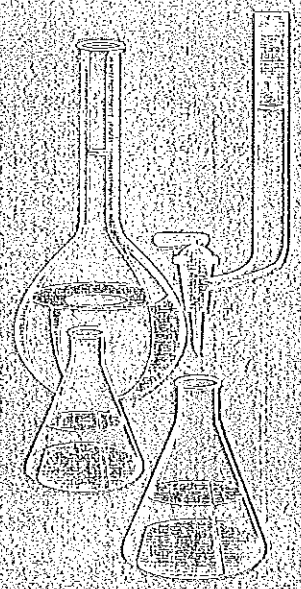
Arizona Disease Control Research Commission



1999 – 2000 Annual Report

January 2001

ARIZONA DISEASE CONTROL RESEARCH COMMISSION

ANNUAL REPORT
1999-2000

Jane Dee Hull, Governor

Henry Reeves, Ph.D., Chairman
Orme Lewis, Jr., Co-chairman

COMMISSION MEMBERS

General Public
C. Eileen Bond, J.D.
Orme Lewis, Jr.
Joseph A. Mislove, J.D.

Medical Community
Betty J. Gale, D.N.Sc.
Eladio Pereira, M.D.
Stuart F. Quan, M.D.

Scientific Research Community
T. Lon Owen, Ph.D.
Henry C. Reeves, Ph.D.
Walter H. Williams, Ph.D., M.D.

Staff
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January 2001

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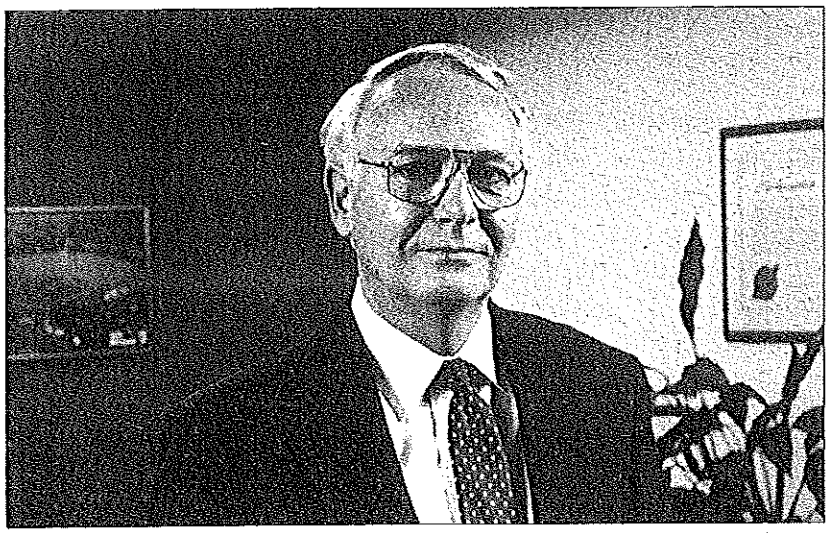


Message from the Chairman

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's Fiscal Year 2000 Annual Report. Established by the Arizona Legislature in 1984, Commission activities contribute to improving the health of Arizonans through scientific research. Funding comes through the Tobacco Tax Initiative, passed by the voters in 1994. The ADCRC receives five percent of the revenues collected from the tax to fund tobacco-related disease research.

In FY 2000 the Commission is reporting on both the tobacco-related disease research program and the anticancer drug discovery program. The researchers have made progress in areas of gene-related therapies, the discovery of compounds with anticancer, antiangiogenesis, antimicrobial, antiviral and antifungal properties, and in new single drug or combination therapies now in Phase II and III clinical trials.

The Commission continues to be involved in technology transfer and the patenting and licensing of discoveries funded with ADCRC monies. This year the Commission worked with representatives of the Arizona Board of Regents and other interested parties to develop a joint technology transfer blueprint for research supported with ADCRC funds.

The Commission has begun preparations for the second and final cycle of funding under the Anticancer Drug Discovery Program. Funds were provided for major equipment purchases in the first cycle of this program. The availability of state-of-the-art technology assisted the University of Arizona and Arizona State University in competing successfully for federal funds. The Commission will be awarding contracts in the spring of 2001.

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. The Commission is divided into three communities – General Public, Medical and Scientific Research. Each community is represented by three Commissioners appointed for three-year terms. Generally, the terms of three members expire each year; Commissioners may be reappointed. The Chairman and Commissioners who served during 1999-2000 are presented below.

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

Commissioner Reeves is a member of the Scientific Community. He 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 Arizona State University, he served as director of the Division of Physiology, Cellular and Molecular Biology at the National Science Foundation from 1976 to 1979. Commissioner Reeves received his B.S. from Franklin and Marshall College and his M.A. and Ph.D. from Vanderbilt University. He was first appointed to the Commission by Governor Symington to complete the term of Commissioner James Bloedel in 1995. He was reappointed in 1996. Governor Hull appointed him to his second full term in 1999. His term will expire in May 2002.

