythropoietin production because of abnormal iron availability.

We speculated that abnormal distribution of iron and other nutrients, associated with poor growth, are responsible for the decreased erythropoietin production seen. We utilized liver cells in culture and have modified laboratory tests for this cell system. We found that removing iron from the cells increases production of erythropoietin and have begun understanding some of these mechanisms. Secondary to our preliminary findings, we became involved with additional projects dealing with iron delivery and erythropoietin production. The findings of all projects will further clarify mechanisms involving iron delivery and erythropoietin production, especially as it relates to premature infants.

Publications:

Kling PJ, Sullivan TM, Leftwich ME, Roe DJ. Score for Neonatal Acute Physiology (SNAP) and Need for Transfusions in Very Low Birth Weight Infants. Archives of Pediatric and Adolescent Medicine, In Press.

Kling PJ, Dragsten PR, Roberts RA, Dos Santos B, Brooks DJ, Hedlund BE, Taetle R. Iron Deprivation Increases Erythropoietin Production *In Vitro*, in Normal Subjects and Patients with Malignancy. British J. Hæmatology 95: 241-248; 1996.

Caselli, M.D., Richard J.

Mayo Clinic, Scottsdale

Award Amount FY 1996: **$21,175**

Kinematic Studies of Upper Limb Apraxia

Apraxia is a severe and poorly understood movement disorder that complicates neurorehabilitation. It is thought to result from impaired spatial and temporal aspects of motor programming. We tested temporal aspects of motor programming, and determined, for the first time, the "kinematic" properties of upper limb apraxia in patients with corticobasal degeneration (CBD).

Our three year study has shown that:

1. Apraxic upper limb movements are generally slower and less spatially accurate than normal.
2. Apraxic open and close their hands faster than normal during reaching movements. (This may provide a clinical test for apraxia; none presently exists.)
3. Both upper limbs are affected in CBD, even when the disease appears unilateral.
4. Despite spatial programming impairment, temporal aspects of the motor program are less impaired. For example the time allotted to reach peak wrist velocity compared to total transport time remains proportionally normal in apraxia.

Publications:

Caselli RJ, Redman EM, Stelmach GE, Timmann D, Lawson M, Osborne D, Moore SB. Progressive Apraxia in Clinically Discordant Monozygotic Twins. Arch Neurol 52:1004-1010; 1995.

Abstracts:

Caselli RJ, Stelmach GE, Timman D, Tadikonda S. Kinematic studies of Upper Limb Apraxia in Patients with Cortical-basal Ganglionic Degeneration: Is Apraxia a Motor Programming Disorder? Presented at the Movement Disorders Society Symposium, Cortical-basal Ganglionic Degeneration and Its Relationship to Other Asymmetric Cortical Degeneration Syndromes, Satellite to the 120th Annual Meeting of the American Neurological Association, Washington, D.C., October 25, 1995. Movement Disorders 11: 346-357; 1995.

Caselli RJ, Stelmach GE, Timman D, Tadikonda S. A Kinematic Test of the Motor Programming Hypothesis in Apraxia. Presented at the 48th Annual Meeting of the American Academy of Neurology in San Francisco, CA, March 28, 1996. Neurol 46 (suppl): A382; 1996.

Montfort, Ph.D., William R. **University of Arizona**

Award Amount FY 1996: **$19,543**

Anticonvulsant (Phenobarbital) Effects on the Developing Nervous System: An *In Vitro* Model

Phenobarbital may adversely affect a number of steps in central nervous development, including early formation of the nervous system, nerve cell proliferation and migration, as well as the initiation of nerve processes and the establishment of contacts on target cells. That therapeutic or even subtherapeutic levels of phenobarbital may have unacceptable deleterious effects on neuronal differentiation is debated. Phenobarbital, however, is the most commonly used anticonvulsant in newborn and young infants during a time when there is still considerable brain development occurring. In these studies we use a tissue culture model of neuronal development to test for the adverse effects of phenobarbital and eventually to study the mechanisms by which such effects might occur. Nerve cells can be treated with increasing concentrations of phenobarbital during a critical time period of normal process development induced by either co-culture with normal companion (glial) cells or by a purified growth factor.

Polt, Ph.D., Robin L. **University of Arizona**

Award Amount FY 1996: **$21,250**

Crossing the Blood-Brain Barrier

Twenty-five years ago, scientists isolated opiate messengers from the human brain which control pain (enkephalins). Much has been learned about the receptors located on brain cells which receive enkephalins. Tragically, the long-sought goal of using the human brain's own messengers as drugs has been blocked by the blood-brain barrier (BBB) which prevents their entry into the brain. We have attached sugar molecules to enkephalins to form glycoconjugates which do penetrate the BBB. The glycopeptides cross the BBB to block pain with potency approaching morphine or Demerol®, two widely prescribed narcotics. By altering the structure of the glycopeptides, we switch on only certain opiate receptors in the brain. Our newly synthesized glycopeptides are even more selective, and offer the possibility of fewer side effects. Second-generation glycopeptides may reduce tolerance and physical dependence during chronic use. Tailor-made glycopeptides could treat opiate addiction by relieving withdrawal symptoms and craving.

Publications:

Polt R, Porreca F, Szabo LZ, Bilsky EJ, *et.al.* Glycopeptide Enkephalin Analogues Produce Analgesia in Mice: Evidence for Penetration of the Blood-Brain Barrier Proc. Nat Aca of Sci 91: 7114-7118; 1994.

Brachova, Ph.D., Libuse

Sun Health Research Institute, Sun City

Award Amount FY 1996: **$21,250**

Functional Correlates of Complement Activation in Alzheimer's Disease

During the three-year-period funding by ADCRC both proposed aims were achieved:
1) We have demonstrated that full complement activation, with its concomitant potential to damage tissue, only occurs in areas of the Alzheimer's disease (AD) brain that sustain significant nerve cell destruction.

2) We have compared inflammatory and classical hallmarks of AD pathology in a group of
(i) putatively nondemented elderly control patients who at the autopsy evidence sufficient amount of neurofibrillary tangles and neuritic plaques to qualify them for the diagnosis of AD patients
(ii) AD patients and
(iii) nondemented elderly patients with little or no AD pathology.

We have demonstrated that the first group of patients represents early pre-clinical stage of Alzheimer's disease where destructive inflammation impact could not deteriorate the brain tissue as well as in patients suffering with AD for many years.

3) We have characterized the final product of classical complement cascade (C5b-9, one of the hallmarks of inflammation) using multiple antibodies, multiple techniques from molecular biology to ultra-structure, and multiple independent laboratories to cross validate results. At the light and electron microscopic levels, we demonstrate co-localization of C5b-9 with neuronal membranes. We show that C5b-9 is detected in the AD but not nondemented elderly brain with multiple anti-C5b-9 antibodies, and that *C5b-9* immunoreactivity in western blots of AD brain parallels that for *in vitro* generated *C5b-9* standard.

Publications:

Lue L-F, Brachova L, Walker DG, Rogers, J. Characterization of Glial Cultures From Rapid Autopsies of Alzheimer's and Control Patients. Neurobiology of Aging 17(3):421-429; 1996.

Lue L-F, Brachova L, Civin WH, Rogers, J. Inflammation $\alpha\beta$ Deposition, and Neurofibrillary Tangle Formation as Correlates of Alzheimer's Disease Neurogeneration. J Neuropath Exper Med. In Press.

Section B

Continuing Contracts

Unrestricted Medical Research

Year Two

Appleton, M.D., Christopher

Mayo Clinic Scottsdale

Award Amount FY 1996: **$26,382**

Experimental Determinants of Transmitral and Pulmonary Venous Flow During Atrial Contraction.

The purpose of this experimental study was to define the factors which alter venous blood flow in the lungs when the heart pressures become elevated. Certain observations have been made through heart ultrasound, which suggests that recognizing when pressures are abnormally elevated might be possible; however, the physiologic reason for these observations has previously not been defined. The reason for this was that it is not possible in human studies to measure venous pressure in the lungs directly.

By using a combination of heart ultrasound methods together with pressure recordings in an animal model, we were able, for the first time, to determine the physiology behind venous blood flow coming from the lungs to the heart. We also found that this flow is altered by changes in heart rate, breathing, and changes in the filling pressures in the heart. The results of this work resolve controversy about the determinants of venous blood flow returning to the heart from the lungs, which has been unresolved for 40 years. We believe the work will improve the non-invasive assessment of heart and lung pressures using cardiac heart ultrasound techniques. The ultimate aim is that these ultrasound methods will replace the need for direct measurement of heart pressures (cardiac catheterization) and thus reduce patient morbidity and mortality from invasive procedures.

Berg,* M.D., Robert

Arizona State University

Award Amount FY 1996: **$26,400**
($3,564)

Regulation of Programmed Cell Death in Human Cancer Cells

This study was designed to determine whether assisted ventilation during simulated, single-rescuer, bystander cardiopulmonary resuscitation improves outcome. The first half of the study was completed and demonstrated that successful initial resuscitation, 24-hr. survival, and neurologically intact outcome was similar after chest compressions only or chest compressions plus assisted ventilation during CPR. Both techniques improved outcome compared to no bystander CPR.

In part two of our study, we plugged the coronary arteries of animals causing a myocardial infarction (heart attack), and then we induced cardiac arrest and provided cardiopulmonary resuscitation with either chest compressions only, chest compressions plus assisted ventilation, or no cardiopulmonary resuscitation, as in part one of the study. Preliminary results of the first 20 animals in this study again suggest that assisted ventilation during CPR does not improve outcome compared to chest compressions alone.

The prospect of mouth-to-mouth ventilation is a significant barrier to the provision of CPR. The implications of this research are clear: If assisted simpler, easier to perform CPR technique, chest compressions alone, may be equally effective and more frequently performed in light of its greater acceptability to the public.

Publications:

Berg RA, Kern KB, Hilwig RW, Berg MD, Sanders AB, Otto CW, Ewy GA. Assisted Ventilation Does Not Improve Outcome in a Porcine Model of Single-Rescuer Bystander CPR. Circulation, In Press.

Burt, Ph.D., Janis M.

University of Arizona

Award Amount FY 1996: **$26,400**

Vascular Smooth Muscle Dysfunction Early in Atherogenesis

Smooth muscle cell (SMC) proliferation is an integral step in atherogenesis. Consequently, identification of factors which cause SMC proliferation and development of strategies to prevent proliferation are logical goals for studies whose ultimate aim is prevention of atherosclerosis. A major risk factor for atherosclerosis is elevated blood levels of low density lipoproteins (LDL). Our data indicate that i) oxidized LDL stimulates proliferation of SMCs, ii) proliferation of SMCs is associated with compromised gap junction function, and iii) oxidized LDL compromises gap junction function as do growth factors. Gap junctions are channels which allow cells to communicate with one another. These channels therapy support coordinated tissue functions like controlled growth (or growth arrest) and maintenance of normal blood pressure. Thus our data suggest that interventions designed to prevent LDL induced loss of gap junction function would also prevent SMC proliferation. If this proves to be the case, then development of strategies to enhance gap junction function or expression would be the next logical goal for therapeutic intervention in this disease.

Fernandez, Ph.D., Marie Luz

University of Arizona

Award Amount FY 1996: **$26,378**

Vitamin C and Cardiovascular Disease Risk

These studies compared adequate *versus* marginal levels of vitamin C intake in combination with polyunsaturated, monounsaturated or saturated fatty acids to determine how the level of vitamin C and the saturation of the fat effects plasma cholesterol and triglyceride concentrations. Guinea pigs were used in these experiments because similar to humans, they cannot synthesize vitamin C. Plasma cholesterol triglyceride levels were higher in animals fed vitamin C deficient diets. In addition, guinea pigs fed the polyunsaturated diet with marginal vitamin C had an LDL particle more susceptible to oxidation with an increased potential for atherogenesis. Animals fed saturated fat had higher plasma cholesterol than those fed mono or polyunsaturated fat. From these studies we concluded that adequate Vitamin C intake reduced LDL susceptibility to oxidation and intake of monounsaturated fat with adequate levels of vitamin C resulted in a plasma lipid profile associated with decreased risk for cardiovascular disease. These studies are meaningful for Arizona residents because they emphasize the importance of eating diets with adequate amounts of vitamin C and suggest that consumption of monounsaturated fatty acids may decrease the risk for coronary heart disease.

Publications:

Abstract:
Montano C, Osman M, Fernandez ML, McNamara DJ. Vitamin C Deficiency Alters VLDL Secretion Rates in the Guinea Pig. FASEB J. 10: A2946; 1996.

Going, Ph.D., Scott B.

University of Arizona

Award Amount FY 1996: **$24,515**

Metabolism of High Density Lipoprotein Cholesterol in Postmenopausal Women

High levels of HDL-C are associated with lower risk of coronary heart disease (CHD). In women, after menopause, natural estrogen and HDL-C levels decrease and the risk of CHD increases. Hormone replacement therapy (HRT) may increase HDL-C and reduce CHD risk. The effects of menopause (estrogen withdrawal) and HRT may be due to changes in two proteins, lecithin cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP), which influence reverse cholesterol transport. This possibility was examined in 190 postmenopausal women of Anglo (n=129) and Hispanic descent (n=55), with about 50% undergoing HRT. Total and regional body fat was also measured since Hispanic women have more central fat than Anglos and central fat influences lipid metabolism. Data analyses are ongoing. Preliminary analysis has shown no difference between HRT users and nonusers in plasma total cholesterol (TC), lipoproteins, and enzyme activities. However, Hispanic women had lower HDL-C, TC/HDL-C, CETP and higher triglycerides and LDL oxidation than Anglo women. CETP and LCAT was significantly correlated with truncal fat in all groups demonstrating the influence of truncal fat on these enzyme activities, HDL-C metabolism and reverse cholesterol transport. Ethnic differences in CETP activity were no longer evident when the effects of truncal fat were controlled.

Thomas, Ph.D., Katherine T.

Arizona State University

Award Amount FY 1996: **$26,398**

Body Mass, Total And Regional Fat Response of Gynoid Woman to 8 Weeks of Exercise

While men respond favorably to endurance training programs, losing both mass and body fat without decreasing caloric intake, women seem to be resistant to weight loss or body composition changes. Based on the literature, women were expected to decrease in waist circumference (C) have stability in gluteal C, decrease in abdominal skinfold (SF) measurements, have stability in gluteal SF, and decrease in waist-to-hip ratio (WHR). Because of similar responses to training in men and android women in previous studies, gynoid women were predicted to remain weight stable in response to training, while it was hypothesized that android women would lose a significant amount of mass. However, all women who completed the training were predicted to lower their percent body fat. Eight weeks of aerobic activity (30 minutes of stair climbing 3 times a week) brought about no significant change in WHR, gluteal C or waist C. Abdominal SF (sum of abdominal and subscapular) decreased significantly (e.s.= .42), as did gluteal-femoral SF (sum of thigh and suprapatellar) (e.s.= 1.03). Percent body fat as measured by hydrostatic weighing decreased significantly (e.s.=.57). Average fat loss was 1.6%, which was consistent with the sum of 11 SF decreasing significantly (e.s.= 1.01) from pre- to post-test. Android and gynoid subjects remained weight stable throughout the 8-week study. No differences between pre- and post-test caloric intake or daily activity as taken from 7-day weighed food logs and 7-day activity logs were recorded. Subjects did increase fitness in 5 of 6 measures taken (e.s. ranging from .31 to 1.77). Drop-outs tended to have higher pre-test VLDL levels (e.s.= -2.93), and lower body fat percentage measurements than adherers (e.s.= 1.06), as well as a tendency to score better on pre-test self-efficacy (e.s. ranging from -1.17 to -2.25). Gynoid women seeking to lose fat and reduce health risk should be encouraged to exercise based on these findings.

Braun, Ph.D., Eldon J.

University of Arizona

Award Amount FY 1996: **$26,400**

Kidney Stone Formation with an Emphasis on the Formation of Uric Acid Containing Stones

Kidney stone disease is a disorder whose incidence is increasing with time. Demographic records show that this disease is more common in hot climates such as Southern Arizona. It is clear from existing reports on hospitalizations that kidney colic has become one of the commonest causes of emergency admissions (10 of 1000 admissions) and therefore it must constitute a major health problem. This disease is a multi-factorial disorder that can arise from any one of several risk factors. A problem is that the formation of kidney stones is not well understood. A factor that has hampered the understanding of this disease is the lack of a model system that can be manipulated to study production of kidney stones. A major drawback of models used thus far is that few if any animals develop kidney stones spontaneously as do humans. The research of this proposal is about an animal that produces small kidney stones as part of its normal pattern of excretion of metabolic wastes. This is the domestic chicken. It has not been widely recognized that small kidney stones are normally found in the urine of birds. Data developed as background for this proposal suggest that the small stones of bird urine have the same chemical composition as those in human urine and therefore can be used to study the development of kidney stones. Kidney stones appear to be a phenomena of the sun belt and their formation parallels the level of heat in the environment. In Arizona, the incidence of stones increases during the summer and decreases during the winter. This disease has a significant economic impact on the state of Arizona above personal pain and suffering. Based on older (1976) census data and hospital records for the same year, the economic impact was about 3.5 million dollars. With the increased hospital costs and the increased incidence of kidney stones, the actual amount today is probably much higher.

Publications:

Casotti B, Braun EJ. Functional Morphology of the Glomerular Filtration Barrier of *Gallus*. J Morphol 228: 327-334; 1996.

Flores, M.D., Carlos A. — **University of Arizona**

Award Amount FY 1996: **$22,879**

Dietary Carnitine Gastrointestinal Metabolism During Development

Carnitine is a nutrient present in breast milk, that appears essential for newborn infants. Its function in breast milk is not known. Critically ill infants in the state of Arizona and elsewhere are provided nutrition which is lacking carnitine.

Work done as part of this project has shown that:

a) The intestine of nursing animals possesses a special capacity for burning dietary fats that far exceeds that of older animals.

b) The capacity for the burning of fats by the intestine is determined by the location, with different functions along different locations of the intestine.

c) Deprivation of carnitine in milk may be associated with a decreased capacity for the use of fat as fuel in newborn animals.

These findings indicate that intestinal metabolism is different in nursing animals when compared to adults and that carnitine may be necessary in the diet of infants.

Garewal, M.D., Ph.D., Harinder — **University of Arizona**

Award Amount FY 1996: **$24,400**

Antioxidant Vitamins for the Secondary Prevention of Cardiovascular Disease

The second year of this study has been very successful. A cohort of patients with existing heart disease was enrolled in a double-blind placebo-controlled study of antioxidant supplementation and followed for one year. Compliance was excellent, and data collection and analysis of blood antioxidant levels, serum lipoprotein and lipoprotein oxidation parameters, and cardiovascular events were completed. Recruitment rates and methods were also analyzed, and used to project the number of potential subjects that would have to be contacted and the feasibility of large scale, long-term study of antioxidant supplementation for patients with existing heart disease.

McCloskey, Ph.D., Laura A. — **University of Arizona**

Award Amount FY 1996: **$27,407**

Violence and Substance Use in Hispanic and Anglo Youth

The goal of our study was to identify risk markers for substance use among Hispanic and Anglo adolescents, especially violence within the family. In our study of 363 children between the ages of 6 and 12 we found relatively few children were experimenting with drugs or alcohol before the age of 12, but those children who were using drugs were likely to be from violent homes. There were no ethnic differences. The strongest predictor in adolescence was family income. The link between early exposure to family violence and later substance use was weak if the family had stabilized. In a separate follow up of 70 adolescents referred to juvenile court, we found few ethnic differences in drug or alcohol use. Hispanics were more likely to use marijuana than Anglos, but no other differences in use surfaced. Anglos were more likely to sell drugs than Hispanic youths (42% vs. 17%). These findings suggest that family functioning and income are important determinants of substance use in children and adolescents, but that Anglos and Hispanics within the same social class display few differences.

Ampel, M.D., Neil M. — **University of Arizona**

Award Amount FY 1996: **$21,596**

Cytokine in Human Coccidioidomycosis

Coccidioidomycosis, known popularly as Valley Fever, is a very common fungal infection in Arizona. While most people who are infected do well, certain people develop severe disease. We have found that blood cells in people with severe Valley Fever fail to make a specific immune substance called interferon-gamma, while the cells from people who are immune to Valley Fever make IL-10, which is known to turn down the immune response in other infections. Finally, we've been able to determine that the number of blood cells actually responding to the fungus is much lower in people with severe Valley Fever than in people who have done well. These studies should allow us to examine if there are ways to alter the immune response and help people control their Valley Fever.

Marchalonis,* Ph.D., John J. — **University of Arizona**

Award Amount FY 1996: **$107,229**
($14,622)

Analysis of Autoantibodies to T-Cell Receptors in Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease characterized by chronic systemic inflammation predominately affecting diarthrodial joints and frequently a variety of other organs. The disease affects 1-1.5% of Americans with a female to male ratio 3:1. The incidence of the disease is increased to approximately 5% in certain tribes of native Americans and the prevalence in the Tucson area is approximately 5% due to the influx of individuals suffering from the disease and the high percentage of native Americans in the area. We found that individuals suffering from RA tend to have increased levels of autoantibodies directed against the recognition molecules on their own thymus-derived lymphocytes. These autoantibodies are predominately of the immune macroglobulin (IgM) class and react with the combining site region of the T-Cell receptor. The autoantibodies against self Tcrs probably play a role in immune regulation and the elevation of these antibodies in RA patients may indicate a dysfunction of normal immunological mechanisms. The central question to be addressed is whether these antibodies are essentially the same ones expressed in low levels by healthy individuals in immunoregulation, or whether they represent a distinct disease-related population that uses different immunoglobulin variable region genes. The antibodies were detected using a novel synthetic immunopeptide approach developed here and their presence offers new approaches for diagnosis and potential therapy for this prevalent and crippling autoimmune disease. Adverse lung complications occur in approximately 20% of rheumatoid arthritis patients. Smoking causes a more rapid progression of lung disease requiring significant increases in clinical intervention. Since a strong correlation has been found between levels of rheumatoid factor and smoking in males, it will be necessary for us to correlate levels of anti-T-cell receptor and gene usage with sex and smoking status in our patient population.

Publications:

Marchalonis JJ, Kaymaz H, Schluter SF, Yocum DE. Naturally Occurring Human Antibodies to Define T-Cell receptors and Light Chain Peptides. *Immunobiology of Proteins and Peptides VII.* (M. T. Atassi, ed.) Plenum Press, N.Y., pp 135-145; 1994.

Marchalonis JJ, Schluter SF, Wang E, Dehghanpisheh K, Lake D, *et. al.* Synthetic Autoantigens of Immunoglobulins and T-cell Receptors: Their Recognition in Aging, Infection, and Autoimmunity. Proc Soc Exp Biol 207:129-147; 1994.

Wang E, Lake D, Winfield JB, Marchalonis JJ. IgG Autoantibodies to "Switch Peptide" Determinants of Tcr α/β in Human Pregnancy. Clin Immunol & Immunopathol 73: 224-228;1994.

Lake DF, Bernstein RM, Schluter SF, Marchalonis JJ. A Method for Generating Diverse Single Chain Proteins Using a Universal $(Gly_4\text{-ser})_3$ Encoding Oligonucleotides. Biotechniques 19: 700-702;1995.

Schluter SF, Wang E, Winfield JB, Yocum DE, Marchalonis JJ. Autoregulation of Tcr V Region Epitomes in Autoimmune Disease. Adv Exp Med Biol 383: 231-236; 1995.

Marchalonis JJ, Lake DF, Schluter SF, Dehghanpisheh K, *et. al.* Autoantibodies Against Peptide Defined Epitomes of T-cell Receptors in Retrovirally Infected Humans and Mice. Adv Exp Med Biol 383: 211-222; 1995.

Lake DF, Lansperger WJ, Bernstein RM, Schluter SF, Marchalonis JJ. Characterization of Autoantibodies Directed Against T Cell Receptors. Adv Exp Med Bio 383: 223-229; 1995.

Landsperger WJ, Schluter SF, Garza A, Yocun DE, Marchalonis JJ. Fine Specificity Analysis of Autoantibodies to T-cell Receptor CDR1 Segments in Rheumatoid Arthritis. Annals N. Y. Academy of Science, In Press.

Posner, Ph.D., Richard G.

Northern Arizona University

Award Amount FY 1996: **$26,210**

Development of Strategies to Inhibit Allergic Responses

Aggregation of cell surface receptors is an important mechanism for initiating responses in cells of the immune system. It is well established that allergic responses are initiated when antibodies (IgE) that are bound to the surface of certain immune cells are cross-linked by the allergen (antigen) to which they are specific. Individuals who are allergic to a specific antigen produce IgE that specifically binds that antigen. When cell surface bound IgE is cross-linked by that antigen, a biochemical cascade is initiated that results in the release of histamine. We have developed an assay that directly measures how a multivalent antigen interacts with cell surface IgE. We are in the process of developing and characterizing a class of model compounds that will block the binding of multivalent antigen to the cell surface. These molecules have the potential to serve as potent inhibitors of specific IgE mediated allergic responses.

Akporiaye, Ph.D., Emmanuel

University of Arizona

Award Amount FY 1996: **$30,000**

Analysis of Tumor-Rejection Responses Using a Retrievable Matrix of Tumor Implantation

Using a gelatin sponge model of tumor implantation, we have characterized lymphocytes associated with progressing tumors (TILS) or with rejected tumors (TRLs). We have shown that the ineffectiveness of TILs to control tumor growth is likely due to local production of immuno-suppressive chemicals. The antitumor activity of TRLs makes them potentially useful in treating tumors following their transfer into tumor-bearing animals. In these studies we have enhanced the antitumor activity of these cells by *in vitro* stimulation and have identified the T cell receptor populations that may be crucial in the anti tumor response. Additionally, we have concluded preliminary cell labeling studies that would enable us to track these T lymphocytes following into tumor-bearing animals. The effectiveness of these cells in abrogating tumors *in vivo* will depend on their ability to specifically home to tumor after *in vivo* injection and to manifest potent tumor-killing ability. These findings form the basis for ongoing and future studies to understand lymphocyte homing mechanisms during tumor destruction.

Publications:

Tsang TC, Harris DT, Akoporiaye ET, Schluter SF, Bowden GT, Hersh EM. Simple Method for Adapting DNA Fragments and PCR Products to All of the Commonly Used Restriction Sites. Biotechniques 20:51-52; 1996.

Tsang TC, Harris DT, Akoporiaye ET, Chu RS, Brailey J, Liu F, Vasanwala FH, Schluter SF. A Mammalian Expression Vector with Two Multiple Cloning Sites for Expression of Two Foreign Genes Biotechniques, In Press.

Moore,* Ph.D., Ana L.

Arizona State University

Award Amount FY 1996: **$25,692**
($3,503)

Carotenofluorophores: Imaging Agents for Diagnosis of Neoplastic Disease

The goal of this investigation is to develop drugs that can be used as markers for early detection of malignancy. Chemicals that fluoresce (fluorophores) have been chosen as potential markers because fluorescence is one of the most sensitive analytical techniques available. Drugs that fluoresce frequently sensitize and damage healthy skin when exposed to sunlight, which is a serious problem particularly germane to Arizona residents. We have previously shown that linking certain derivatives of β-carotene to fluorophores prevents the skin sensitization. These new drugs are called carotenofluorophores. During this contract period three new carotenofluorophores have been synthesized and their photophysical properties determined. The fluorophores are two hematoporphyrin derivatives and a phthalocyanine derivative. The new drugs have structural modifications which should facilitate their elimination from the liver. The first generation of carotenofluorophores had the shortcoming of accumulation and retention in this organ.

As a result of the research carried out under this contract, funding from Mallinckrodt Medical has been secured. Our contract with Mallinckrodt covers the synthesis of new carotenofluorophores and *in vivo* testing. It is an important step forward in this project to systematically screen the new drugs by *in vivo* testing in order to characterize the tumor localization and fluorescence properties of these compounds in living tissue.

Publications:

Abstracts:
Moore AL, Gust D, Moore TA, Lidell PA, *et. al.* Carotenoids in Tumor Detection and Imaging. Photochem Photobiol 63: 97S; 1996.

Moore AL, Gust D, Moore TA, Lidell PA, *et. al.* Carotenoids in Tumor Detection and Imaging. Presented at the 11th International Symposium on Carotenoids, August 18-23, 1996, Leiden, The Netherlands.

Pettit,* Ph.D., George R.

Arizona State University
Award Amount FY 1996: **$241,875**
($187,640)

Discovery and Development of New Anticancer Drugs

As in previous years, the Arizona Disease Control Research Commission support for the Arizona State University Cancer Research Institute was primarily focused on further development of new anticancer drugs for treatment of human cancer. The potential and powerful antiangiogenesis anticancer drugs, combretastatin A-4 and pancratistatin continue to be under development in our Institute and elsewhere. Combretastatin A-4 prodrug syntheses have been expanded and pancratistatin supplies are being pursued from isolation by farming techniques through synthesis (from other natural products) and prodrug studies. Another thirteen promising anticancer drugs discovered at the ASU-CRI, *e.g.* , bryostatin 1; DABIS maleate; phyllanthoside; dolastatins 10 and 15; auristatins C, PE, PYE, and M; cephalostatins 1 and 7; halichondrin B and halistatin 1 are at various stages of preclinical and clinical development. Development of such new anticancer drugs from marine animals and plants, fungi, and microorganisms continues as the sharply focused objective of the ASU Cancer Research Institute.

Publications:

Pettit GR, Xu JP, Cichacz ZA, Williams MD, Chapuis JC. Antineoplastic Agents 323. Isolation and Structure of Phakellistatin 6 from a Chuuk Archipelago Marine Sponge. Bioorganic & Chemistry Letters 4(22): 2677-2682; 1994.

Pettit GR. Marine Animal and Terrestrial Plant Anticancer Constituents. Pure and Applied Chemistry 66: 2271-2281; 1994.

Pettit GR, Thorton TJ, Mullaney JT, Boyd MR, *et. al.* The Dolastatins; 20: A Convenient Synthetic Route to Dolastatin 15. Tetrahedron 50: 12097-12108; 1994.

Pettit GR, Srirangam JK, Herald DL, Hamel E. The Dolastatins; 21: Synthesis, X-ray Crystal Structure, and Molecular Modeling of (*6R*)-Isodolastatin 10. Journal of Organic Chemistry 59:6127-6130; 1994.

Pettit GR, Singh SB, Herald DL, Lloyd-Williams P, *et. al.* The Dolastatins; 17: Synthesis of Dolaproine and Related Diastereoisomers. J Organic Chem 59: 6287-6295; 1994.

Pettit GR, Xu J-P, Ichihara Y, Williams MD, Boyd MR. Antineoplastic Agents 285. Isolation and Structures of Cephalostatins 14 and 15. Can J of Chem 72: 2260-2267; 1994.

Pettit GR, Kantoci D, Herald D, Barkoczy J, Slack JA. Procedures for the Analysis of Dolastatins 10 and 15 by High Performance Liquid Chromatography. J Liquid Chromatography 17(1): 191-202; 1994.

Stanwell C, Gescher A, Bradshaw TD, Pettit GR. The Role of Protein Kinase C Isoenzymes in the Growth Inhibition Caused by Bryostatin 1 in Human A549 Lung and MCF-7 Breast Carcinoma Cells. International Journal of Cancer 56: 585-592; 1994.

Pettit GR, Xu J-P, Williams MD, Christie ND, Doubek DL, Schmidt JM. Isolation and Structure of Cephalostatins 10 and 11. Journal of Natural Products 57(1): 52-63; 1994.

Pettit GR, Delbert RT, Herald DL, Cerny RL, Williams MD. Antineoplastic Agents; 277: Isolation and Structure of Phakellistatin 3 and Isophakellistatin 3 from a Republic of Cosmos Marine Sponge. The Journal of Organic Chemistry 59(7): 1593-1595; 1994.

Pettit GR, Singh SB, Srirangam, JK, Pierson FH-P, Williams MD. The Dolastatins; 19: Synthesis of Dolaisoleuine. The Journal of Organic Chemistry 59(7): 1796-1800; 1994.

Pettit GR, Gao F, Cerny RL, Doubek DL, Tackett LP, Schmidt JM, Chapuis JL. Antineoplastic Agents; 278: Isolation and Structure of Axinastatins 2 and 3 from a Western Caroline Island Marine Sponge. Adv ACS Abstracts 94: 1837-1165; 1994.

Pettit GR, Barkoczy J, Srirangam JK, Singh SB, *et. al.* The Dolastatins; 22: Synthesis of Boc-dolaproinyl-dolaphenine and Four Related Chiral Isomers. The Journal of Organic Chemistry, 59(11):2935-2938; 1994.

Pettit GR, Pettit GR, III, Groszek G, Backhaus RA, *et. al.* Antineoplastic Agents; 301: An Investigation of the *Amayllidaceae* Genus *Hymenocallis.* J Natural Products 58(5): 756-759; 1995.

Pettit GR, Buter MS, Bass CG, Boubeck DL, *et. al.* Antineoplastic Agents; 326: The Stereochemistry of Bastadins 8, 10, and 12 from the Bismarck Archipelago Marine Sponge. J Natural Products 58(5): 680-688; 1995.

Pettit GR, Holman JW, Boland GM. Synthesis of the Cyclic Heptapeptides Axinastatin 2 and Axinastatin 3. J Chem Soc; 1996.

Pettit GR, Hoard MS, Doubek DL, Schmidt JM, *et. al.* Antineoplastic Agents; 338: The Cancer Cell Growth Inhibitory Constituents of *Terminalia Arjuna* (Compretaceae). J Ethnopharmacol; 1996.

Zhang X, Zhang R, Zhao H, Cai H, *et. al.* Preclinical Pharmacology of the Natural Product Anticancer Agent Brystatin 1, and Activator of Protein Kinase C. Cancer Research 56: 802-808; 1996.

Szallasi Z, Du L, Levine R, Lewin NE, *et. al.* The Bryostatins Inhibit Growth of B16/F10 Melanoma Cells *In Vitro* through a Protein Kinase C-independent Mechanism: Dissociation of Activities Using 26-Epi-Bryostatin 1. Cancer Research 56: 2105-2111; 1996.

Pettit GR, Gao F, Blumberg PM, Herald CL, *et. al.* Antineoplastic Agents; 340: Isolation and Structural Elucidation of Bryostatins 16-18. J Natural Products 59: 286-289; 1996.

Kamano Y, Zhang H, Morita H, Itokawa H, *et. al.* Conformational Analysis of a Marine Antineoplastic Macrolide, Bryostatin 10. Tetrachedron 52(7): 2369-2376; 1996.

Kraft AS, Woodley S, Pettit, GR, Gao, F, Coll JC, Wagner F. Comparison of the Antitumor Activity of Bryostatins 1,5, and 8. Cancer Chemotherapy Pharmacology 37: 271-278; 1996.

Kamano Y, Zhang, H-P, Hino A, Yoshida M, *et. al.* An Improved Source of Bryostatin 10, *Bugula Neritina* From the Gulf of Aomori, Japan. J Natural Products 58(12): 1868-1875; 1995.

Pettit GR, Xu J-P, Dorsaz A-C, Williams MD, *et. al.* Isolation and Structure of the Human Cancer Cell Growth Inhibitory Cyclic Decapeptides Phakellistatins 7,8, and 9. Bioorganic Med Chem Letters 5(13): 1339-1344; 1995.