General Public

C. Eileen Bond, J.D. Prescott
Private Practice Specializing in Child Welfare Law

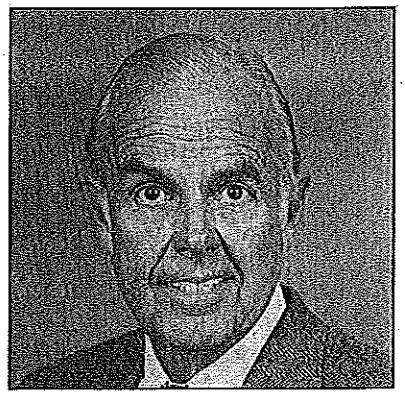
Commissioner Bond received her B.A. in History (Far Eastern Studies) and Master of Library Science from U.C.L.A. She received her J.D. from Arizona State University in 1971. Commissioner Bond retired from the Arizona Attorney General's Office in 1996 and is in private practice in Prescott, Arizona, where she specializes in the area of child welfare law. She serves on the Board of Directors of Child Haven, the Yavapai County Child Crisis Center, and as an advisor to the Yavapai County Family Drug Court. Commissioner Bond serves as a Disciplinary Hearing Officer for the Arizona State Bar Association, and as a due process hearing officer for the Arizona Department of Education. Commissioner Bond was appointed by Governor Hull in May, 2000. Her term expires in May 2003.

Lois Emden, M.S. Paradise Valley
Nutritional Counselor,

Commissioner Emden received a B.S. in 1963 and an 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 an advocate representative for Scientific Peer Review with the 1995 and 1997 Department of Defense Breast Cancer Research Program. She is a Phoenix Art Museum Docent. Commissioner Emden was appointed by Governor Symington in 1994 and reappointed in 1997. Her term expired in May 2000.

General Public

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



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, Boyce Thompson Southwestern Arboretum, and the Polycystic Kidney Research Foundation. He 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 Legislatures. He received a B.S. in Economics in 1958 from the University of Arizona. Commissioner Lewis was appointed to the Commission by Governor Symington in 1995 and reappointed by Governor Hull in 1998. His term will expire in May 2001.

Joseph A. Mislove, J.D. Phoenix
Coppersmith, Gordon, Schermer, Owens & Nelson, PLC

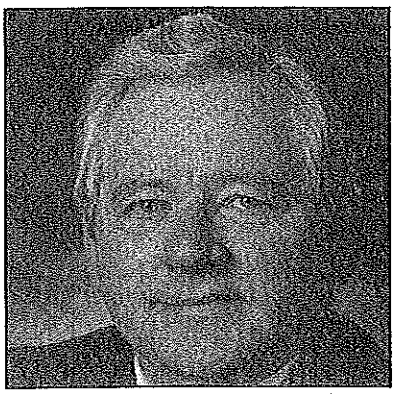
Commissioner Mislove has practiced health care law in Arizona since 1989, in both private practice and as in-house counsel for a managed-care organization with more than 350,000 members. He advises clients on legal issues concerning licensure, certification, and accreditation; arrangements that implicate physician self-referral, anti-kickback, and other fraud and abuse laws; EMTALA and general compliance programs; payor and medical service contracts; Medicare, Arizona Health Care Cost Containment System, and other public programs; and corporate matters. Commissioner Mislove received B.S. and M.B.A. degrees from Arizona State University in 1981 and 1986, and his J.D. degree from the University of Arizona in 1986. He is a member of the American Health Lawyers Association and the Arizona Association of Health Care Lawyers. Commissioner Mislove served as President of the Arizona Association in 1996-97. He was appointed to the Commission by Governor Hull in August 1999. His term expires in 2002.

Medical Community

Betty J. Gale, D.N.Sc. Tempe
Professor and Chair, Division of Community Health Nursing
Arizona State University

Commissioner Gale has her D.N.Sc. from the University of San Diego and her M.S. in Community Health Nursing from Arizona State University. Commissioner Gale is Division Chair and Professor at the Arizona State University, College of Nursing. She is also a Clinical Associate Professor at the University of Arizona. Her practice and research focus on health promotion programs with low income older adults. Commissioner Gale recently completed a study on the sociocultural influences on functional health in older Anglo and Hispanic Women that was funded by the National Institutes of Health. Current projects, in collaboration with other partners, are Community-oriented Primary Care and Muscular Fitness in Older Adults. She is the Nurse Administrator of The Escalante Health Partnerships. Appointed to the Commission in August, 1999 by Governor Jane Hull, her term expires in May 2001.

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



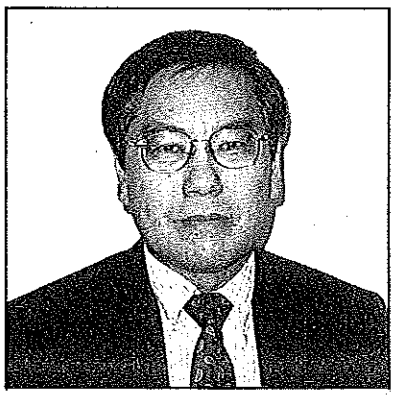
Commissioner Oakley received his A.B. from Washington University, St. Louis and an M.D. from the University of Missouri College of Medicine. He completed a rotating internship and four-year residency in General Surgery at St. Louis County Hospital. A member of the Arizona Medical Association for 38 years, Commissioner Oakley has also served as its 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 reappointed in 1997. His term expired in May 2000.