Pettit GR, Srirangam JK, Herald DL, Xu J-P, *et. al.* Isolation and Crystal Structure of Stylopeptide 1: A New Marine Porifer Cycloheptapeptide. J Organic Chem 60: 8257; 1995.

Chaplin DJ, Pettit GR, Parkins CS, Hill SA. Antivascular Approaches to Solid Tumor Therapy: Evaluation of Tublin Binding Agents. Brit J Cancer 74(XXVII): S86-S88; 1996.

Pettit GR, Temple C, Jr, Narayanan VL, Varma R, *et. al.* Antineoplastic Agents; 322: Synthesis of Combretastatin A-4 Prodrugs. Anti-Cancer Drug Design 10: 299-309; 1995.

Pettit GR, Xu J-P, Schmidt JM, Boyd MR. Isolation and Structure of the Exceptional Pterobranchia Human Cancer Inhibitors Cephalostatins 16 and 17. Bioorganic Chem Letters 5(17): 2027-2032; 1995.

Pettit GR, Ichihara Y, Wurzel G, Williams, MD, Schmidt JM, Chapuis J-C. Isolation and Structure of Halistatin 3 from the Western Pacific (Chuuk) Marine Sponge Phakellia sp. J Chem Soc: 383-385; 1995.

Pettit GR, Tan R, Ichhara Y, Williams MD, *et. al.* Antineoplastic Agents; 325: Isolation and Structure of the Human Cancer Cell Growth Inhibitory Cyclis Octapeptides Phakellistatin 10 and 11 from Phakellia sp. J Natural Products 58(6):961-965; 1995.

Pettit GR, Srirangam JK, Barkoczy J, Williams MD, *et. al.* Antineoplastic Agents 337. Synthesis of Dolastatin 10 Structural Modifications. Anti-Cancer Drug Design 10: 529-544; 1995.

Pettit GR, Taylor, SR. Synthesis of the Marine Sponge Cycloheptapeptide Stylopeptide1. J Organic Chem 61: 2322-2325; 1996.

Pettit GR, Orr B, Herald DL, Doubeck DL, *et. al.* Isolation and X-ray Crystal Structure of Racemis Xestospongin D from the Singapore Marine Sponge Niphates sp. Bioorganic Med Chem Letters 6(12): 1313-1318; 1996.

Aherene GW, Hardcastle A, Valenti M, Bryant A, *et. al.* Antitumor Evaluation of Dolastatins 10 and 15 and Their Measurement in Plasma by Radioimmunoassay. Cancer Chemotherapy Pharmacology 38: 225-232; 1996.

Pettit GR, Burkett DD, Williams MD. Dolastatins 23: Stereospecific Synthesis of Dolaisoleuine. J Chemistry Soc: 853-858; 1996.

Pettit GR, Srirangam JK, Singh SB, Williams MD, *et. al.* Dolastatins 24: Synthesis of (-)-Dolastatin 10: X-ray Molecular Structure of N,N-Dimethylvaly-Valyl-Dolaisoleuine Tert-Butyl Ester. J Chem Soc: 859-863; 1996.

Pettit GR, Burkett DD, Barkoczy J, Breneman GL, Pettit WE. The Dolastatins; 18: Stereospecific Synthesis of Dolapronine. Synthesis 6: 719-726; 1996.

Powis,* D.Phil., Garth

University of Arizona
Award Amount FY 1996: **$598,982**
($81,679)

Arizona Cancer Center Multidisciplinary Research Program

Work at the Arizona Cancer Center has led to significant advances in both understanding the mechanisms of cancer causation and response to cancer preventive and therapeutic drugs. The frequency of specific gene mutations in animal models of carcinogen-induced colon cancer have been investigated showing that the models are appropriate for studies of early but not late carcinogenesis in humans. This is important for studies of agents to inhibit colon cancer. Several such agents are being studied, including some that prevent the development of cancer by causing the early cancer cell to undergo programmed cell death. Studies are underway to identify and isolate the gene responsible for resistance of cancer cells to the anticancer drug mitoxantrone, and to study the mechanism of action of a new drug that can reverse this resistance. Preclinical and animal model studies of approaches to gene therapy of cancer have been conducted. Several clinical studies developed from the basic studies are underway or have been completed. Phase I and II clinical studies to investigate high dose chemotherapy as a means of overcoming cancer drug resistance are underway. Phase I and II trials involving the intratumoral injection of HLA-B7 and IL-2 genes in patients with various solid tumors have been conducted.

Publications:
(Please Note: This is a program-project contract. The name of the Principal Investigator does not appear as an author on all publications relating to the contract.)

Federico M, Alberts D, Garcia DJ, Emerson J, Fanta P, Liu R, Salmon S. *In Vitro* Drug Testing of Ovarian Cancer Using the Human Tumor Colony-Forming Assay: Comparison of *In Vitro* Response and Clinical Outcome. Gynecologic Oncology 55: S156-S163; 1994.

Glasser L, Dalton WS, Fiederlein RL, Cook P, Powis G, Vogler WR. Response of Human Multiple Myeloma-Derived Cell Lines to Alkyl-lysophospholipid. Experimental Hematology 24:253-257; 1996.

Gallegos A, Gasdaska JR, Taylor CW, Paine-Murrieta GD, *et. al.* Transfection with Human Thioredoxin Increases Cell Proliferation and a Dominant-negative Mutant Thioredoxin Reverses the Transformed Phenotype of Human Breast Cancer Cells. Cancer 56: 5765-5770; 1996.

Scheck,* Ph.D., Adrienne C.

St. Joseph's Hospital, Phoenix
Award Amount FY 1996: **$21,148**
($2,884)

Identification of Genes Associated with BCNU/Resistance in Human Malignant Gliomas

Approximately 15,600 Americans will be diagnosed with malignant brain tumors this year, with an estimated 300 cases from Arizona. This figure is likely to worsen, as the incidence of gliomas (the most common form of brain tumor) in people over the age of 65 is increasing. Currently available therapies including surgery, radiation and chemotherapy kill 90% of tumor cells, but the cells that survive regrow, rapidly resulting in patient mortality. Improved treatment requires the design of new therapies based on a better understanding of factors that contribute to therapy resistance. Our work is designed to understand the basis for resistance to the most commonly used chemotherapeutic agent in brain tumors–1, 3-bis(2-chloroethyl)-1-nitrosourea (BCNU or carmustine). We have isolated a number of genes that may be involved in previously unidentified mechanisms of chemotherapy resistance. Our future work will identify these genes and confirm their role in therapy resistance.

Weinert, Ph.D., Ted A.

University of Arizona
Award Amount FY 1996: **$26,396**

Checkpoint Genes and Genomic Instabilities

We study genes involved in cell cycle controls called checkpoints that block eucaryotic cell division after DNA damage. All normal cells stop cell division when their chromosomes are damaged, and damaged cells then repair their chromosomes before cells resume dividing. Cancer cells are notably defective for checkpoints; they continue to divide even when damaged. As a consequence of cell division with damaged chromosomes, the damaged cells end up with abnormal chromosomes and a phenomenon termed "genomic instability." This leads to rearrangements of chromosomes, often times activating key cancer genes that lead directly to cancer.

Checkpoint genes are relevant to cancer because when defective they cause genomic instability. The exact forms and mechanisms of instability are poorly understood. Our continuing goal discussed in this summary is to unravel the mechanisms of genomic instability, using the highly experimentally tractable yeast cell as a model. A basic understanding of mechanisms of genomic instability may lead to useful insights into how cancer evolves and how to better diagnose its onset.

We have now partially characterized genomic instability in yeast cells and find an association between checkpoint mutations, chromosome loss and mitotic recombination. More importantly, we have identified and characterized an instance of genome instability (we call "unstable 7") useful in understanding how genomic instability can arise in checkpoint mutants. This form of genomic instability is probably related to a breakage-fusion-bridge cycle, one mechanism by which cancer cells may undergo genome rearrangements.

Wilson, Ph.D., Jean M.

University of Arizona

Award Amount FY 1996: **$26,151**

Membrane Dynamics in Colon Carcinoma Cells

There are two problems in the area of the chemotherapy of cancer: the presence of multidrug resistance and the inefficient killing by immunotoxins. We are studying how molecules move through the cells to discern how to prevent cancer cells from avoiding their toxic effects. To accomplish these goals we have studied a novel protein that is present in the intracellular membranes of intestinal cells. We have determined that this protein is synthesized as a large molecule that is processed and lives for several hours before degradation. These results may help to evaluate how quickly an immunotoxin conjugate will be delivered to the lysosomal compartment after binding to the cell surface. We also found that very little of this protein is present on the cell surface, suggesting that cycling between the endotubin-containing compartment and the cell surface is minimal. Therefore, accumulation of chemotherapeutic drugs in this compartment would not necessarily allow the cell to extrude them into the extracellular fluids.

Heidenreich, M.D., Randall A. **University of Arizona**

Award Amount FY 1996: **$29,931**

Galactose-1-Phosphate Uridyltransferase Gene Regulation

Galactosemia is a genetic disorder in which affected individuals have lost the ability to breakdown the dietary sugar, galactose. The most common cause of galactosemia is due to deficient activity of galactose-a-phosphate uridyltransferase (GALT). Since galactose is a major sugar in milk, accumulation of blood galactose begins soon after infants begin to feed. This galactose is toxic and infants become ill within the first week of life. If not diagnosed properly, they may die or be left with permanent neurologic damage. With prompt diagnosis and prescription of a galactose-free formula, infants recover rapidly from their acute illness and stay well as long as they adhere to a galactose-restricted diet.

However, despite these efforts, chronic complications of galactosemia still occur. These include ovarian failure in females, verbal dyspraxia (difficult speaking) in about half of patients, tremor and ataxia (difficult walking) in some patients. These particular problems were unexpected by physicians. Our goal in this project was to understand the basis for these complications and believe an answer lies in the regulation of the GALT gene. To achieve this goal we investigated regulation of expression of the rat GALT gene.

Johnson, M.D., Mary I.

University of Arizona

Award Amount FY 1996: **$19,543**

Anticonvulsant (Phenobarbital) Effects on the Developing Nervous System: An *In Vitro* Model

Phenobarbital may adversely affect a number of steps in central nervous system development, including early formation of the nervous system, nerve cell proliferation and migration, as well as the initiation of nerve processes and the establishment of contacts on target cells. That therapeutic or even subtherapeutic levels of phenobarbital may have unacceptable deleterious effects on neuronal differentiation is debated. Phenobarbital, however, is the most commonly used anticonvulsant in newborn and young infants during a time when there is still considerable brain development occurring. In these studies we use a tissue culture model of neuronal development to test for the adverse effects of phenobarbital and eventually to study the mechanisms by which such effects might occur. Nerve cells can be treated with increasing concentrations of phenobarbital during a critical time period of normal process development induced by either co-culture with normal companion (glial) cells or by a purified growth factor.

Publications:

Loegering MD, Johnson MI. Phenobarbital Inhibits Dendritic Development in Cultured Sympathetic Neurons. Ann Neurol 36: 494-495; 1994.

Loegering MD, Rueger D, Johnson MI. Phenobarbital Inhibits dendritic development Induced by Osteogenic Protein-1 (OP-1) in Culture of Sympathetic Neurons. Ann Neurol 38: 507; 1995.

Kappen, Ph.D., Claudia

Mayo Clinic, Scottsdale

Award Amount FY 1996: **$26,392**

Analysis of Transgenic Mice Expressing the Precursor Protein of Alzheimer's Disease B-Amyloid

The goal of our studies is to investigate in which way the protein that is deposited in Alzheimer's patients' brains affects the survival of brain cells. To this end, we are using mice as a model system. We are genetically manipulating these mice so that they express the human protein in their nervous system. This allows us to analyze how cells in the intact animal respond to this protein. In particular, we wish to determine whether the protein causes neuronal cell death or decreased survival of cells in the brain or in specific regions of the brain. If the mice show pathologies similar to those found in human patients, they would represent an excellent model system for the development of drugs that may slow down cellular degeneration in the brain of Alzheimer's disease patients.

Kurth, M.D., Ph.D., Janice H.

St. Joseph's Hospital, Phoenix

Award Amount FY 1996: **$21,967**

Genomic Localization of the Cavernous Malformation Gene

Cavernous malformations (CM) are abnormalities of the blood vessels, often presenting with headaches, seizures, strokes and/or progressive abnormalities of the nervous system. The defect causing familial CM has not been identified. Our research goal is to localize the gene causing CM within human DNA (the genetic material).

Blood samples from 64 individuals within 7 CM families from Arizona have been collected, and DNA isolated. These samples were used to localize the abnormality causing CM to a small area within DNA. These results have been published and presented at international meetings. Our future work will involve collection of additional family members and ascertainment of new families to decrease the interval of abnormality for identification of the specific defect.

Most CM families are Hispanic, many from Arizona. Localization of the CM defect will make genetic counseling and early diagnosis possible, lead to identification of the gene, will increase understanding of the pathology and provide new knowledge about blood vessel development, which may allow therapeutic intervention and/or correction of the genetic defect.

Publications:

Dubovsky J, Zabramski JM, Kurth JH, Rich SS, *et. al.* A Gene Responsible for Cavernous Angiomas of the Brain Maps to Chromosome 7q. Hum Molec Genet 4:453-358; 1994.

Johnson EW, Green ED, Rich SS, Orr HT, Gil-Nagel A, Kurth JH, *et. al.* Refined Localization of the Cerebral Cavernous Malformation Gene (CCM1) to a 4-cM Interval of Chromosome 7q Contained in a Well-defined YAC Contig. Genome Research 5: 368-380; 1995.

Abstracts:

Kurth JH, Dubovsky J, Zambramski JM, Weber JL. Genetic Linkage of the Familial Cavernous Malformation (CM) Gene to Chromosome 7q. Am J Genet (Suppl) 55: A15; 1994.

Zambramski JM, Kurth JH, Dubovsky J, Weber JL, Spetzler RF. Familial Cavernous Malformations: Results of Human Chromosome Linkage Analysis in Two Large Arizona Families. Proceedings of the Congress of Neurological Surgeons, Chicago IL, October; 1994.

Johnson EW, Green ED, Rich SS, Orr HT, Gil-Nagel A, Kurth JH, *et. al.* A Gene for Familial Cerebral Cavernous Malformations Maps to 4 cM Region of Chromosome 7q in Several affected Families. Am J Hum Genet 57: A194; 1995.

Bloom,* M.D., John W.

University of Arizona
Award Amount FY 1996: **$25,649**
($3,498)

Development of a Recombinant Glucocorticoid Receptor with Constitutive Activity-Potential Therapy for Asthma

Asthma is the most important cause of chronic illness in children and affects 5% of adults. This illness is a particularly serious problem in Arizona. The prevalence rate for asthma in Arizona is twice the national average, and the death rate is 1.5 times the national rate. Glucocorticoids (*e.g.*, cortisone, prednisone, "steroids") are the most effective medications for asthma but may have serious adverse side effects. The effects of glucocorticoid medications such as cortisone are produced through proteins termed glucocorticoid receptors that are found in all cells in the body. Our research goal is to develop an effective treatment for asthma that is devoid of serious side effects. We have developed glucocorticoid receptors that are activated in the absence of cortisone as therapy for asthma. This therapy should have all of the beneficial effects of cortisone but avoid the harmful side effects.

Publications:

Behr FD,LeVan TD, Adkins KK, Miesfeld RL, Bloom JW. Cross-signaling Between the Glucocorticoid Receptor and the AP-1 and NfkB Pathways in Human Bronchial Epithelial Cells. Am J Resp Crit Care Med 153: A227; 1996.

LeVan TD, Behr FD, Adkins KK, Miesfeld RL, Bloom JW. Amino-terminal Deletions in the Glucocorticoid receptor (GR) Uncouple Transrepression of NF-kB Signaling from GR-mediated Transcriptional Activation. Am J resp Crit Care Med 153: A228; 1996.

Rider,* M.D., Evelyn D.

University of Arizona
Award Amount FY 1996: **$24,050**
($3,280)

Pulmonary Surfactant Degradation in Newborn Rabbit Lung Lysosomes *In Vivo*

In the previous funding period, lysosome isolation techniques developed for adult rabbit lungs were adapted for 3 day- and 3 week-old rabbits. Biochemical characterization of lung lysosome preparations from 3 day- 3 week- and 6 week-old rabbits were completed. Significant differences in peak lysosome enzyme specific activities between the 6 week adult rabbits and the 3 day- and 3 week-old animals were demonstrated. Peak lysosomal enzyme activity in the younger rabbits was 50% lower than in the 6 week rabbits. The 3 day-and 3 week-old rabbits had peak lysosomal activity measured at lighter densities compared to the 6 week-old group. This may be significant as data in the literature indicate that more immature lysosomes isolate at lighter densities than more mature lysosomes. Morphologic characterization of these fractions by electron microscopy are in progress. Experiments during the year include studies designed to track radioactivity labeled surfactant phospholipids in lung lysosome preparations from 3 day-old and 3 week-old rabbits. The next series of studies will help define the role of lung lysosomes in surfactant degradation in developing lungs.

Section C

Continuing Contracts

Tobacco Related Research

Year One

Heimark, Ph.D., Ronald L.

University of Arizona

Award Amount FY 1996: **$29,707**

Smoking and Pericytes: Their Roles in Angiogenesis

Microvessels are formed by endothelial cells which line all blood vessels, surround the vessels and control, blood flow. Angiogenesis which is the process of formation of blood vessels from existing vessels is fundamental to wound healing and is impaired by products of cigarette smoking. Nicotine released during smoking constricts blood flow and this is probably from effects on pericytes. Angiogenesis also plays a pivotal role in tumor growth and metastasis. Lung tumors, for example, can exist as small groups of cells until they are activated to recruit blood vessels and then are able to grow. The increase in the number of blood vessels in tumors correlates with increased probability of metastasis as tumor cells detach from the mass and enter the vasculature to move to another site. Very little is known concerning the molecular basis of the functions of pericytes in angiogenesis and blood flow. Pericytes are the focus of this proposal and characterized by the composition of their contractile apparatus to examine regulation by nicotine. We have established a cell culture model of angiogenesis to characterize the role of pericytes in regulation of angiogenesis. This is the first model that maintains the *in vivo* cellular organization of blood vessels.