Medical Community

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

Commissioner Pereira received his B.S. in Chemistry from Georgia Tech in 1979. He graduated *Magna Cum Laude* from Emory University School of Medicine in 1983. After completing his Internal Medicine Residency at the same institution, he joined the staff of Mariposa Community Health Center as a Scholar of the National Health Service. He returned to Emory University from 1990-1992 as Assistant Professor of Medicine and Director of the Intensive Care Unit, Grady Memorial Hospital, Atlanta. He has been a Fellow of the American College of Physicians since 1993. In February, 1998 he was named Chief of the Medical Staff and Clinical Services at Mariposa, overseeing an 11-physician group that provides 60 per-cent of the medical care in Santa Cruz County. He was appointed to the Commission by Governor Symington in 1995 to complete the term of Commissioner Carlos Gonzales whose term expired in May 1996. He was reappointed in 1996. Governor Hull appointed him to a second full term in 1999. His term expires in May 2002.

Stuart F. Quan, M.D. Tucson
Professor of Medicine and Anesthesiology
University of Arizona College of Medicine



Commissioner Stuart Quan received his A.B. in Psychology from the University of California, Berkeley, in 1970 and an M.D. from the University of California, School of Medicine, San Francisco, in 1974. He completed an internship and residency at the University of Wisconsin, Madison, and then did three Fellowships: Emergency Medicine, Critical Care, and Pulmonary Medicine. He is currently Professor of Medicine and Anesthesiology and Chief of the Pulmonary/Critical Care Section at the University of Arizona College of Medicine. Commissioner Quan is the founder of the Sleep Laboratory at University Medical Center and served as president of the American Academy of Sleep Medicine in 1999-2000. He is the principal investigator for two sleep research studies funded by the National Institutes of Health. Commissioner Quan was appointed by Governor Jane Hull in May, 2000 and his term expires in 2003.

Scientific Research Community

T. Lon Owen, Ph.D. — Flagstaff
Professor of Medical Anatomy and Physiology
Northern Arizona University

Commissioner Owen received his B.A. in Zoology from the University of California, Davis; a master's degree in Biology from California State University; Sacramento, and his Ph.D. in Physiology from U. C. Davis. He was a National Institutes of Health Postdoctoral Fellow at Michigan State University and Visiting Associate Professor in the Pharmacology Department of the University of Arizona College of Medicine. He has chaired the Research Committees of the American Heart Association at both the Arizona Affiliate and Southwestern Regional levels. His publications are in the areas of cardiovascular, aging and environmental physiology. Commissioner Owen was appointed to the Commission in June 1998 by Governor Hull and his term expires in May 2001.

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

Commissioner Williams received his B.S. with majors in Chemistry and Physics from the University of Missouri in 1963, 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 study medicine. From 1985 to 1987, he was a clinical instructor in the Joint Program for Radiology and Nuclear Medicine at Harvard. He has authored numerous publications in the areas of physics and medicine. Commissioner Williams was appointed by Governor Symington in 1994 and reappointed in 1997. Commissioner Williams was appointed to a third term by Governor Hull in May 2000 and his term expires in 2003.

Commission Staff

Dawn C. Schroeder, D.D.S., M.A.
Executive Director

Dr. Schroeder received her B.A. in Biology and Psychology from Augustana College in 1969, her D.D.S. with Thesis Honors from State University of New York at Buffalo in 1975 and her M.A. in Health Services Administration from Webster University, St. Louis, Missouri in 1981. She completed a residency in Oral and Maxillo-facial Surgery at the Naval Hospital, Oakland, California in 1985. Dr. Schroeder has held her present position since September of 1992.

Damika D. Brock
Executive Staff Assistant

Ms. Brock joined the Commission staff in October of 1993. She was promoted to her current position in September, 2000. Ms. Brock is responsible for purchasing, travel and payroll as well as contract review. She is also a part-time student at Phoenix College where she is pursuing a business management degree.

Debra Young
Accountant II

Ms. Young came to the Commission in November, 1999 from the Arizona Department of Health Services where she had worked for seven years. Ms. Young is responsible for contract expenditure and other contract related accounting. She is a part-time student at Arizona State University where she is a senior, majoring in accounting.

Commission Staff

Daniel Powell
Fiscal Services Specialist II

Mr. Powell has been with the Commission since June of 1994 and accepted his current position in March of 1997. He is responsible for anticancer drug discovery contract expenditures and accounts payable. Mr. Powell spent four years in the U.S. Navy, including a tour of duty aboard the *U.S.S. John F. Kennedy*, prior to joining the Commission staff. He is pursuing a liberal arts degree at Phoenix College where he is completing his sophomore year.