Jacobs, Ph.D., Bertram L.

Arizona State University

Award Amount FY 1996: **$30,000**

Regulation of Programmed Cell Death in Human Cancer Cells

Our work has been involved in characterizing how a variant virus (VV) can induce human HeLa cancer cells to commit suicide. Since HeLa cells, like most cancer cells, have mutations that prevent induction of suicide by standard chemotherapeutic agents, we think this virus may be able to overcome one of the main blocks to ridding the body of cancer. The variant of VV that induces suicide in cancer cells has been engineered to remove a gene: the E3l gene. Thus, this gene appears to prevent VV from inducing cancer cells to commit suicide. We have now inserted several well characterized mutants of the E3L gene back into VV to determine how this gene functions in preventing VV from inducing cancer cells to commit suicide. Our results suggest that it functions by inhibiting the well characterized interferon system. These results reinforce the role of this system in fighting cancer.

Publications:

Jacobs B, Langland JO. When Two Strands are Better Than One: The Mediators and Modulators of the Cellular Response to Double-Stranded RNA. Virology 219:339-349; 1996.

Abstracts:

Kibler KV, Langland JO, Shors T, Mallley G, Jacobs BL. Introduction of apoptosis in HeLa Cells Infected with variants of Vaccinia Virus. Presented at the Annual Meeting of the American Society for Virology, Austin, Texas; 1995.

Kibler KV, Langland JO, Shors T, Zeman CC, Jacobs BL. A double-stranded RNA-binding protein Is Necessary to Inhibit Induction of Apoptosis and for Interferon-resistance in Vaccinia Virus Infected Cells. Presented at the Xth International Congress of Virology, Jerusalem, Israel.

Larkey, Ph.D., Linda

University of Arizona

Award Amount FY 1996: **$29,300**

Curbing the Trend of Tobacco Related Cancer Deaths: Identifying Factors Influencing Late Presentation of Symptomatic Hispanics

Although smoking cessation and proper diet are key to preventing cancer, the second line of defense is regular visits to the doctor. This line of defense is weakened among Hispanics in Arizona by a pattern of postponing visits to the doctor when symptoms appear (also termed, "late presentation") leading to late detection of treatable cancers. In the first year of the study, we have identified ten issues that affect Hispanics' decision to visit the doctor when facing symptoms: other resources/remedies, trust in caretaker, scheduling/finding time, insurance/money, denial/emotions about symptoms, faith/God's will, talking with others, embarrassment or discomfort in going to the doctor, desire for a certain type/characteristics of doctor, severity of symptoms. Using focus groups and culturally-sensitive qualitative methodology, these issues have been identified and describe important factors to be measured and quantitatively assessed in the second year. Once the strengths of contributing factors are assessed, prevention campaigns may be designed to address these and provide a more culturally appealing approach for Hispanics in Arizona.

Nelson, Ph.D., Mark A.

University of Arizona

Award Amount FY 1996: **$29,361**

Functional and Clinical Evaluation of the p-16 Protein in Melanoma and Human Lung Cancers

This project involves evaluating the frequency of p-16 gene alteration in human lung cancer and melanoma specimens and correlation of these data to clinical variables. We have developed PCR-based methods for analysis of p-16 gene alterations and applied these techniques to archival lung cancer and melanoma specimens. We are also evaluating if straining of the p-16 protein product is a surrogate for determining p-16 gene inactivation in melanoma and lung cancer cases.

The p-16 gene function is also being studied. We have characterized the p-16 protein in melanoma and lung cancer cell lines and determined that the p-16 protein is often absent or has altered mobility. These observations will be correlated with experiments designed to evaluate p-16 gene alterations in the same cell lines. In other studies, gene transfer techniques are utilized to replace the mutant p-16 with a normal p-16 gene.

Publications:

Nelson MA, Wymer J, Clements N Jr. Detection of K-ras Gene Mutations in Non-neoplastic Lung Tissue and Lung Cancers. Cancer Letters 103: 115-121; 1996.

Payne, Ph.D., Claire M. — **University of Arizona**

Award Amount FY 1996: **$30,000**

Evaluation of a Novel Biomarker for Individuals at Risk for Colon Cancer: Resistance to Apoptosis

The results that we have obtained so far indicate that the resistance to apoptosis bioassay is able to identify some patients that may be at risk for colon cancer. We have obtained biopsies from a total of 55 human subjects undergoing colonoscopy for medical reasons, of which 152 biopsies from 36 patients have processed and quantitated for resistance to apoptosis. All normal patients and patients with low risk tubular adenomas showed a high induction of apoptosis using the bile salt, sodium deoxycholate. Of 14 patients with previous colon cancer, 71% showed a low induction of apoptosis. Quality control studies were also performed to determine site-to-site variability within the colon of individual patients in different risk groups. Duplicate biopsies were obtained from the cecum, the descending colon and the sigmoid colon. Inter-observer variability studies on apoptosis quantitation indicated that the bioassay is most reliable (r=0.86).

Yamamura, Ph.D., Henry I. — **University of Arizona**

Award Amount FY 1996: **$29,700**

Tobacco, Cancer Pain and Opioids: Determination of the Ligand Binding Domains of the Human Delta Opioid Receptor

Opiates such as morphine have been used to manage the pain of tobacco induced cancers in the state of Arizona. The effects of opioid drugs are mediated through specific opioid receptors called mu, delta and kappa receptors. Drugs acting through the delta opioid receptors produce analgesia with minimal side effects. A basis for rational drug design is the biochemical characterization of the human delta opioid receptor. The tools of molecular biology have enabled us to create cell lines expressing the human opioid receptors, to modify the structure of the receptor protein and to study its effects on receptor function. The third extracellular loop of the human delta opioid receptor determines the selectivity of drugs acting at this receptor.

Publications:

Li X, Varga EV, Stropova D, Zalewska T, Malatynska E, Knapp RJ, Roeske WR, Yamamura HI. Delta Opioid receptor: The Third Extrcellular Loop Determines Naltrinole Selectivity. Europ J Pharmacol 300: R1-R2; 1996.

Drumm, Ph.D., Denise A. — **St. Joseph's Hospital, Phoenix**

Award Amount FY 1996: **$24,793**

Glycemia, Subarachnoid Hemorrhage, Neurobehavioral Outcome in Smokers, Passive Smokers and Nonsmokers

Project progress has been favorable following a late start due to SJHMC administrative problems and technological difficulties at this Institution. Both have been remedied. The project is progressing as planned--it was designed for long-term tracking of patients over two years. Since this is the first year, data are not available for analysis. Repeated observations of test performance suggest: (1) Individuals continue to exhibit cognitive deficits despite designations of good neurological outcome; (2) Few individuals return to work by 3- and 6-month assessments; (3) Individuals report that they are still in need of rehabilitation at 6 months; (4) Caregivers and patients are not always aware of existing cognitive impairments setting up possible serious consequences, e.g., difficulty with attention, directionality and route finding; yet feel they are ready to drive. It is too early to analyze these scant data; however if these findings are substantiated at the end of the study, they will impact Arizona residents.

Fernandez, Ph.D., Maria Luz — **University of Arizona**

Award Amount FY 1996: **$29,700**

Cardiovascular Disease Risk Reduction by Dietary Fiber

These studies were conducted to determine the mechanisms by which pectin, guar gum and psyllium, three sources of soluble fiber, decrease plasma LDL, the atherogenic lipoprotein. Guinea pigs were used as the animal model due to their lipoprotein profile and responses to dietary fiber which are similar to humans. Animals fed pectin, guar gum and psyllium-based diets had lower plasma LDL cholesterol concentrations compared to control animals fed cellulose, a source of insoluble fiber. Soluble fiber intake reduced the secretion of apo B VLDL, the precursor of LDL in plasma, increased direct removal of VLDL and decreased conversion rates to LDL in the plasma compartment. In addition, soluble fiber reduced plasma LCAT and CETP activities indicating alterations in the intravascular processing of lipoproteins. All these effects of soluble fiber on lipoprotein metabolism contributed to the reduced levels of plasma cholesterol. These studies are important for the American population and the residents of Arizona for they address specific alterations in lipoprotein metabolism induced by dietary soluble fiber which reduce plasma LDL cholesterol concentration and the associated risk for cardiovascular disease.

Publications:

Fernandez ML, Vergara-Jimenez M, Conde K, Behr T, Abdel-Fattah G. Regulation of VLDL-LDL apo B Metabolism by Dietary Fiber: Specific Effects of Pectin, Guar Gum and Psyllium. FASEB J 10: A 1418; 1996.

Gamble, Ph.D., Debra — **University of Arizona**

Taken over by: **Kay**, Ph.D., Marguerite M.B. — *Award Amount FY 1996:* **$30,000**

Aging, Free Radicals and Nutritional Interventions

The purpose of this proposal is to investigate the role of free radicals, such as those generated by cigarette smoke in the aging of brain band 3, a major anion transporter. Antioxidants may play an important role in preventing free radical damage associated with aging by interfering directly in the generation of radicals and/or by scavenging them. We found that vitamin E but not β-carotene delayed/prevented oxidative damage to brain and lymphocytes during aging. Since increased aged band 3 and decreased anion transport are initial steps in band 3 aging that culminate in the generation of SCA and cellular removal, vitamin E prevents and/or delays aging of band 3 related proteins in lymphocytes and brain.

Publications:

Kay MMB, Lake D, Cover C. Band 3 and Its Peptides During Aging, Radiation Exposure, and Alzheimer's Disease: Alterations and Self-recognition. Adv Exp Med Biol 383: 167-193;1995.

Poulin J, Cover C, Guftafson M, Kay MMB. Vitamin E Prevents Oxidative Modifications of Band 3 related Proteins and Subsequent generation of Senescent Cell Antigen During Aging. Proc Natl Acad Sci 93: 5600-5602; 1996.

Kay MMB, Cover C, Goodman J. Posttranslational Modifications of Brain and Erythrocyte Band 3 During Aging and Disease. Cell Mol Biol, In Press.

Kay MMB. Aging and Immunity. *The Thymus* (J. March and M. Kendall, eds.) CRC Press, In Press.

Massia, Ph.D., Stephen P. — **University of Arizona**

Award Amount FY 1996: **$27,995**

Underlying Mechanisms for Tenascin-Stimulated Smooth Muscle Cell Migration in Response to Vascular Injury

During the past year, this research has verified that the extracellular matrix (ECM) protein tenascin plays a functional role in stimulating vascular smooth muscle cell (SMC) migration following tissue injury. This migratory response to injury is the driving force behind why blocked coronary arteries which are opened by balloon angioplasty treatments reclose. SMCs migrate from inside the artery to form scar tissue on the inner surface of arteries which leads to reclosure after angioplasty (restenosis). Our research has looked at how tenascin, a protein that is produced in artery tissue in response to injury, facilitates SMC migration and restenosis. We have determined how SMCs recognize tenascin and have shown that this recognition process stimulates migration. Such findings could lead to improved methods, during or following angioplasty treatments.

Morkin, M.D., Eugene

University of Arizona
Award Amount FY 1996: **$91,785**

Actions of Diiodothyropropionic Acid in Heart Failure: Pharmacology and Cardiac Biochemistry

The major objective of this study is to investigate the actions of diiodothyropropionic acid (DITPA), a thyroid hormone analogue, in the treatment of heart failure. Despite advances in the prevention and treatment of heart disease, heart failure remains a major public health problem. In fact, it is the only form of heart disease that seems to be increasing. American Heart Association data suggests that there are 3 to 5 new cases per 100 persons per year with an overall incidence of 10 per 1,000. Development of betters drugs for the management of this condition is essential. Experimental studies indicate that DITPA improves the performance of the heart after infarction, but the mechanism for this action is largely unknown. In this multidisciplinary program, investigators in *Section 1* are studying the time that the drug spends in the body after intravenous or oral administration. Such information is important to determine correct dosage and dosing intervals. In addition, they plan to distinguish the effects of DITPA on the peripheral circulation from the direct actions of DITPA on the heart by studies to be carried out in isolated cardiac muscle preparations. Investigators in *Section 2* are assisting in the analysis of levels of the drug and its breakdown products in the blood and tissues. Also, they are studying the actions of DITPA on intracellular calcium transport in the failing heart. Calcium uptake and release by intracellular components is often depressed in heart failure, and they hope these studies will determine whether these changes can be reversed by treatment with the drug.

Publications:

Mahaffey KW, Raya TE, Pennock GD, Morkin E, Goldman S. Left Ventricular Performance and Remodeling in Rabbits after Myocardial Infarction: Effects of a Thyroid Hormone Analogue. Circulation 91(3): 794-801; 1995.

Morkin E, Pennock GD, Raya TE, Bahl JJ, Goldman S. Development of a Thyroid Hormone Analogue for the Treatment of Congestive Heart Failure. Thyroid 6(5): 521-526; 1996.

Rogulski, Ph.D., Michel M.

University of Arizona
Award Amount FY 1996: **$29,954**

Interactive Three-Dimensional Display for Evaluation of Coronary Artery Disease

Coronary artery disease is the leading cause of death in the United States. The elderly population, a important part of the population of the state of Arizona, is particularly at risk. This project is focused towards improving diagnosis of early detection of coronary artery disease to provide more appropriate, prompter and more cost-effective patient management.

To do so, we are developing interactive three-dimensional rendering displays of three-dimensional radioisotope myocardial perfusion images, a usually difficult type of image-display, to comprehend and interpret. This interactive display is based on standard desktop personal computer technology and will be tested for accuracy.

During first year of this contract most of the software has been written, the rest is scheduled for the second year and test cases are being developed. Early pilot work has been completed and the main part of the project is nearly ready to be implemented.

Slepian, M.D., Marvin J.

University of Arizona

Award Amount FY 1996: **$29,645**

The Role of Beta-3 Integrin Expression in Arterial Smooth Muscle Cell Migration Following Injury

The migration of smooth muscle cells (SMCs), the constituent cells of the arterial wall, into the lumen of the artery leading to arterial narrowing, is a vital mechanism in the development of atherosclerosis. It has recently been recognized that cell-extracellular matrix interactions, mediated through specialized cell surface adhesion receptors known as "integrins" are vital in cell migration.

We previously demonstrated that blockade of SMC β3 integrin-matrix interactions resulted in reduced SMC migration. The present proposal is designed to identify mechanisms responsible for the predominant role of β3 integrins in SMC migration. Over the past year we determined that rat aortic SMCs express detectable levels of β1 and β3 integrins on their surfaces. Further we have shown that migrating SMCs preferentially express β3 integrins on their leading migratory edge. These findings support our working hypothesis that β3 integrins are preferentially expressed in migrating cells, acting as the "sticky feet" needed for forward locomotion. Defining the role of integrins in SMC migration will open the door for Arizona citizens to new therapies which limit SMC migration and therefore interfere with the development and progression of atherosclerosis.

Publications:

Slepian MJ, Massia SP. Antagonism of Beta-3 integrin-Matrix Interactions limits Smooth Muscle Migration *In Vitro* and *In Vivo*. Circulation 94(8) Suppl 1: 1-42; 1996.

Stopeck, M.D., Alison

University of Arizona

Award Amount FY 1996: **$28,424**

Genetically-Modified Endothelial Cells in Vascular Biology

Atherosclerosis and its complications, including coronary artery disease, myocardial infarctions (heart attacks), peripheral vascular disease (hardening of the arteries), and strokes are the leading cause of death in Arizona. The underlying disease process involves abnormal or increased growth of smooth muscle cells, the major cell type in blood vessels. Endothelial cells line blood vessels and play a major role in regulating smooth muscle cell growth and function. Our results prove that endothelial cells are capable of controlling smooth muscle growth in culture. We have genetically manipulated human endothelial cells to produce a protein or substance that inhibits the growth of human smooth muscle cells in culture. Our results are important in that they suggest a possible way to prevent smooth muscle cell growth in diseased and damaged blood vessels. The use of genetically-modified endothelial cells may be a future therapy for patients with atherosclerotic disease or damaged blood vessels.

Publications:

Hersh E, Stopeck A, Warneke J, Briley J, Parker S, Burman D, Norman J. Transfection (T) of Tumor Cell Lines (TCL) and Fresh Tumor Cells (FTC) with Plasmid DNA/Catonis Lipid Complexes. AACR, 13; 1996.

Stopeck AT, Geissner A, Miller TP, Hersh EM, Johnson CF, Grogan TM. Cellular Adhesion Molecules (CAMs) Predict Tumor Infiltration T Lymphocyte Response (T-TIL) in B Cell Diffuse Large Cell Lymphoma. AACR 15; 1996.

Stopeck A, Hersh E, Wameke J, Unger E. Results of a Phase I Study of Direct Gene Transfer of Interleukin-2 (IL-2) Formulated with Catonic Lipid Vector, Leuvectin, in Patients with Metastatic Solid Tumors. AARC 15; 1996.

Wang, Ph.D., Yi Ran

University of Arizona

Award Amount FY 1996: **$29,729**

Apolipoprotein A-I Gene Promoter: Functional Change in a Common Point Mutation

Apolipoprotein A-I (apo A-I) is the major protein of high density lipoproteins (HDL). Plasma levels of apo A-I and HDL are inversely correlated with cardiovascular disease risk factors (*i.e.,* smoking). The promoter elements that control hepatic expression of apo A-I gene located at nucleotide -256 to -41 downstream from the transcription start site. A common apo A-I polymorphism, with a frequency of 10-20%, resulted from a point mutation, a single nucleotide substitution at -78 position of apo A-I gene promoter region, with or without the point mutation, were inserted and expressed in human hepatoblastoma (Hep G2) cells. Our results indicate that the G to A substitution at -78 position significantly increases the expression of apo A-I gene promoter activity in Hep G2 cells.