Ismene Quintanilla
Administrative Secretary I

Mrs. Quintanilla came to the Commission in January 1998. She was promoted to her current position in September 2000. She is the receptionist and is responsible for routine clerical tasks as well as maintaining the mailing and peer reviewer databases. Mrs. Quintanilla is a part-time student at Phoenix College where she is pursuing a degree in accounting.

Summary of 1999-2000 Commission Activities

The Commission had 85 contracts in two programs, tobacco-related research and anticancer drug discovery, with medical and health researchers in Arizona as of July 1999. The section headings list the program and whether the project is in its first, second or third year of funding. Research abstracts outlining the progress made during the year are contained in Sections A-C and F. Citations for scientific publications and abstracts arising out of the research are also listed.

Lay summaries for new tobacco related research projects made in 1999 can be found in Section E. These projects are scheduled to begin in FY 2000. The summaries provide an overview of the new research.

Approximately 1012 Requests for Proposals (RFPs) for 1999-2000 awards were mailed to potential applicants in September 1998. The amount available for new tobacco-related research contract awards was approximately $1,900,000. In response to the RFPs, the Commission received 58 proposals. Section D lists the research proposals received in response to the RFP.

In November and December the proposals were sent to a panel of national scientific and medical experts for peer review and evaluation. In January, February and March the Commission received approximately 177 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 19 award-winning research projects from among the applications. During 1999-2000 the ADCRC will be managing 85 contracts.

SECTION A

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR ONE

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY 2000: $148,892

Effect of Antioxidant Nutrients and Smoking on Type-Specific HPV Persistence

During the past year, we have collected data that will be used to identify cervical dysplasia risk factors, nutritional factors and smoking, in order to modify these factors and decrease the overall disease risk. Laboratory assays have been initiated on archived cervical and plasma samples and results entered into the recently developed databases.

Preliminary data analysis from this study suggests that dietary intake of vitamin E, lutein, and vegetables may be protective against HPV persistence, an early stage of cervical cancer. Vitamin E, lutein and vegetable consumption may influence HPV persistence through their roles as antioxidants, which prevent damage caused by oxidative stress. Oxidative stress could produce a decrease in immune function and increase in viral load. These preliminary results suggest that Arizona women residents can reduce their risk of HPV persistence by consuming diets rich in foods containing vitamin E (i.e., vegetable oils), lutein (i.e., spinach and broccoli), and vegetables.

Iman Hakim, M.D.

University of Arizona
Award Amount FY 2000: $125,061

The Role of High Tea Consumption in the Modulation of DNA Oxidative Damage in Smokers

Changes in dietary habits, with the intake of more cancer-chemopreventive agents, is potentially a practical approach for cancer prevention in smokers, with tea as one promising agent. The role of tea drinking as a potential inhibitor of carcinogenesis merits careful evaluation. The main aim of the study is to compare high consumers of green or black tea to the control group for the effect of tea consumption on oxidative DNA damage, as measured through urinary 8-OhdG. Adherence and biologic response to the high tea consumption were measured in all randomized participants. Levels of urinary 8- OhdG are determined monthly and will be the endpoint measurements. During year I of this grant, data collection and subject recruitment procedures were established and 50 regular smokers were recruited, randomized to a green or black tea or a control (water) group, and successfully completed the 4-month intervention trial. This study will continue recruitment and implementation over the next two years to complete the intervention for 150 individuals.

Jesse Martinez, Ph.D.

University of Arizona
Award Amount FY 2000: $49,990

p53 Dependent Apoptosis in Lung Cancer

Lung cancer is the number one cancer killer in the United States, with 150,000 new cases diagnosed every year. Its epidemiology and predisposing factors have been intensively studied, and tobacco consumption has clearly emerged as the single most important risk factor for the development of lung cancer. As with other cancers, lung cancer is thought to develop due to the accumulation of multiple genetic mutations that collectively dysregulate cell proliferation. A frequent target of those mutational events is the p53 gene which functions to maintain stability of the genome. Loss of p53 function is thought to lead to an increase in the frequency of genetic mutations that may lead to cancer and a decrease in the processes that can eliminate cells with a high potential for neoplastic transformation. Hence, p53 normally functions to suppress tumor formation.

However, our most recent results suggest that, in the context of at least some lung cancers, wild-type p53 may actually function to support tumor growth. We have found in studying a lung cancer cell line that expresses a wild-type p53 that the presence of the p53 protein makes the cells more resistant to killing by ionizing radiation. Elimination of wild-type p53 actually makes the cells more sensitive to killing by radiation. This is a novel finding and suggest a previously undiscovered function of p53. More significantly, it means that in those lung cancers that continue to express wild-type p53, depletion of the cells of their p53 protein may be an effective means of sensitizing them to conventional anti-cancer therapies.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY 2000: $150,000

Aberrant Expression of Redox-Associated Proteins NF-κB(p65) Thioredoxin and Inducible Nitric Oxide Synthase as Biomarkers of Colon Cancer Risk

We have determined that the normal-appearing flat mucosa of patients with colon cancer is resistant to DOC-induced apoptosis, a controlled form of cell death, using a novel *ex vivo* live cell bioassay developed in our laboratory. Immunohistochemical staining of NF-κB (p65) (a transcription factor that binds to DNA and turns on gene expression), NOS2 (the inducible form of nitric oxide synthase and a downstream target of NF-κB) and thioredoxin (a protein that modulates the binding of NF-κB in the nucleus and decreases colonic cell differentiation) was performed on 113 biopsy specimens from 16 excised colons obtained during surgery. All three of these redox-related, anti-apoptotic proteins were significantly up-regulated in the region of proliferation and differentiation within the colonic crypts, and may prove useful as more practical biomarkers of colon cancer risk on an individual basis.

Publications:

Bernstein C, Bernstein H, Garewal H, Dinning P, Jabi R, Sampliner RE, McCuskey MK, Panda M, Roe DJ, L'Heureux L, Payne C. A bile acid-induced apoptosis assay for colon cancer risk, and associated quality control studies. **Cancer Res** 59:2353-2357, 1999.

Bernstein H, Payne C, Bernstein C, Beard S, Schneider J, Crowley C. Activation of the promoters of genes associated with DNA damage, oxidative stress, ER stress and Protein, malfolding by the bile salt, deoxycholate. **Toxicol Letters** 108:37-46, 1999.

Washo-Stultz D, Hoglen N, Bernstein H, Gerner EW, Garewal H. Role of nitric oxide and peroxyntrite in bile salt-induced apoptosis. **Nutrition and Cancer** 35:180-188, 1999.

Payne CM, Bernstein C, Bernstein H, Gerner EW, Garewal H. Reactive nitrogen species in colon carcinogenesis. Review. **Antioxidants & Redox Signaling** 1:449-467, 1999.

Crowley C, Payne CM, Bernstein H, Bernstein C, Roe D. NAD+ precursors, nicotinic acid and nicotinamide protect cells against apoptosis induced by a multiple stress induces. **Cell Death and Differentiation** 7:314-326, 2000.

Washo-Stultz D, Crowley C, Payne CM, Bernstein C, Marek S, Gerner EW, Bernstein H. Increases susceptibility of cells to inducible apoptosis during growth from early to late log phase: An important caveat for *in vitro* apoptosis research. **Toxicology Letters** 116:199-207, 2000

Bernstein C, Bernstein H, Payne CM, Garewal H. (2000). Field defects in progression to adenocarcinoma of the colon and esophagus. **Electronic J Biotechnol** 3:1-17, 2000.

Donato Romagnolo, Ph.D.

University of Arizona
Award Amount FY 2000: $48,675

Transcriptional Repression of the Breast Cancer Gene BRCA-1 by Tobacco Polycyclic Aromatic Hydrocarbons

The long-range goal of this project is to investigate the role of tobacco smoking in the etiology of sporadic breast cancer. The central hypothesis of this project is that polycyclic aromatic hydrocarbons, compounds found in tobacco smoke, may contribute to breast carcinogenesis through the inhibition of the tumor suppressor gene, BRCA-1. We postulate that the degree of damage caused by PAHs is a function of 1) intensity and duration of exposure, and 2) estrogen receptor status. At non-cytotoxic concentrations (=chronic exposure), PAHs may contribute to breast tumorigenesis through chronic inhibition of DNA repair functions requiring BRCA-1, thus favoring the fixation of mutations. On the other hand, the exposure to cytotoxic concentrations of PAHs (=acute exposure) may allow proliferation of resistant cells with low BRCA-1 and containing mutations or chromosomal aberrations. These effects may be mediated by the presence of the estrogen receptor in breast cells.

Publication:

Jeffy BD, Chen EJ, Gudas JM, Romagnolo D. Disruption of cell cycle kinetics by benzo[a]pyrene: inverse expression patterns of BRCA-1 and p53 in MCF-7 cell arrested in A and G2. **Neoplasia** October Vol. 2, No. 5, 2000.

Seth Rose, Ph.D.

Arizona State University
Award Amount FY 2000 $50,000

Enzyme Active Site Tailored Anticancer Drugs

Cancer cell division in approximately one-third of lung cancers is triggered by a faulty protein. This protein can only exert its detrimental effect after it is modified by a particular enzyme in the cancer cell. Thus, prevention of the enzyme from modifying the faulty protein has become an aggressively pursued anticancer strategy. We are designing, preparing, and testing chemical compounds aimed at blocking the enzyme involved in cancer growth. Our chemical compounds are tailored to fit into the enzyme and chemically react with it, destroying its activity. In cell-free test tube experiments with the enzyme, several of our compounds were found to potently eradicate enzyme activity within minutes. Arrangements have been made with a pharmaceutical company for testing of these compounds against cancer cells grown in culture. These studies may lead to the development of effective anticancer agents for the benefit of Arizona residents.

Publications:

Rose SD, Hartman RF, Ottersberg SR, Okolotowicz KJ, Lefler SR, Kim AY, Lee W-J, Robinson DE. Anticancer strategy based on prevention of ras farnesylation by irreversible inactivation of farnesyl transferase. **Signal Transduction** 2000.