Bloedel, M.D., Ph.D., James — **St. Joseph's Hospital, Phoenix**

Award Amount FY 1996: **$30,000**

Genetic Engineering of Receptors for Nicotine

Nicotinic acetylcholine receptors (nAChR) mediate important forms of chemical signaling between many different cell types in the nervous system. nAChR also are targets of nicotine, thought to be the addictive substance in tobacco products. An improved understanding of how nicotine affects the nervous system and contributes to habitual use of tobacco products requires an improved understanding of the properties and function of nAChR and their interactions with nicotine.

In this project, we used powerful, genetic engineering techniques to establish properties of a simple form of nAChR, α7-nAChR. We learned and refined ways to create human cells that reliably synthesize "normal" or mutant forms of α7-nAChR for our studies. We found that one mutant form of α7-nAChR differing by only one amino acid from the normal form binds nicotine usually tightly and does not go through a "fail-safe" process that ordinarily limits the strength of chemical signaling initiated by that binding. We also have found that cells expressing this mutant α7-nAChR have much lower capacity for survival than do cells expressing normal α7-nAChR. This opens the possibility that minor differences in genes that eventually code for α7-nAChR may predispose individuals to greater or lesser sensitivity to nicotine, thereby perhaps contributing to tobacco product usage patterns. Cells in these individuals also may have altered susceptibility to toxic or traumatic injury, as might contribute to degeneration of cells and neuronal circuits in which nAChR regulate chemical signaling.

French, Ph.D., Edward D. — **University of Arizona**

Award Amount FY 1996: **$27,429**

Marijuana, Nicotine and Dopamine Reward Systems: A Unifying Hypothesis of Dependence

Tobacco use has long been considered a gateway drug to other substance abuse, and nicotine has been suggested to play a major role in the abuse liability profile of tobacco and tobacco related products. The reinforcing effects of most drugs of abuse have been found to involve the activation of dopamine, a major neurotransmitter in the brain. Thus, it is reasonable to suspect that nicotine may produce its addictive effects via activation of a select population of dopamine neurons within the central nervous system. Moreover, the repeated activation of this pathway may produce long-lasting changes which could augment the rewarding effects of other drugs of abuse, such as marijuana. Using electrophysiological and behavioral methods in rats we have found that nicotine is a potent stimulator of dopamine neuronal activity within the ventral tegmentum. Nicotine also results in elevated levels of gross behavioral activation. Moreover, the psychoactive ingredient in marijuana, delta-9-tetrahydrocannabinol (THC) stimulates the same neurons. Furthermore, once daily administrations of nicotine will result in an augmented behavioral response to nicotine, or sensitization. Presently, we are testing whether this behavioral sensitization is reflected by long-term neuronal sensitization, and if the stimulatory effects of THC are potentiated. This latter aim is particularly important given the fact that marijuana is the most highly abused illicit drug in Arizona. Thus, prior tobacco use may facilitate the acquisition of marijuana abuse by increasing the sensitivity of reward mechanisms within the brain.

Lynch, Ph.D., Ronald M.

University of Arizona

Award Amount FY 1996: **$29,902**

Regulation of Insulin Secretion from Individual Beta Cells

Diabetes afflicts 9 million Americans and is often associated with obesity. In Diabetes, insulin secreting beta cells lose sensitivity to glucose leading to changes in blood insulin which then causes insulin sensitive cells to become insulin insensitive. Chronic exposure to nicotine also causes insulin insensitivity. Therefore, loss of normal glucose and insulin sensing may form the basis for changes in body weight after smoking cessation. We have begun studies to determine how excitation of beta cells initiates insulin secretion. Probes are being developed to measure insulin secretion from single cells on a microscope stage which will facilitate these studies. In addition, beta cell lines have been developed which do not sense glucose normally. In the coming year, the factors required for translating cell excitation into insulin secretion will be studied which may shed light on alterations in these processes which occur during development of Type II Diabetes and long term nicotine exposure and withdrawal.

Publications:

Zaguilan RM, Tompkins LS, Lynch RM. Time Resolved Laser Spectroscopy in Biochemistry IV. The International Society for Optical Engineering 2137: 17-28; 1994.

Lee, Ph.D., Nancy A.

Mayo Clinic, Scottsdale

Award Amount FY 1996: **$30,000**

Transgenic Mouse Models of Asthma and Other Inflammatory Diseases

Our research is focused towards identifying the molecular causes of inflammatory diseases such as asthma. At present, we are concentrating our efforts on characterizing one particular cell, the eosinophil, which is a type of white blood cell whose presence at sites of inflammation has been repeatedly documented. The numbers of these cells rise dramatically in instances of parasitic infestation or allergic/asthmatic reactions. Therefore, although believed to play a role in host defenses against parasites, eosinophils may also, if inappropriately stimulated, cause allergic/asthmatic disease. Many Arizonans suffer from these diseases (*e.g.*, >200,000 residents have asthma). Our experimental results examine the consequences of creating high levels of eosinophils in an animal and of recruiting eosinophils to specific tissues, such as the lung and skin. These results show that eosinophil infiltration of tissues can cause pathologies that are relevant to human diseases such as asthma.

Publications:

Larson KA, Horton MA, Madden BJ, Gleish GJ, Lee NA, Lee JJ. The Identification and Cloning of a Murine Major Basic Protein Gene Expressed in Eosinophils. J Immunology 155:002-3012; 1995.

Horton MA, Larson KA, Lee JJ, Lee NA. Cloning of the Murine Eosinophil Peroxidase Gene (mEPO): Characterization of a Conserved Subgroup of Mammalian Hematopoietic Peroxidases. Journal of Leukocyte Biology, In Press.

Lien, Ph.D., M.D., Y. Howard

University of Arizona

Award Amount FY 1996: **$30,000**

Gene Therapy in Carbonic Anhydrase II Deficient Mice: Role of Carbonic Anhydrase in CO2 Exchange and Acid-Base Homeostasis

We investigated whether Carbonic anhydrase II deficiency is associated with respiratory acidosis in CA II-null mice, and whether intravenous liposome-mediated gene therapy can correct this defect. Arterial blood samples were obtained from carotid artery in CA II deficient and normal mice and analyzed with blood gas analyzer immediately. CA II deficiency is associated with a lower pH and [HCO_3], and CO_2 retention, consistent with combined metabolic and respiratory acidosis. Liposome and human CA II expression plasmid mixture was injected via the jugular vein in CA II deficient mice. The human CA II gene was detected most strongly in the lung, followed by spleen, heart, liver and kidney. Arterial blood gas analyses were performed 7-12 days later. The gene therapy was associated with a significant decrease in CO_2 retention with no change in [HCO_3]. Our results indicate that CA II deficiency in mice is associated with respiratory acidosis and intravenous liposome-mediated gene therapy corrects the respiratory, but not metabolic, acidosis.

Martin, Ph.D., Arnold R.

University of Arizona

Award Amount FY 1996: **$29,470**

New Antituberculer Agents: Prodrugs and Isosteres of Isoniazid and Pyrazinamide

Twenty (20) analogs of the standard antitubercular drugs isoniazid (INH) and pyrazinamide (PZA) were synthesized and evaluated against a standard strain of *Mycobacterium tuberculosis in vitro*. The basis for this effort is to design antitubercular drugs that are effective against strains of the tubercle bacillus that are resistant to INH and/or PZA. The concept is to discover new biochemical mechanisms to activate our drugs that differ from those that activate INH or PZA. (Resistant strains of *M. tuberculosis* appear to lack the required activation mechanisms for INH and PZA). The emergence of resistant strains is a major factor contributing to the alarming upturn in reported cases of tuberculosis in the State of Arizona, the United States and worldwide.

Four of our compounds have shown promising activity in the test tube against the tubercle bacillus. The more promising compounds are prodrugs similar to PZA, in that they are apparently converted to the active forms inside the bacterial cell. Their potencies are approximately ten (10) times that of PZA. Compounds that have exhibited promising activity *in vitro* will be tested *in vivo* in infected mice and their toxicities in mice will be determined. If the results of the *in vivo* tests are promising then patenting and commercial development of selected compounds will be considered.

Ahmad, Ph.D., Nafees

University of Arizona

Award Amount FY 1996: **$30,000**

Influence of Tobacco Smoking on the Molecular Mechanisms of HIV-1 Transmission from Mother to Infant

AIDS in children is increasing at a significant pace in the United States and the State of Arizona. This is as a result of HIV-1 infection among woman in childbearing age group. The mechanisms of transmission and factors associated with maternal transmission are not known. In addition to viral and host factors, tobacco smoking during pregnancy increases the rate of mother-to-infant transmission of HIV-1 by 3- to 5-fold. The state of Arizona has the highest rate of smoking in the country. Smoking will increase the rate of HIV-1 transmission from mothers to infants and convert more HIV-1 infected individuals from asymptomatic to full blown AIDS. To further elucidate the molecular mechanisms of HIV-1 mother-to-infant transmission, we have performed a systematic analysis of the genetic and biological properties of HIV-1. The envelope V3 region sequences directly derived from the DNA of the peripheral blood mononuclear cells (PBMC) from infected mothers displayed a heterogeneous population. In contrast, the infants' sequences were less diverse than those of their mothers'. In addition, the sequences from the younger infants' PBMC DNA were more homogeneous than the older infants' sequences. All infants' sequences were different but displayed a similar pattern as seen in their mothers'. In the mother-infant pairs' sequences analyzed, a minor genotype or subtype found in the mothers predominated in their infants. The biological properties of the V3 region isolate from mothers and infants following perinatal transmission were found to be macrophage-tropic. In conclusion, a minor genotype macrophage-tropic maternal virus is transmitted from mothers to their infants. This finding may be helpful in developing strategies for prevention and treatment of HIV-1 infection that will also be beneficial to Arizona residents.

Publications:

Ahmad N. Maternal-Fetal Transmission of Human Immunodeficiency Virus. Biomedical Science 3: 38-250; 1996.

Castro, Ph.D., Felipe G. **Arizona State University**

Award Amount FY 1996: **$29,938**

The Evaluation of a Peer health Worker Model: Is it Effective in Preventing Cigarette Smoking in Promoting Women's and Children's Preventative Health Care

The principle aims of this project are to examine the manner in which the interventions of Lay Health Workers (Promotoras) may aid in improving the birth outcomes and self-care of women who participate in the Health Start Program. These interventions include the aid provided by Lay Health Workers in the form of outreach, education, and social support which is offered to participating mothers. Secondary goals of this project are to: (1) develop procedures to evaluate the activities and interventions of the Lay Health Workers, and (2) to assess the effect of the Health Start Program in enhancing the confidence, self-esteem, and self-efficacy of the Lay Health Workers themselves. The significance of this research for Arizona residents is that study findings are expected to offer information to facilitate the design and development of improved Lay Health Worker programs that will better serve the needs of pregnant women and their children.

Overall, this study seeks to answer four questions. In summary form, these questions are: (1) do the mothers and infants who participated in this program, relative to a *comparison group* of mothers and infants who have *not* participated in the Health Start Program, exhibit better birth outcomes as indicated by fewer medical complications? (2) do the participating mothers, relative to the comparison group mothers exhibit higher levels of knowledge and higher levels of service utilization? (3) what are the activities, strategies, and techniques of the Lay Health Workers, as these actions may increase client knowledge and utilization of preventive health services? (4) what is the prevalence of cigarette smoking among the Health Start mothers, relative to the prevalence for the comparison group, and how are these difference manifested as examined by ethnic/racial status?

Galgiani, M.D., John G. — **University of Arizona**

Award Amount FY 1996: **$30,000**

Structure of a Protein Antigen from *Cryptococcus Neoformans*

Complications of smoking tobacco include both scarring and cancer of the lungs. These consequences make smokers more prone to infections with unusual fungi such as *Cryptococcus neoformans* which normally can not infect the lungs and is resisted by normal human immunity. We are interested in determining the specific antigens that stimulate immune protection against *C. neoformans,* and that interest has led to the current work.

In preliminary studies we have determined that immunity develops against a protein about 200 amino acids in length. Our current work is to identify and sequence the gene that encodes this protein. Thus far, we have determined small parts of the amino acid sequence biochemically. Current efforts are focused on using this information to retrieve the entire gene by molecular biologic procedures. Once this is done, we can test the protein produced by this gene for activity in producing immunity in mice.

Graves, Ph.D., Joseph L. — **Arizona State University, West**

Award Amount FY 1996: **$29,700**

Comparative Genetics and Biology of Aging II: Selection Studies and Resistence to Toxic Compounds

Five *Drosophila* species (*melanogaster, pseudoobsura, arizonae, hydei and virilis*) are in their second year of laboratory artificial selection for delayed reproduction. Selection for delayed reproduction has shown to result in both the postponement of senescence (greater life span) and resistence to a wide variety of environmental toxicants (such as ethanol vapor resistance). Assays of age specific fecundity, longevity, and stress resistance are under way for these species. Results show a significant differentiation for longevity has already occurred in the *Drosophila pseudoobscura* stocks (a result we did not expect until next year). The delayed reproduction *pseudoobscura* stock showed a statistically significant difference at the 0.02 level for females and 0.0008 in males.

Lorton, Ph.D., Dianne

Sun Health Research Institute

Award Amount FY 1996: **$29,677**

Neuroamine Involvement in the Progression of Experimental Arthritis

Rheumatoid arthritis (RA) is a disease of the joints in which cartilage and bone is destroyed. RA is characterized by inflammation of joints possibly resulting from an abnormal immune response. We propose that the nervous system modulates disease progression by altering immune responses. We have shown that decreasing the availability of the neurotransmitter, norepinephrine (adrenaline) in nerves distributing to immune organs increases severity of experimental arthritis. This proposal examines mechanisms by which destruction of nerves that supply immune organs change the destructive processes of arthritic joints.

The incidence of RA increases with age. Since two of the most concentrated geriatric communities in the world are in the state of Arizona, RA is likely to represent a significant health problem for many of its residents. The ultimate goal of this research is the development of novel therapeutic approaches involving modulation of neurotransmitter systems in the control and prevention of RA.

Publications:

Lorton D, Bellinger DL, Fleten SY, Felton DL. Application of 6-Hydroxydopamine into the Fat Pads Surrounding the Draining Lymph Nodes Exacerbates Adjuvant-Induced Arthritis. J Neuroimmunol 64: 103-113; 1996.

Section D

PROPOSALS RECEIVED

TOBACCO RELATED RESEARCH

FY 1996

Alberts	University of Arizona	A Study of Genetic Alterations and Recurrence in Colorectal Polyps Associated with Smoking	$149,926 145,258 146,837
Ampel	University of Arizona	Cellular Reactivity and Cytokine Production in Human Coccidioidomycosis	$36,108 37,129
Baldwin	University of Arizona	What Cellular Mechanisms are Responsible for Histamine-Induced Alterations in Microvascular Permeability?	$46,861 50,000 49,707
Bernstein	University of Arizona	The Role of Apoptosis in Colon Carcinogenesis and Chemoprevention	$50,000 50,000 50,000
Brown	University of Arizona	Effects of *In Utero* Passive Smoke Exposure on Early Human T-helper Cell Differentiation	$50,000 48,683 49,950
Burgoon	University of Arizona	When Gains Go Up in Smoke: Explaining Adolescents' Negative Reactions to Smoking Prevention Campaigns	$89,961 59,711
Camilli	University of Arizona	Low-Dose Smoking Among Mexican-Americans	$122,535 80,836
Campbell	University of Arizona	Efficacy of Aerosolized Aminophylline in Obstructive Airways Disease	$25,529
Carter	University of Arizona	Synergism Between Smoking and Arsenic Exposure in Lung Injury	$138,340 117,889 122,344
Cesarotti	Arizona State University	Smoke Exposure in Children at Risk for Asthma and Middle Ear Infection: Predictors Interventions and Outcomes	$71,203 71,798
Consroe	University of Arizona	Antiemetic Drug Development for Cancer Treatment	$49,522 49,522 49,522

Davis	University of Arizona	Determination of the Role of Neutral Endopeptides in the Development of Small Cell Lung Cancer	$45,884 46,705 46,682
Davis	Arizona State University	The Effects of Smoking and Oral Contraceptive Use on Physiological Stress Responses in Young Women	$43,264
DeLuca	University of Arizona	HIV Infection of the Developing Thymus *In Vitro*	$103,093 106,495 110,542
DeLuca	University of Arizona	Organ Culture Approaches for Transplantation of Human Stem Cells	$50,000 50,000 50,000
Dembroski	University of Arizona	Biobehavioral Risk Profile of Smokers	$131,657 122,988 127,711
Dodge	University of Arizona	Sleep Disturbances in Persons Exposed to Enviromental Tobacco	$133,647 142,909 66,364
Dyer	Northern Arizona University	The Effect of Nicotine on Ovarian Steroid Hormone Production	$46,863 44,669
Enright	University of Arizona	Spirometry for the Detection of High-Risk Cigarette Smokers	$50,000 50,000 50,000
Espy	University of Arizona	Prenatal Tobacco Exposure, Breast Feeding & Neurobehavioral Development in Hospitalized Preterm Infants	$45,293 49,908
Flink	University of Arizona	Cardiac Cell-Cycle Progression and Terminal Differenttiation	$39,507 40,271 32,513
Friedman	University of Arizona	Identification and Characterization of M. Tuberculosis Genes Involved in Survival Within Macrophages	$50,000 50,000 50,000