Rose SD, Lefler SR, Ottersberg SR, Kim AY, Okolotowicz KJ, Hartman RF. Anticancer agents based on regulation of protein prenylation. **U.S. Patent and Trademark Office** 2000.

Ottersberg SR. The development of α-dicarbonyl reagents as potential chemotherapeutic agents. **Master's Thesis** Arizona State University, 1999.

Lefler SR. Farnesyl transferase inhibitors and inactivators: potential anti-cancer chemotherapeutic agents. **Dissertation** Arizona State University, 2000

Danny L. Brower, Ph.D.

University of Arizona
Award Amount FY 2000: $48,781

Genetic Probes for the Study of Integrin Structure-Function Relationships

Heart attacks are caused by the cross-linking of aggregates of blood platelets into an occluding clot; the platelets are linked to one another (and to the artery wall) by proteins that are members of the integrin family of cell surface receptors. Our studies are aimed at generating and characterizing a set of mutations in integrin genes. These mutations will provide tools for subsequent studies designed to understand how the structures of integrins relate to integrin function. This basic information can then be used to assist in the intelligent design of agents for the therapeutic inhibition of integrin function. Using a genetic screen in the model organism Drosophila, we have to date identified 25-35 new integrin mutations and have determined the molecular defect in some of these. We continue to create and characterize more potentially informative mutations in the gene encoding the betaPS integrin protein.

Mary C. Davis, Ph.D.

Arizona State University
Award Amount FY 2000: $47,156

The Effects of Smoking and Menopause on Physiological Stress Responses in Middle-aged Women

This project examines the potentially damaging physiological consequences of smoking when it coincides with menopause and stress. To date, approximately 230 women have been contacted to determine their eligibility for participation in the project. Of these, 16 pre- and post-menopausal women have successfully completed the laboratory stress session, during which cardiovascular and stress hormone measures are collected. Most women screened out of participation were ineligible because they were 1) not clearly either pre- or post-menopausal, or 2) taking medications that affect physiological stress responses. The sample recruited is comprised of smokers and nonsmokers, and Caucasians and women of color that appears to be representative of mid-life women in Arizona. As expected, preliminary evaluation of physiologic responses during the laboratory session suggests that the protocol is eliciting a strong reaction in women, which should allow for powerful comparisons between the stress responses of pre- and post-menopausal smoking groups.

Eugene Morkin, M.D.

University of Arizona
Award Amount FY 2000: $150,000

Grafting of Stem Cell-Derived Cardiomyocytes to Repair Myocardial Infarction

Coronary artery disease causes heart attacks (myocardial infarctions), which may result in death or disability from congestive heart failure, and is a major tobacco-related public health problem. After myocardial infarction the heart is unable to repair itself because heart cells in adults have lost their ability to divide. The damaged heart is unable to maintain an adequate output of blood and heart failure follows. The relationship of tobacco smoking to this deadly chain of events is well established and parallels in many ways the association between smoking and cancer. The goal of this proposal is to develop a strategy for repair of myocardial infarction using cardiomyocytes grafting. The aims are to 1) investigate the control of the cardiac cell cycle with the goal of discovering ways to stimulate heart cells to divide, 2) determine the survival of heart cell grafts in animals with experimental myocardial infarction, and 3) determine whether the grafts improve cardiac function.

Publication:

Maitra N, Flink IL, Bahl JJ, Morkin E. Expression of α and β integrins during terminal differentiation of cardiomyocytes. Cardiovascular Research 47:715-725, 2000.

Alexander M. Simon, Ph.D.

University of Arizona
Award Amount FY 2000: $50,000

Role of Gap Junction-Mediated Communication in Preventing Endothelial Dysfunction

There is evidence that smoking contributes to endothelial dysfunction, a key part of the atherosclerotic process. The mechanism by which smoking leads to diminished blood vessel responses is not completely understood. Smoking could lead to a deficiency of gap-junction mediated communication in vascular cells. Gap junctions are aggregations of intercellular channels that connect adjacent cells. They are composed of a family of related proteins called connexins. The objective of this project is to examine the effects of disrupting intercellular communication in the blood vessel wall, using mice which lack specific connexin proteins. In the first year, we examined communication patterns in these mice by injecting tracers and found that endothelial cell communication is partially reduced when Cx37 or Cx40 is missing and may be eliminated when both of these proteins are ablated. We also found that eliminating one connexin causes a reduction in the other connexin, indicating that they may normally interact.

Abstracts

Simon AM, McWhorter AR, Sellitto C, Goodenough DA, Paul DL. Generation of vascular connexin double knockout mice. **The FASEB Journal** 14 (4):p A541, abstract no. 377.2, 2000.

Ronald J. Lukas, Ph.D.

St. Joseph's Hospital
Award Amount FY 2000: $149,954

Molecular Bases for Nicotine Dependence

Nicotine dependence is thought to drive the habitual use to tobacco products by an estimated one million individuals in the State of Arizona alone at enormous economic and personal costs. Nicotine's powerful and multi-faceted effects on the brain and body must begin with its actions at its principal targets, the diverse group of chemical signaling molecules called nicotinic acetylcholine receptors (nAChR). This project seeks to establish how extended nicotine exposure affects individual forms of nAChR.

Work completed in the first year of this renewed project supports our hypothesis that extended exposure to nicotine induces long-lasting changes in numbers and function of nAChR. Doses and time-dependencies of these effects differ across nAChR subtypes. We have found that different nicotine-like drugs mimic or block the effects of nicotine at specific forms of nAChR. We also have made the surprising finding that the loss in nAChR have been extended to examination of nAChR found in neurons, and chemical studies have investigated genetically engineered nAChR like those that predominate in the brain. A new focus of our work is on the numerically most abundant forms of nAChR in the brain. Our studies are identifying nAChR forms that are most powerfully affected by chronic nicotine exposure, and our work is uncovering drugs that mimic or block those effects. This information is of potential use in the design of strategies and therapies to block nicotine dependence or to relieve unpleasant effects of nicotine withdrawal, thereby promoting cessation to tobacco use and control of tobacco-related disease. Our findings prompt new hypotheses that long term losses in nAChR function contribute to collateral changes in brain chemistry and function, thereby contributing to nicotine dependence.

Abstracts:

Fryer JD, Eisenhour CM, Ke L, Lukas RJ. Onset of and recovery from nicotine-induced functional inactivation of nicotinic acetylcholine receptors (nAChR). Soc Neurosci Abst 25 1721, 1999.

Gentry CL, Krishnan C, Eisenhour CM, Lucero L, Lukas RJ. Anesthetics noncompetitively inhibit nicotinic acetylcholine receptor function. Soc Neurosci Abst 26, in press, 2000.

Publication:

Pacheco MA, Pastoor TE, Lukas RJ, Wecker L. Nicotine-stimulated calcium influx in SH-EP-1 cells transfected with $\alpha 4\beta 2$ nicotinic receptors. FASEB J, in press, 2000.

Ronald M. Lynch, Ph.D.

University of Arizona
Award Amount FY 2000: $49,936

Effect of Nicotine on Hypothalamic Glucose-Responsive Neurons

A primary reason smokers choose not to stop smoking is weight gain following cessation of nicotine dosing. Specialized neurons within the hypothalamus of the brain detect changes in nutrients and hormones to regulate metabolic homeostasis and body weight. Our experiments provide a basis for studying the effects of nicotine on these neurons in rodents, since rodents are an accepted model for nicotine exposure and metabolic homeostasis. Initially, we identified markers specific for nutrient sensitive neurons and analyzed their distribution in the hypothalamus. We next learned to isolate these neurons from rodent brains for analysis and growth in cell culture. In the coming year, we will develop techniques for purifying populations of these neurons from the adult brain to study the effects of chronic nicotine exposure on the function of nutrient sensing neurons. These studies will ultimately provide a better understanding of the physiological basis of this important aspect of nicotine addiction. One of the primary reasons smokers choose not to stop smoking is the psychological barrier associated with weight gain which follows the cessation of nicotine dosing. There are specific regions in the hypothalamus of the brain where specialized neurons respond to changes in nutrients and hormones to regulated metabolic homeostasis and, thereby, body weight. Proposed are experiments that set a basis for studying the effects of nicotine on these nutrient-sensing neurons isolated from these hypothalamic nuclei. By analogy with the known changes in glucose sensing by pancreatic β-cells, we propose that the effects of nicotine may be mediated through altered hypothalamic control. The neuron type central to his mechanism responds to increases in ambient glucose by becoming activated. In the proposed experiments, these Glucose-Responsive Neurons will be selected and characterized, and the effects of nicotine on their functional response to glucose will be determined. With an enhanced understanding of the effect of chronic and acute challenges with nicotine on the function of the hypothalamic control centers, ultimately, it may be possible to understand the precise physiological basis of an important aspect of nicotine addiction and its consequences from cellular to the systems level of relevance.

Publication:

Lynch RM, Tompkins LS, Brooks HL, Dunn-Meynell AA. Levin and B.E. Localization of glucokinase gene expression in the rat brain. **Diabetes** 49 (5):639-700, 2000.

Ian D. Bier, ND MS

IB Scientific, LLC
Award Amount FY 2000: $146,666

Auricular Acupuncture, Education and Smoking Cessation: A Randomized, Sham-Controlled Trial

In the 5th quarter of the first year of the project, all goals projected in the extension request have been accomplished. The treatment of the first group commenced, as scheduled, on September 11, 2000. Radio ads are in place and will be airing for recruitment of the second and third groups scheduled for October and November start dates.

Objectives of the study are as follows:

1. Determine the effects of sham acupuncture as compared to true acupuncture given alone and in combination with a smoking cessation educational program on the following dependent variables:
   (a) number of cigarettes smoked during treatment and 3, 6, 9, 12, 15, and 18 months later;
   (b) level of cigarette craving during treatment and 3, 6, 9, 12, 15, and 18 months later;
   (c) participant's health and psychological status at 3, 6, 9, 12, 15, and 18 months following treatment;
   (d) participant drop-out rate from treatment.
2. Determine the efficacy of acupuncture alone, versus in combination with a smoking cessation educational program.
3. Identify predictor variables to determine who might or might not respond to acupuncture and educational intervention based on psychological and health status characteristics predictive of outcome in conventional treatment (*i.e.*, anxiety, depression).
4. Identify possible adverse effects of acupuncture treatment.