Gervay	University of Arizona	The Synthesis of C-Glycoside Sulfones as Potential Cancer Therapeutics	$29,212 29,777 29,700
Giuliano	University of Arizona	Effects of Smoking on Persistent HPV Infection Among Reproductive Age Women	$149,538 149,333 149,995
Gmitro	University of Arizona	A Fiber-Optic Confocal Microscope for *In Vivo* Imaging	$50,000 50,000 50,000
Gorman	Northern Arizona University	Respiratory Virus-host Cell Interactions: The Specificity of the Viral Attachment Protein	$28,537
Habib	University of Arizona	The Effect of Micronutrient Antioxidants on Exhaled Ethane in Cigarette Smokers	$38,052 35,867 36,820
Harris	University of Arizona	Smoking and Epithelial Skin Cancer: What is the Relationship?	$49,552 34,540
Heiserman	St. Joseph's Hospital & Medical Ctr.	Clinical Utility of High Performance Gradient Carotid Magnetic Resonance Angiography	$46,115 16,154
Hill	University of Arizona	Effects of Smoking Cessation on Micronutrient Levels and Body Composition in Hispanics	$48,750
Kling	University of Arizona	Iron Lipid Peroxides and Erythropoietin Production	$30,000 30,000
Kramer	University of Arizona	An *In Vitro* Model for the Study of Glaucoma and Ocular Hypertension; Tobacco Associated Diseases	$49,400 49,640 50,006
Kurth	St. Joseph's Hospital & Medical Ctr.	Nicotine Acetycholine Receptors as Genetic Markers for Nicotine Dependence	$148,282 148,466 147,983

Lake	University of Arizona	Generation of Recombinat Antibodies from Lung Tumor-Infiltrating Lymphocytes (TIL-B)	$49,985 49,807 49,981
Lantz	University of Arizona	Effect of Environmental Tobacco Smoke on Lung Growth and Development	$109,383 94,505 98,079
Lee	Mayo Clinic, Scottsdale	Tumor Surveillance and Regression: Assessment of Immune Reactions Mediated by Eosinophils	$50,000 50,000 50,000
Lippiello	Harrington Arthritis Research Ctr.	Impaired Osteosynthesis in Skeletal Fractures of Smokers	$49,785
Lukas	St. Joseph's Hospital & Medical. Ctr.	Molecular Basis for Nicotine Dependence	$149,313 149,718 149,383
Lukas	St. Joseph's Hospital & Medical Ctr.	Properties of a Novel Receptor for Nicotine	$49,903 49,818 49,909
Macia	Arizona State University	Noninvasive Measurement of Respiratory Resistance	$29,946
Malan	University of Arizona	Role of c-fos in the Regulation of Neuropathic Pain	$46,609 47,879 49,767
Manseau	University of Arizona	Functional Analysis of a Drosophila Gene that Shares Structural Domains with Oncogenes	$47,997 48,707 49,431
Martinez	University of Arizona	Reactivation of p53 Function and Anti-Cancer Therapy	$49,988 49,988 49,988
Mason	VA Medical Center	Morbidity of Diabetic Foot Lesions in Smokers and Non-Smokers with Abnormal Plantar Foot Pressures	$49,445 48,895 48,895

McDonald	Mayo Clinic, Scottsdale	Molecular Genetic Analysis of Fibronectin Binding Integrins	$50,000 50,000 50,000
McQueen	University of Arizona	Genetic Variation in N-Acetyltransferase and the Developmental Toxicity of Aromatic Amines	$46,346 47,876 49,696
Metzger	Radiation Safety Engineering	Po-210 Activity in Cigarette Tobaccos and Mainstream Smoke and a Assessment of Radiation Dose to the Lung from Smoking	$50,000 50,000 50,000
Miller	University of Arizona	Reduction in Tobacco Use Among Adolescents Participating in an Incentive Based Prenatal Care Program	$105,266 149,263 149,522
Muramota	University of Arizona	Effects of Long Term Nicotine Replacement on Harm Reduction and Health Care Utilization	$149,402 149,966 140,823
Pajor	University of Arizona	Cloning and Expression of a Renal Na/Nucleoside Cotransporter	$33,359 34,460 35,769
Peng	University of Arizona	Effect of Increased Fruit and Vegetable Intake on Plasma Carotenoid Levels and Oxidative DNA Damage in Smokers	$50,000 51,626 53,558
Perry	Arizona State University	Vascular Reactivity Endothelial Function and Ovarian Hormones in Hypertension	$42,967 40,222
Pomeroy	Good Samaritan Reg. Med. Ctr.	Effects of Smokeless Tobacco on Semen Quality	$20,920
Porecca	University of Arizona	Delta Opioid Receptor Agonists may Improve Survival and Outcome of Cigarette Smoking-Induced Stroke	$30,300 27,753 29,850
Racowsky	University of Arizona	Investigation of the Mechanisms Underlying the Deleterious Effect of Cigarette Smoking on Human Fertility	$50,000 49,995 49,993
Regan	University of Arizona	Molecular Mechanisms Underlying the Pathophysiology of Ocular Hypertension	$49,610 48,574 48,661

Remers	University of Arizona	Design of Non-cross Resistant Agents for Lung Cancer	$84,147 86,932 90,226
Romagnolo	University of Arizona	Influences of Tobacco Derivatives on Regulation of Expression of the Breast and Ovarian Cancer Susceptibility Gene BRCA-1	$49,500 51,132 53,072
Rose	Arizona State University	Chemotherapy by Contravention of Oncogenesis Smoking-Induced Lung Cancer	$47,882 48,840 49,817
Santosham	Johns Hopkins University	Assessment of Tobacco Use and Exposure in Children, Youth and Childbearing Women in the Gila River Pima-Maricopa Indian Community	$49,530
Schauss	S. W. College of Naturopathic Medicine	Acupuncture in Smoking Cessation: A Randomized Placebo-Controlled Trial	$49,037 48,850 41,960
Schultz	Sun Health Research Institute	Development of a Method for Tumor Therapy Selection in Prostate Cancer	$50,000 50,000 40,000
Sherrill	University of Arizona	Assessment of Genetic Markers Associated with Development of Chronic Obstructive Airway Disease	$46,412 47,942
Smith	Arizona State University	Anti-Proliferative Actions of Interferon on Small Cell Lung Carcinoma Cells	$50,000 50,000 50,000
St. John	University of Arizona	Nicotine Receptors and Neuronal Death in the Spinal Cord	$137,377 141,913 147,303
Taetle	University of Arizona	Transgenic Models for Leukemia and Myelodysplasia (Preleukemia)	$32,757 34,067 35,423
Tsang	University of Arizona	Development of New Vectors and Gene Transfer Techniques for Gene Therapy	$50,000 50,000 50,000

Wegner	University of Arizona	Preservation of Microvascular Integrity and Left Ventricular Function in Hypothermically Preserved Rat Hearts	$37,969 37,861 37,380
Wright	University of Arizona	Passive Smoke Exposure Immunologic Function and Lower Respiratory Tract Illness in Infancy	$147,706 139,141
Wu	Mayo Clinic, Scottsdale	Roles of Integrins in Fibronectin Matrix Assembly	$50,000 50,000 50,000
Yool	University of Arizona	Influence of Nicotine on Glucose-Sensitive Neurons of the Hypothalamus	$49,952 49,793 49,853

Section E

New Contract Awards

Tobacco Related Research

Beginning in FY 1997

Alberts, M.D., David S.

University of Arizona

Award Amount FY 1997: **$115,330**

A Study of Genetic Alterations and Recurrence in Colorectal Polyps Associated with Smoking

Colorectal cancer is the second leading cause of cancer death in the United States with an estimated 50,000 deaths and 150,000 new cases diagnosed yearly. Research has shown that multiple factors, including family history, metabolic characteristics and dietary factors, play a role in the etiology of this disease. Early studies investigating the effect of tobacco use on colorectal cancer failed to consistently show an association; however, two recent studies have reported a relationship between the number of cigarettes smoked and risk of colorectal cancer. Because cigarette smoking and colorectal cancer are both common, even a weak association between the two would have a major impact on public health.

Sporadic adenomatous colorectal polyps are believed to be the most important precursors for most colorectal cancers. Improved information about the risk factors for the occurrence and recurrence of these adenomas would strengthen our understanding of the etiology of colorectal cancer. Several studies have associated cigarette smoking with a 2- to 3- fold increased risk for colorectal adenomas. Of the two published studies investigating the effect of cigarette smoking on adenoma recurrence, the most recent showed that the risk of recurrence was higher among smokers.

The molecular biology of colorectal cancer has been studied extensively and is among the best understood of all common human cancers. As adenomas advance to cancer, there are an increasing number of genetic changes detected; however, not all of these changes occur in every tumor and the order of these events is variable. Only one study has attempted to look at the relationship of these changes to tobacco smoke. Additional research investigating the effects of smoking at specific stages in the development of colorectal cancer is needed.

Baldwin, Ph.D., Ann L.

University Of Arizona

Award Amount FY 1997: **$46,861**

What Cellular Mechanisms are Responsible for Histamine-induced Alterations in Microvascular Permeability?

An important property of blood vessels is their ability to retain large molecules such as blood proteins, within the circulation and to regulate the passage of smaller molecules, such as water, across their walls from blood to tissue. The primary anatomic site of this selective barrier is the single layer of cells, or endothelial damage and subsequent leakage of larger molecules into the surrounding tissue. The basic cellular mechanisms responsible for this effect remain obscure. Endothelial damage is thought to contribute to atherogenesis, or hardening of the arteries, the incidence of which is significantly increased in smokers. In Arizona 25% of the population smokes, and in 1991 cardiovascular disease was responsible for 38% of total deaths. A better understanding of endothelial cell biology may lead to development of methods for controlling endothelial damage and for ultimately reducing the incidence of disease to which endothelial damage contributes.

Bier, M.S., N.D., L. Ac., Ian

Southwest College of Naturopathic Medicine

Amount Awarded FY 1997: **$49,995**

Acupuncture in Smoking Cessation: A Randomized, Placebo-Controlled Trial

The 1988 Report of the US Surgeon General asserts that the use of tobacco products is not a matter of free choice, but is the result of an addiction as scientifically valid as the addiction to heroin and other narcotics. For the most recent year of available data, 1989, Arizona lost 76,021 productive years of life due to deaths associated with smoking. The economic cost of smoking-related morbidity and mortality in Arizona in 1992 alone hit $706 million. In 1994, 23 percent or 928,466 Arizonans smoked cigarettes, of which 324,963 were between the ages of 18 and 24. More than 70% of this population stated they would like to quit. That same year, Arizonans spent $896,492,902 in smoking attributable costs including health care, morbidity and mortality. Given these statistics, a reduction in the number of Arizonans that smoke could benefit the state's productivity, economy, and the health of its citizens.

Acupuncture is becoming increasingly popular as a method of helping smokers break their addiction. Research has demonstrated that acupuncture is effective for many smokers who fail to quit by other methods. In general, acupuncture is painless, non-traumatic, economical, and easily accepted by most smokers. Therefore, a smoking cessation protocol involving acupuncture could be valuable in the treatment of smokers who have failed to quit by other methods.

In the United States, research on acupuncture as a treatment for addictions is still in an early stage of development. The treatment protocols tested have been based on clinical experience rather than systematic research, and only a handful of the studies have adequate follow-up data. This proposal follows the successful model for chronic alcoholism set forth by Bullock, *et. al.* and is designed to evaluate the efficacy of a multi-component treatment program involving acupuncture alone, and in combination with a smoking cessation educational program, over a significant follow-up period.

Carter, Ph.D., Dean E.

University of Arizona

Award Amount FY 1997: **$120,417**

Synergism Between Smoking and Arsenic Exposure in Lung Injury

The adverse effects of tobacco smoke on human lung function and disease have been well described; the lung has reposes to smoke that range from chronic inflammation to lung cancer. Less well described are the effects of airborne arsenic on lung disease but it is clear that arsenic also causes effects that range from chronic inflammation to lung cancer. Studies on arsenic smelter workers showed that cigarette smoking increased the number of arsenic-induced lung cancer cases. These findings are important to Arizona because arsenic is a major emission from copper smelters and coal-fired power plants and this may be an additional risk to tobacco smokers. Of additional concern is that our groundwater and surface drinking water sources contain substantial amounts of arsenic and this provides additional exposure to arsenic.

Consroe, Ph.D., Paul

University of Arizona

Award Amount FY 1997: **$49,522**

Antiemetic Drug Development for Cancer Treatment

Tobacco smoking is the leading cause of cancer in the United States. Arizona is no exception. Over 25% of Arizonans surveyed in 1990 were smokers. Anticancer drugs are the treatments of choice when the cancer spreads. While these drugs are mostly effective, their use almost always causes severe and continuous nausea and vomiting, more appropriately called "horrible retching." About 5 years ago, a new approach to treatment of this retching was introduced; two cannabinoids (Dronabinol and Nabilone) were approved for prescription use in the treatment of nausea and vomiting in cancer patients. While these drugs are definitely effective, both of these have undesirable psychoactive side effects similar to marijuana. If we know more about how these drugs work in the brain, we could design better antiemetic drugs from these unique chemical compounds. The present study will research the effects of cannabinoid drugs in mice. We will study their effects on mouse behavior and brain cannabinoid receptors, in order to determine where and how these drugs are acting. The results of this study will allow us to design more specific antivomiting drugs for cancer patients without marijuana-like side effects.

Davis, Ph.D., Mary C.

Arizona State University

Award Amount FY 1997: **$43,264**

The Effects of Smoking and Oral Contraceptive Use on Physiological Stress Response in Young Women

Although smoking itself is a potent risk factor for coronary heart disease (CHD), the health consequences of smoking in combination with other CHD risk factors are even more alarming. For example, women who smoke heavily have 4 times the risk of nonsmokers for heart attack, but that risk increases to 20 times when they use oral contraceptives as well. Smoking may also combine with stress to affect cardiovascular health. Both stress and smoking have profound physiological effects, and when stress and smoking coincide, their effects add together. This plays an important role in CHD progression.

Roughly 25% of women in Arizona smoke. Women report that they smoke more when under stress, and also select oral contraceptives more often than any other method of birth control. Although tobacco, oral contraceptives and stress each have important biological effects, little information is available regarding their combined impact on physiological systems.

Davis, Ph.D., Thomas P. — **University of Arizona**

Award Amount FY 1997: **$45,884**

Determination of the Role of Neutral Endopeptidase in the Development of Small Lung Cancer

Lung cancer and other respiratory diseases caused by cigarette smoke are common in the state of Arizona. The number of Arizona deaths attributed to smoking in 1992 was 5,186, representing 15.5% of the total number of deaths for that year. In addition, smoking has been identified as the most important cause of chronic obstructive pulmonary disease, the fourth leading cause of death in Arizona (Source: Arizona Cancer Registry Data). Small cell carcinomas of the lung (a cancer which occurs almost exclusively in cigarette smokers secrete growth hormones which act upon the cells to stimulate growth. An enzyme called neutral endopeptidase (NEP), exists on the surface of normal lung cells and cleaves and inactivated the growth hormones, thereby limiting their concentration and duration of action. However, tobacco smoke has been shown to decrease the level of NEP while at the same time increasing the level of growth hormones. It is suspected that the altered expression of growth hormones and degradative enzymes caused by cigarette smoke leads to the development of lung cancer. The research problem we confront is to determine whether a decrease in the expression of NEP leads to an increase in the proliferation of lung cancer cells, and to identify genetic alteration in the NEP gene which lead to the inappropriate expression of NEP in lung cancer cells. This knowledge will facilitate a better understanding of the events leading to the development of lung cancer.

DeLuca, Ph.D., Dominick — **University of Arizona**

Award Amount FY 1997: **$50,000**

Organ Culture Approaches for Transplantation of Human Stem Cells

Tobacco Smoking has been associated with increased rates of lung cancer for many years. A coalition has developed to support the strongest possible public position against smoking. For the first time lung cancer incidence has leveled off in white males; women and minorities continue to be a major target for smoking cessation programs. However, with no decline in smoking rates since 1991, use of tobacco will significantly affect the development of lung cancer. Thus, lung cancer is a problem in Arizona, and tobacco smoking will result in a greater number of people with this disease. This will have a greater impact on the Arizona Health Care System with all of its attendant increase in costs for care of those afflicted. Lung cancer is very difficult to treat; the 2- to 3-year survival is still only 10% to 25% for patients with limited disease and 1% to 2% for those with extensive disease. The most successful treatment regimens involve the use of high doses of toxic drugs to destroy the tumor. However, these drugs also destroy the blood-forming cells of the bone marrow, including the thymus-derived cells (T-cells) of the immune system. Therefore, drug treatment must be followed up with expensive bone marrow transplants (BMT) to "rescue" the blood-forming cells of the BM from the drugs. New measures, namely the substitution of normally-discarded human cord blood (HUCB) or human peripheral blood (HUPB) in place of BMT, would make transplantation a much more widely available procedure. The HUCB from the 2 million births a year in the U.S. is normally discarded and could provide a racially balanced source of hematopoietic stem cells (HSCs) for transplantation. However, little work has been done to determine if these approaches can support the development of the T-cells that are lost during the drug treatment procedure. Another serious problem is the limited amount of HUCB from a given source which requires the expansion and long-term storage of HSCs to allow for multiple transplants. In this proposal, we wish to develop new approaches to dealing with these problems.

Dyer, Ph.D., Cheryl A.

Northern Arizona University

Award Amount FY 1997: **$46,863**

The Effect of Nicotine on Ovarian Steroid Hormone Production

The deleterious direct effects of cigarette smoking on general health and the increased risk for lethal diseases has received much research attention. However, an under-investigated area is the indirect effect of smoking on women's health. Specifically, the effect of nicotine on ovarian steroid hormone production. Ovarian-derived estrogen protects women from their number one killer, heart disease due to atherosclerosis, and their number one crippler, osteoporosis. But women who smoke are estrogen deficient and their risk for these diseases is doubled or even tripled. In addition, a steroid made by the ovary acts on the brain to alleviate anxiety similar to the effect of Valium. It is possible that nicotine stimulates the ovary to make more of this neuroactive steroid and may explain why women are more addicted to smoking than men, who do not make this neuroactive steroid. Also, the sudden withdrawal of nicotine when a women tries to quit smoking could cause a large drop in the level of the neuroactive steroid causing her to experience more severe withdrawal symptoms and contribute to her not being successful at quitting. This may help explain why women are less successful at quitting than men. The information that will be gained by conducting these studies will help define the effects of nicotine: 1) on ovarian estrogen production, which will give women more information about the problems associated with using nicotine from cigarettes and nicotine supplementation by patch or chewing gum during cigarette withdrawal, and 2) on ovarian neuroactive steroid production which may explain why women are more addicted and less successful at quitting then men and who may benefit from neuroactive steroid supplementation during cigarette withdrawal to alleviate the withdrawal symptoms.

Flink, Ph.D., Irwin L.

University of Arizona

Award Amount FY 1997: **$39,507**

Cardiac Cell-Cycle Progression and Terminal Differentiation

The long-term goal of this research is to define the biochemical events which control cardiomyocyte terminal differentiation and cardio-specific gene expression.

Smoking is the leading cause of death in individuals beyond childhood in the state of Arizona and is associated with several biochemical changes in the heart and vascular system. The retinoblastoma tumor suppressor protein (pRb) is the prototype tumor suppressor which regulates normal cellular growth and differentiation processes. The chemical components of cigarette smoke cause mutations of genes including tumor suppressors. Molecular changes in pRb cause unregulated cellular growth (tumor formation). pRb inhibits normal cell division (tumor suppression) by modulating the function of several protein factors which are operative in a cellular replicative growth process called the cell cycle. A delineation of the steps involved in cell cycle progression and gene expression will help us to further understand the cascade of events which lead to cardiac disease and cancer.

Friedman, Ph.D., Richard L. **University of Arizona**

Award Amount FY 1997: **$50,000**

Identification and Characterization of *M. Tuberculosis* Genes Involved in Survival Within Macrophages

Tuberculosis is probably the most important infectious disease of humans. The World Health Organization estimates that one third of the world's population is infected with the tubercle bacillus, that ten million new cases of tuberculosis occur annually, and that over three million people die of tuberculosis every year. For citizens of the state of Arizona, tuberculosis is a serious and rapidly increasing health problem. The Arizona Department of Health Services Tuberculosis Elimination Section reported a more than 8% increase in new cases for 1994 as compared to 1993. Asians and Native Americans were about 10 times more likely to develop tuberculosis than Caucasians, whereas Hispanics were 5 times the greater risk. For the first half of 1995 as compared to a similar period in 1994, the situation is even more alarming--a 59% increase in total number of new cases have been reported with a distressing 23% increase among children up to 14 years of age.

In cigarette smokers, the risk of contracting tuberculosis and the severity of the ensuing disease is significantly increased. This has been the consistent finding in numerous investigations carried out over a span of several decades in the U.S.A. and in several other countries.

Gervay, Ph.D., Jacquelyn **University of Arizona**

Award Amount FY 1997: **$29,212**

The Synthesis of Glycoside Sulfones as Potential Cancer Therapeutics

Traditionally, carbohydrates have been viewed as important food sources – if you are low on energy, eat a candy bar. Only recently, it was discovered that carbohydrates serve another important biological role that facilitates communication between cells. For example, disease fighting white blood cells are transported from the blood stream to damaged tissue through the interaction of cell surface proteins and carbohydrates. This process does not involve metabolism of the proteins and carbohydrates; rather these biological molecules provide lock-and-key mechanisms for cells to move throughout the body. Proteins found on the surface of cells lining the blood vessel serve as locks that can only be opened by specific carbohydrates found on white blood cells. The carbohydrate opens the door to a cascade of events culminating in white blood cell transport from the blood stream to the tissue where damage has occurred. This recognition process is termed lymphocyte homing because white blood cells (lymphocytes) are directed to a specific site. In 1990, researchers discovered that a carbohydrate named Sialyl Lewis X is involved in lymphocyte homing. Interestingly, in 1984 it had been shown that this very same carbohydrate is present on the surface of cancer cells. These two facts suggest that tumor metastasis (spreading of cancer from one site of the body to another) may be facilitated by normal recognition processes, i.e., lymphocyte homing mechanisms.

Sialyl Lewis X is made up of four different carbohydrate subunits, two of which are critical for efficient lymphocyte transport. White blood cells, treated with enzymes that remove either of these subunits from their surface, are no longer recognized by proteins lining the blood cell. Consequently, the transport mechanisms breaks down. There are at least two approaches that one could take in order to prevent cancer cells from being transported. Development of a drug that removes the subunits before transport can take place is reasonable.

However, cell selectivity could be a problem since healthy white blood cells may be adversely affected. Alternatively, a drug that prevents the subunits from ever being present on the cancer cell surface in the first place could be developed. The latter approach could also impact upon healthy cells. However, since cancer cells typically replicate much faster, there is a potential for selectivity. The proposed research is directed to the development of cancer therapeutics that will prevent cell surface expression of the carbohydrate subunits required for tumor metastasis.

Giuliano, Ph.D., Anna R. **University of Arizona**

Award Amount FY 1997: **$149,538**

Effects of Smoking on Persistent HPV Infection Among Reproductive Age Women

Over one million cases of cervical dysplasia, the precursor lesion to cervical cancer, are diagnosed each year in the U.S. Reproductive-aged women (18-35 yrs.) are at the greatest risk of developing cervical dysplasia. In Arizona, the number of women diagnosed with this disease has increased significantly in the last decade. Research has definitely shown that infection with the human papilloma virus (HPV) is a cause of most cases of cervical cancer. Although, a women's risk of cervical cancer is 10- to 20-fold higher if she has HPV infection, HPV infection alone is insufficient to cause cervical cancer. Approximately 28% of women who are infected with the HPV virus develop cervical dysplasia or cancer. The women at highest risk for cervical dysplasia and cancer are those who consistently test positive for HPV infections over time (persistent infection). However, there is little information currently available on what factors allow HPV infections to persist and progress to cervical cancer.

A major risk factor for cervical cancer is smoking. The majority of studies conducted investigating the association between smoking and cervical dysplasia/cancer found smoking to increase a woman's risk for these diseases by 200-400%. There is extensive research demonstrating the negative effect of smoking in general, and the damage to cervical tissue specifically. However, no studies have investigated whether smoking is related to HPV infection persistence. Therefore, more research investigating the effects of smoking at particular stages in the development of cervical cancer is needed.

Gmitro, Ph.D., Arthur F. **University of Arizona**

Award Amount FY 1997: **$50,000**

A Fiber-Optic Confocal Microscope for *In Vivo* Imaging

Histologic evaluation of biopsy samples is the primary means for the diagnosis of cancer. The extraction of a good biopsy sample is, however, fraught with difficulty, especially for disease deep within the body. Misdiagnosis because of improper tissue selection is an enormous problem for the patient and costly to our society as a whole. A variety of methods, such as ultrasound or CT image guidance, exist for directing the physician to the appropriate site for tissue extraction. While these methods are adequate for large homogeneous tumors, they lack sufficient accuracy for precise localization in heterogeneous tumors, small tumors, of diffuse disease. Endoscopic imaging has improved the ability to localize disease for biopsying many forms of cancer but is still relatively coarse from the standpoint of the cellular level where disease occurs. Significant advance would be if the physician could direct himself to the site of disease and then perform a microscopic evaluation *in situ*. This might obviate the need to taking a biopsy sample altogether, or at the very least, allow more precise selection of tissue for biopsy. The microscopic visualization of lining tissue is not trivial. Fluorescence imaging with a confocal microscope is a relatively new technique that allows greatly improved imaging of this biological samples. The development of a catheter-based confocal microscope is the subject of this research. Such an instrument will have a significant impact on the practice of medicine as it concerns the diagnosis of cancer, including lung cancer, esophageal cancer, bladder cancer, and other forms of cancer related to smoking.

Habib, M.D., Michael P. **University of Arizona**

Award Amount FY 1997: **$38,052**

The Effects of Oral Micronutrient Antioxidants on Exhaled Ethane in Cigarette Smokers

Chronic obstructive lung disease (COPD) is one of the major causes of morbidity and mortality in Arizona. Our dry climate attracts many patients with respiratory ailments imposing a large financial burden on the individual patient, the family, the community and the State. Although cigarette smoking is mostly responsible for COPD, only 15-20 percent of smokers will develop clinically significant disease. Antioxidants may play a role in determining which smokers are at risk. Oxygen free radicals in cigarette smoke, tar and white blood cells contribute to the inflammation and tissue destruction associated with tobacco-induced lung disease. Oxygen free radical activity results in peroxidation of lipid membranes and the production of ethane gas which can be measured in the exhaled breath. We have shown that exhaled ethane is highest in smokers, lowest in non-smokers and intermediate in ex-smokers. Work from our lab indicates that simultaneous intake of Vitamin C, vitamin E and β carotene reduce the ethane found in the breath of smokers, and, importantly, that the greater the fall in ethane levels induced by these antioxidants, the better preserved is the lung function of these smokers. This technique therefore may identify those smokers at risk of developing clinically significant COPD. Concentration of our efforts on this group of smokers for disease prevention would then follow. This proposal will examine the effects of vitamin C alone, Vitamin E alone and β carotene alone on the exhaled ethane three groups of 30 smokers. We will examine the relationship between lung function and the effect of these antioxidants on exhaled ethane. Identification of antioxidant(s) reducing exhaled ethane and associated with preservation of lung function in smokers may have important implications for the prevention of COPD and in reducing its cost to the state.

Heiserman, M.D., Ph.D. , Joseph

Barrow Neurological Institute

Award Amount FY 1997: **$46,115**

Clinical Utility of High Performance Gradient Carotid MR Angiography

Among the health problems that plague cigarette smokers, stroke is one of the most common and most devastating. A stroke occurs when an area of the brain stops receiving a sufficient supply of blood. Cigarette smokers suffer a significant increase in stroke compared to nonsmokers. These strokes result in death in many cases, but just as importantly leave many survivors unable to work or even care for themselves.

Atherosclerotic narrowing of the carotid arteries which supply blood to the brain is associated with as many as one-half of all strokes, and smokers suffer more of this narrowing than the general population. Two therapies can reduce the risk of stroke. When only mild narrowing of the carotid arteries is present, simple treatment with aspirin is sufficient. However, patients with severe narrowing benefit from a minor surgical procedure, carotid endarterectomy, which can add high quality years to their lives by preventing future strokes.

Lee, Ph.D., James J.

Mayo Clinic, Scottsdale

Amount Awarded FY 1997: **$50,000**

Tumor Surveillance and Regression: Assessments of Immune Reactions Mediated by Eosinophils

The understanding of malignant diseases has progressed tremendously in the past 20 years. Despite these advances, current treatment strategies are still complex and usually include surgery, radiation and/or chemotherapy. In addition, some types of cancers have remained resistant to treatment and spontaneous tumor regression is a rare event. The cause of most cancers can be attributed both to environmental (e.g., tobacco smoke) and/or genetic factors (e.g., the gene marker for familial breast cancer, BRCA1). Although these genetic mutations or environmental exposures should predispose individuals to malignancies, many remain cancer-free. These puzzles illuminate a prevailing, yet unanswered, question in cancer immunology, how does the immune system normally "patrol" the body to prevent or eliminate tumors? A potential mechanism implicated in "tumor surveillance" involves the eosinophil, a type of blood cell that is also known to be important in discharging parasitic infestations from the body. Since the presence of eosinophils at the site of tumor growth is indicative of a good prognosis, it is believed that these cells may play a role in the destruction of tumor cells in a manner similar to the destruction and clearance of parasitic organisms. The goal of this research proposal is to determine if the eosinophil is involved in patrolling the body for malignancies and targeting tumor cells for destruction.

As a leading cause of death in Arizona, cancer represents a significant health risk to its citizens. In addition, the costs for treatment alone consume substantial tax revenues and utilize invaluable medical resources (e.g., available hospital space). Improved diagnostics and better treatments represent an immeasurable benefit to our citizenry.

Lukas, Ph.D., Ronald J.

Barrow Neurological Institute
Award Amount FY 1997: **$149,303**

Molecular Basis for Nicotine Dependence

Addiction to nicotine is thought to drive the habitual use of tobacco products by an estimated one million individuals in the state of Arizona alone. Habitual tobacco use causes a variety of illnesses and over 400,000 deaths in the United States each year. The financial and human costs of nicotine addiction are enormous to citizens, the state, and the nation. The biological targets of nicotine's actions are complex molecules called nicotinic acetylcholine receptors (nAChR). nAChR exists in a number of different forms. Each nAChR form has a unique sensitivity to nicotine. Different forms of nAChR play essential roles in many normal brain and body functions. Nicotine probably has its powerful and multi-faceted effects on brain and body function by affecting nAChR. However, our understanding of how these effects lead to nicotine addiction requires improved knowledge about how exposure to nicotine affects individual forms of nAChR. This proposed studies will provide this kind of knowledge. These studies are significant for many reasons. For example, they will identify nAChR forms that are most powerfully affected by chronic nicotine exposure. They will also identify drugs that block or mimic nicotine's effects on specific forms of nAChR. These or related drug could be used to block the addictive effects of nicotine or to relieve unpleasant effects of nicotine withdrawal. Therefore, this work is potentially useful in the design of therapies to prevent or promote cessation of the habitual use of tobacco products. These therapies could eventually lead to the control of tobacco-related disease.

Macia, Ph.D. Narciso F.

Arizona State University
Award Amount FY 1997: **$29,946**

Noninvasive Measurement of Respiratory Resistance

Asthma is a highly prevalent respiratory disease in Arizona, a state that has one of the highest asthma mortality rates in the nation. Pulmonary function testing to assess severity of asthma and response to therapy require patient cooperation. Children under 7 years of age typically have difficulty in performing the required respiratory maneuvers, because of their lack of understanding and motor skills. Without pulmonary function testing, objective assessment of degree of illness and of response to therapy is very difficult.

Unfortunately, pulmonary function testing for patients who are unable to cooperate is only done in a few centers with highly sophisticated, expensive equipment, by highly trained technical personnel. The Quick Obstruction Diagnostic Device (QODD), which measures respiratory resistance non-invasively and does not require patient cooperation, will enable us to test respiratory resistance in a population ranging from newborns to older adults. This new method of testing will allow the assessment of pulmonary function and early detection of the narrowing of the airways in all patients.

This research effort will produce a diagnostic tool that can be used to evaluate the effect of second-hand smoke and other pulmonary tobacco-related disease. This is urgently needed, since presently available diagnostic tests require intubation, the insertion of a tube with a small balloon at the end into the child's throat. Due to the high level of discomfort and high level of expertise required in performing the maneuver, it is rarely done.

Our present prototype of the QODD needs to be made more user-friendly, and to be tested and validated by comparing results with one of the sophisticated contentional methods, the body plethysmography.

Malan, Ph.D., M.D., T. Philip — **University of Arizona**

Award Amount FY 1997: **$46,609**

Role of c-fos in the Regulation of Neuropathic Pain

The proposed research studies a mechanism which may regulate neuropathic pain (chronic pain caused by injury to nerves, disease of nerves or abnormal functioning of nerves). We will test whether one of the functions of a gene called c-fos, (a gene which regulated the function of a number of other genes), is to cause substances to be made in the nervous system which decrease the intensity of neuropathic pain. Neuropathic pain is relevant to tobacco abuse since approximately 75% of patients with advanced lung cancer suffer from pain. Damage of nerves by tumor (one cause of neuropathic pain) is the second most common cause of cancer pain and it has been estimated that neuropathic pain causes 50% of cancer pain. Neuropathic pain is important to all citizens of Arizona because 33% of the population may suffer from chronic pain (pain lasting for more than one month), either from cancer or from other causes. In addition to the human cost, 50 to 60% of those with pain suffer temporary or permanent disability, resulting in a significant economic impact. Neuropathic pain causes an important fraction of all chronic pain.

McDonald, Ph.D., M.D., John A. — **Mayo Clinic, Scottsdale**

Award Amount FY 1997: **$50,000**

Molecular Genetic Analysis of Fibronectin Binding Integrins

The human lung has a surface area about the size of two-thirds of a singles' tennis court. This large surface area is required to remove carbon dioxide from the blood, and add oxygen to the blood. This surface is completely lined with a sheet of living cells, termed "epithelium." This epithelium can be thought of as similar to tiles on a floor. In fact, similar to floor tiles, the epithelium must be kept clean (in fact sterile), and injured and/or aged cells (broken tiles) must be replaced with fresh ones. This means that there is a programmed, dynamic, ongoing process of cell growth and change that starts with the initial development of the lung, and proceeds throughout our adult lives. Because the lung must move vast quantities of air and blood, about 2,000 gallons of air and blood are pumped through the lung every 24 hours in a resting individual. The lung is a natural target of environmental pollutants, including tobacco smoke components, infectious agents like viruses (for example, influenza) and bacteria, and mineral products such as asbestos. The epithelium and thin overlying fluid layer forms the barrier between these injurious agents and the body. Thus, any process affecting epithelial cells plays a major role in lung disease. We are interested in molecules found on the surface of the epithelial cells called "integrins" that are hypothesized to be critical for epithelial cell attachment, growth, and specialization into their final functional state. Lung disease (cancer, asthma, emphysema, chronic bronchitis, and fibrotic lung diseases) are high on the list of morbidity and mortality nationwide, including Arizona.

McQueen, Ph.D., Charlene A. **University of Arizona**

Award Amount FY 1997: **$46,346**

Genetic Variation in Nacetyltransferase and the Development Toxicity of Aromatic Amines

How an individual responds to a chemical is due to the interaction between his/her genetic make-up and environmental factors. The proposed research will investigate the role of genetic variation in fetal susceptibility to tobacco smoke toxicity. One component of tobacco smoke, a chemical called 4-aminobiphynly (4AB) which causes cancer and birth defects, will be studied. Pregnant women can be exposed to this compound if they smoke or through second-hand smoke. 4AB is found in babies exposed prenatally to tobacco smoke. Higher levels are found in babies born of smoking mothers. There is a genetic difference in how 4AB is detoxified and individuals are classified as having high or low ability. In Arizona, about 50% of the population have a low ability. Studies are planned in an animal model to investigate if genetic variation in how this chemical is handled by the mother and/or fetus alters the risk of developing 4AB induced birth defects. This would demonstrate if a susceptible group with increased risk is present in the population. Such information would be important in developing preventive strategies.

Miller, M.D., Hugh S. **University of Arizona**

Award Amount FY 1997: **$90,944**

Reduction in Tobacco Use among Adolescents Participating in an Incentive Based Prenatal Care Program

Tobacco consumption among adolescents is receiving national attention, as evidenced by the introduction of Senator Bradley's legislation designed to curb adolescents' access to tobacco products by increasing excise taxes and restricting sales via vending machines in public areas. While we wait for national and state legislation to be implemented across the country, adolescent pregnancy continues to be complicated by the rising incidence of tobacco use. Tobacco alone is responsible for a variety of pregnancy complications, including low birth weight (LBW), antepartum hemorrhage and preterm birth (PTB). Coincidentally, adolescent pregnancy itself is often associated with similar complications, particularly low birth weight and preterm birth. Whether the perinatal outcomes experienced by adolescents are a function of reduced participation in prenatal care (PNC), substandard nutrition, poor social situations, or tobacco use is unclear. The state of Arizona has recently been identified as having the highest *per capita* adolescent pregnancy rate in the nation, which makes this question a specific priority for this state. The objectives of the proposed research are to determine the prevalence of smoking among pregnant adolescents and to establish the role that smoking plays in their perinatal outcomes. Efforts to improve perinatal outcomes among adolescents would undoubtedly be fostered by effective smoking cessation programs, which must be initiated early in pregnancy.

Pajor, Ph.D., Ana M. **University of Arizona**

Award Amount FY 1997: **$33,359**

Cloning and Expression of a Renal Na/nucleoside Cotransporter

Nucleoside drugs are widely used in the treatment of cancers and viral infections. Examples of these drugs include Floxidine (used to treat breast cancer and tumors of the gastrointestinal tract), AZT (used to treat AIDS), Cytarabine (used to treat leukemias), and Gemcitabine (in trials for treatment of lung cancer). In order for these drugs to act, they must cross the membranes of the target cells. This is accomplished by specific nucleoside transporter proteins on the cell membrane. The presence of a transporter in the membrane determines whether a nucleoside drug can act on that cell, and the specificity of that transporter protein determines whether it can carry a particular chemical structure. Therefore, it is important to know the types of nucleoside transporters that are present on a given cell, and also the types of nucleoside drugs that can be carried by these transporters. By using techniques of molecular biology, it is possible to isolate the cDNA coding for a single nucleoside transporter protein. We can then use this isolated protein as a model system to design drugs that it prefers to carry, and also to determine what cells in the body contain this transporter. The results of this research should provide better information on what drugs would be most useful during cancer and antiviral therapy.

Peng, Ph.D., Yeh-Shan **University of Arizona**

Award Amount FY 1997: **$50,000**

Effect of Increased Fruit and Vegetable Intake on Plasma Carotenoid Levels and Oxidative DNA Damage in Smokers

Smoking has been implicated as a major cause of certain human cancers as well as respiratory, cardiovascular and many other chronic diseases. The single most effective means to prevent these diseases would be to eliminate tobacco use from society; however, this is currently an unrealistic goal. For many smokers, quitting smoking is difficult. Even if successful, their risk for cancer would still remain high. According to one study, there may be more than a 10-year lag between quitting cigarette smoking and a substantial reduction in lung cancer risk. A national survey (1993) indicates that there are approximately 92 million adult smokers and ex-smokers in the U.S., which constitute nearly 50% of the adult population. Smoking costs the U.S. approximately $50 billion in direct medical costs every year. In Arizona, approximately 20% of adults (ages 18 and up) are current smokers and statistics show that many of them will also suffer from smoking-related diseases. Thus, a prevention strategy targeting this large segment of the high risk population is needed. Smokers and ex-smokers have lower levels of carotenoids in the body than nonsmokers. Since carotenoids are part of the body's defense system against the harmful substances produced by cigarette smoke, the low level of carotenoids may mean the body's defense system is weaker. This may result in genetic damage and possible cancer. A weakening of the body's defense system may also be responsible for the development of some other smoking-related diseases. Thus, to reduce these risks, it may be necessary to restore the levels of carotenoids in the body. The goal of the proposed study is to develop a prevention strategy that will effectively increase the levels of carotenoids in smokers.

Pomeroy, Ph.D., Kimball O. — **Good Samaritan Regional Medical Center**

Award Amount FY 1997: **$20,920**

Effects of Smokeless Tobacco on Semen Quality

While consumption of most tobacco products has declined, use of smokeless tobacco has increased almost three-fold from 1972 to 1991. Usage among young men in the U. S. is estimated at 4.44%. We believe the usage in Arizona to be significantly higher. Although studies have examined the effect of smoking on male reproduction, only one study has examined the effect of smokeless tobacco on male reproduction (India). In this study, we will determine whether chewing tobacco has a negative effect on sperm and whether there is any correlation of the severity of these effects to levels of nicotine or cotinine (tobacco products) in semen. This study would be the first study to use the most advanced and sensitive methods available for studying toxic effects of tobacco on the male reproductive system. A recent development, computer assisted semen analysis, will be used to measure the effects of smokeless tobacco on sperm. Fifty tobacco users and 25 non-users will have their sperm tested manually and by computer to determine if tobacco usage has produced subtle changes in sperm.

Racowsky, Ph.D., Catherine — **University of Arizona**

Award Amount FY 1997: **$48,571**

Investigation of the Mechanisms Underlying the Deleterious Effect of Cigarette Smoking on Human Fertility

In Arizona, approximately one-third of women of reproductive age, or 250,000 women, smoke cigarettes. Population studies have provided consistent evidence that cigarette smoking increases the risk of reduced reproductive capacity in women. Indeed, the more cigarettes a woman smokes, the greater her risk of impaired reproductive potential. However, the mechanisms underlying this deleterious effect of cigarette smoking remain to be elucidated. Accordingly, the proposed research is designed to elucidate the adverse effects of cigarette smoking on reproductive capacity in women.

Knowledge regarding the mechanisms by which cigarette smoking impairs reproductive potential in women would be invaluable both to family planning counselors in the guidance of these women as they seek to establish families, and to infertility specialists in the treatment of their patients. In addition, such knowledge could be provided to young unmarried women to help maintain their reproductive health. Thus, it is anticipated that the information generated from this project will enhance the lives of a very considerable number of women in Arizona, either as they currently seek to have children, or as they may wish to preserve their reproductive health for future reproduction.

Remers, Ph.D., William A. **University of Arizona**

Award Amount FY 1997: **$84,147**

Design of Non-Cross Resistant Agents for Lung Cancer

There will be approximately 2400 deaths from lung cancer and 2500 new cases of lung cancer in Arizona in 1995 according to *Cancer Facts and Figures*. Cigarette smoking will be responsible for 85% of these cases. Lung cancer can be treated by surgery, radiation and chemotherapy, but significant advances are needed in all of these treatment modalities. At this time, two agents, etoposide and methotrexate, are approved for use against small cell lung carcinoma. No agents are approved for non-small cell lung carcinoma, although some are used with limited effect. In this application, we propose to develop new chemotherapeutic agents that may provide useful alternatives to the existing ones and which might be active against non-small cell lung carcinoma. A serious problem with lung cancers is their development of resistance to many antitumor agents. We are especially interested in designing new compounds with activity against these resistant cancers.

Romagnolo, Ph.D., Donato **University of Arizona**

Award Amount FY 1997: **$49,500**

Influences of Tobacco Derivatives on Regulation of Expression of the Breast and Ovarian Cancer Susceptibility Gene BRCA-1

The state of Arizona ranks 16th in the United States in deaths (5,700 in 1990) attributable to smoking with estimated years of potential life lost approaching 67,000 years and a state medical care expenditure related to smoking of about $52,000/death. The association of smoking with diseases of the respiratory and circulatory systems, such as lung cancer, vascular diseases, chronic obstructive lung disease, and myocardial infarction has been studied in great detail. Recent findings substantiate that tobacco usage is important in the etiology of breast and ovarian cancer. Cigarette smoking and other tobacco usage expose women of reproductive age to carcinogenic chemicals and increase the risk of developing these malignancies later in life. Strategies designed to preventing the onset of these tumors require an understanding of the biochemical events induced by exposure to tobacco derivatives. In turn, new knowledge can improve the awareness of the public, in particular of adolescent smokers, and assist in clarifying possible interactions of tobacco exposure with therapeutic interventions.

Rose, Ph.D., Seth D.

Arizona State University
Award Amount FY 1997: **$47,882**

Chemotherapy by Contravention of Oncogenesis in Smoking-Induced Lung Cancer

Recently, it has become known that lung cancer caused by tobacco smoke inhalation is in many cases the result of alterations of a cell's genetic information, or DNA. This specific alteration of the DNA is due to the action of cancer-causing components of the tobacco smoke. The altered DNA is observed in 33% of lung cancer cases known as adenocarcinomas. There were 1,927 new lung cancer cases diagnosed in Arizona in 1991. This amounted to 13.8% of all cancer cases diagnosed in the state that year. The annual death rate in Arizona from lung cancer is 44.9 per 100,000 of population, which makes it a significant cause of death and suffering in the state. The altered DNA causes cells to produce a faulty protein that in turn makes the cell go into a state of unrestrained cell division. Interference with the altered protein's action serves as a strategy for the control of the excessive cell division in the cancer.

Santosham, M.D., Mathuram

Johns Hopkins University
Award Amount FY 1997: **$49,530**

Assessment of Tobacco Use and Exposure in Children, Youth, and Childbearing Women in the Gila River Pima-Maricopa Indian Community

Tobacco is the number one preventable cause of death in the United States and is associated with many diseases including cancer, emphysema, heart disease, and ulcers. In American Indians, many articles have been published showing a significant prevalence of tobacco use.

Tribal leaders of the Gila River Indian Community (GRIC) have raised concerns regarding the use of tobacco products by children, youth and pregnant women and its impact on the health of the community. While these concerns exist among community leaders there are no actual data on prevalence and impact of these issues on health. The only known information is from a survey of 298 women from GRIC who gave birth in 1994 indicating that 16.7% admitted to being smokers on their first prenatal visit. In pregnant women, smoking has been associated with many conditions affecting the unborn baby including decreased birth weight, increased death rate and high rates of abortions. Children under five years of age, when exposed to tobacco smoke have a higher attack rate of lower respiratory diseases.

Before any intervention programs can be developed and implemented, it is necessary to know the frequency of smoking in this population, particularly, among the youth and women of childbearing age since babies exposed to smoking are at highest risk for the bad effects of smoke. Early intervention and education programs can save millions of dollars in long term health care.

Sherrill, Ph.D., Duane

University of Arizona

Award Amount FY 1997: $46,412

Assessment of Genetic Markers Associated with Development of COPD

Several respiratory diseases, particularly chronic obstructive pulmonary disease (COPD), are known to be strongly linked to cigarette smoking. COPD currently represents a major health problem from both a morbidity and mortality standpoint with prevalence rates in Arizona generally being above the national average because of the large retirement population. For many years scientists have known that both environmental and genetic contributions were important in determining the natural history of the disease. Most smokers do not develop COPD, and thus it has been suggested that some individuals may be more susceptible than others to the harmful effects of cigarette smoke and that such susceptibility could be inherited. Since COPD is considered a late onset disease most studies for assessing the degree of heritability have used other disease markers. Such markers are used in place of a COPD diagnosis because they are thought to be early predictors of individuals likely to develop the disease. The most commonly used marker is measured by spirometric testing (PFT) and assesses how well the lungs are able to move air. Other candidate markers include measures of immune status, that reflect the degree in inflammation in the lungs (IgE) and measures of the lungs response or reaction to inhalation of chemicals known to constrict lung tissue (BHR). The primary objective of the proposed study is to determine if selected biological markers for COPD (PFT, IgE or BHR, described above) show patterns of Mendelian inheritance among families.

Taetle, M.D., Raymond

University of Arizona

Award Amount FY 1997: $32,757

Transgenic Models for Leukemia and Myelodysplasia (Preleukemia)

The commonest form of acute leukemia in adults (acute myelogenous leukemia: AML) and a related set of disorders called, myelodysplastic syndromes (MDS) or "preleukemias" occur with increased frequency in smokers. These disorders also increase with age, and are thus relatively more common in Arizona. Both disorders lead to decreased production of normal blood cells, and severe infections or bleeding. The vast majority of adults with AML and all patients with MDS eventually die from their disease. There is no effective therapy for MDS other than supportive care. Like other forms of cancer and related diseases, AML and MDS are caused by genetic abnormalities occurring in a single cell, in this case, in the bone marrow. Understanding mechanisms by which chemicals in tobacco might cause these diseases and developing therapies for MDS have been limited by the absence of relevant animal models. A normal gene called EVI-1, is not usually expressed in normal bone marrow or blood cells, but appears in a subset of AML and MDS patients. Several lines of evidence suggest EVI-1 is important in causing these cancers. To understand the role(s) of this gene in AML and MDS, and to develop new therapies for these diseases, requires a stable, small animal (mouse) model. Once such a model is available, it can be used to assess whether chemicals in tobacco increase the number of animals developing AML and MDS or severity of these diseases.

Wright, Ph.D., Anne L.

University of Arizona
Award Amount FY 1997: **$119,152**

Passive Smoke Exposure, Immunologic Function and Lower Respiratory Tract Illnesses In Infancy

Lower respiratory tract illnesses (LRIs) such as bronchiolitis, bronchitis and pneumonia, are major causes of infant illness and hospitalization; in Arizona, over 20,000 infants have LRIs annually, hundreds of whom are hospitalized. Research which identifies risk factors for this common type of illness, particularly those which are preventable, could have a major impact on the health of infants and on health care costs in Arizona as well as other parts of the nation.

Virtually all studies show that infants passively exposed to cigarette smoke are significantly more likely to have LRIs. Adult smokers differ from nonsmokers in both lung function and immune function, either of which could be responsible for their higher rates of respiratory illness. Although it has been shown that infants born to mothers who smoke have altered lung function, whether they differ also in immunologic function has been virtually unexplored. Further, there is controversy regarding whether breast-feeding in conjunction with maternal smoking, increases exposure to nicotine, which might elevate illness rates, or protects the infant from the deleterious effects of smoking. Smoking-associated differences in infant immunologic function and development could have long lasting impact on susceptibility to all types of illnesses. Documenting immunologic alterations will assist in targeting groups in which asthma interventions may be most beneficial, developing of clinical recommendations for smoking mothers who wish to breast-feed, and providing additional impetus at a pivotal time to assist mothers to stop smoking.

Wu, Ph.D., Chuanyue

Mayo Clinic, Scottsdale
Award Amount FY 1997: **$50,000**

Roles of Integrins in Fibronectin Matrix Assembly

Tobacco smoking is associated with an increased risk of lung diseases, including respiratory tract infections, chronic airway disease and lung cancer. These diseases cause severe disability and death of many people residing in Arizona. Most cells help create a specialized, local environment by producing and organizing complex molecules around themselves called "extracellular matrix." This extracellular matrix helps control cell growth and death. Amazingly, the matrix also helps inform the cells about how they are supposed to function. The lung contains a remarkable assortment of extracellular matrices. The extracellular matrices are essential for lung development. In adult lung, they provide appropriate structural integrity and support plastic deformation during respiration. Alterations in the structure and formation of extracellular matrices are often associated with lung diseases, inducing those caused by tobacco smoking. We have discovered certain proteins on cell surface called Integrins are very important during the formation of the extracellular matrix. We propose to study their role in creating the extracellular matrix environment, and to determine how cells control this process. The results obtained from these studies should help us understand the mechanism of lung development and the cause of certain forms of lung injury, which could translate into novel approaches to modify the matrix formation process and consequently the outcome of the lung diseases involving abnormal formation of extracellular matrix.

Yool, Ph.D., Andrea J.

University of Arizona

Award Amount FY 1997: $49,952

Influence of Nicotine on Glucose-sensitive Neurons of the Hypothalamus

Cessation of chronic nicotine exposure is correlated with changes in feeding behavior and caloric intake in humans and other animals. Neuronal centers in the hypothalamic region of the brain integrate sensory input to provide optimal nutrient intake. It is likely that alterations in the activity of these centers underlie the feeding changes which lead to weight gain and obesity. A corollary of nicotine-induced feeding changes is observed in association with diabetes. It has been appreciated for over three decades that specialized neurons in the hypothalamus respond to elevated blood glucose by decreasing the activity of neurons in the "feeding center." It is likely that changes in sensitivity of these neurons to glucose underlie the frank obesity that is often observed with diabetes. Moreover, we propose that chronic nicotine exposure also alters the normal sensitivity of the glucose-sensing neurons, which thereby elicits at least a portion of the changes in feeding drive which are observed following cessation of smoking. One of the primary obstacles to quitting smoking is the resultant weight gain experienced by many individuals. Moreover, smoking is a significant concern which likely influences weight gain observed in diabetics. Our studies will provide basic information regarding the role-specific hypothalamic neurons may play in linking these events.

Principal Investigators, Continuing Contracts

Principal Investigators, New Awards