Denise J. Roe, Dr. P.H.

University of Arizona
Award Amount FY 2000: $44,924

The Nutritional Impact of Smoking Cessation in Hispanics

The overall goal of this project is to describe the nutritional status of a sample of Mexican-American smokers pre- and post-smoking cessation. We measured changes in energy intake (diet), specific macronutrients (diet), and selected micronutrient (diet and plasma) in 108 Hispanic smokers pre-quit and 39 successful quitters after 10 weeks. We found lower levels of essential micronutrients at baseline and found increases, especially for females, after 10 weeks of continued abstinence from smoking. We found very weak correlations among micronutrients reported in the diet and analyzed in the plasma, especially at study baseline. Our findings corroborate other studies' findings that smokers consume fewer servings of fruits and vegetables known to contain these nutrients. It appears that the dietary and plasma levels of micronutrients are the same as measured in non-Hispanic whites. Important differences were observed among gender, body mass index and smoking categories.

Paul A. St. John, Ph.D. (Mary Johnson)

University of Arizona

Award Amount FY 2000: $46,779

Effect of Nicotine on Neuronal and Glial Development: Interaction with Ethanol

Newborn infants may be exposed to two drugs, nicotine via smoking and alcohol. In Arizona, yearly 8500 pregnant women who smoke will deliver infants three times more likely to be premature with increased mortality and morbidity. Conservatively, 200-300 newborns will be born yearly with significant effects of prenatal exposure to alcohol. Because drinking and smoking are strongly linked, many infants will have co-exposure to both nicotine and alcohol. Our studies in the past year have shown that very low concentrations of nicotine slow the growth of nerve cell processes. In addition, similar low levels of nicotine also significantly reduce the numbers of the glia, the supporting cells in the nervous system responsible for nerve cell development and function. Co-exposure of cultures to both nicotine and alcohol have not shown a significant interaction of the two drugs at a few selected concentrations.

Thomas G. Beach, M.D

Sun Health Research Institute
Award Amount FY 2000: $36,025

Smoking as a Disease Modifier in Alzheimer's Disease: An Autopsy Study

The purpose of this project is to assess whether the age of onset, duration or severity of Alzheimer's disease (AD) differs between smokers and non-smokers. This is to be accomplished using data and brain tissue from AD patients at the Sun Health Research Institute. During the first year of this project, we have completed 64 telephone interviews to the next-of-kin of the research subjects. The histologic work has also been completed during this first year. This work involved the collection of tissue from 60 cases and staining of sections from 3 regions from each case with 2 different methods, the Gallyas method for tangles and Aβ immunohistochemistry for plaques. The work remaining for the final year of the project consists of counting the tangles and plaques, performing the statistical analyses, and publishing the results.

Dianne Lorton, Ph.D.

Sun Health Research Institute
Award Amount FY 2000: $50,000

Nicotine-Induced Effects of Immune Functions: Neural-Immune Mechanisms

Rheumatoid arthritis (RA) represents a significant health problem for Arizona residents. Data supports that nicotine in tobacco smoke may adversely affect RA by changing sympathetic and hypothalamic outflow to alter release of NE (epinephrine) and cortisol levels. These nervous system pathways modulate immune functions that may contribute to disease pathology. Our studies examine whether nicotine increases arthritis severity and whether nicotine does so by altering nervous system pathways that modulate immune functions. We observed an increase in disease severity measures following chronic nicotine treatment. We propose that chronic nicotine treatment will shift T helper cell cytokine production toward a Th1 cytokine profile that would be expected to promote inflammation in arthritic joints. These data suggest that chronic nicotine exposure as a result of smoking or treatments to quit smoking exacerbates arthritis. Our preliminary findings indicate that one way nicotine promotes inflammation in arthritis is to shift T helper cell cytokine patterns toward a Th1 cytokine profile.

SECTION B

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR TWO

Anne E. Cress, Ph.D.

University of Arizona
Award Amount FY 2000: $48,900

Targeting of Lung Cancer Cells with Anti-Adhesive Agents

Our project is to test if anti-adhesive agents (peptides) can be developed which could prevent human lung tumor cells from adhesion to a protein called laminin in the lung. As reported previously, we have obtained pure peptide reagents that are present in mg amounts. We have discovered that the peptides are biologically active as determined by the human lung cell adhesion test. The peptides can inhibit human lung tumor cell adhesion to laminin. We have unexpectedly made three discoveries. One peptide promotes adhesion of the cells to laminin. This reagent may have the potential to promote laminin adhesion in other cell types, that contain laminin receptors. This may be useful for the improved adhesion of normal human cells to implants, such as vascular grafts. We have also discovered a variant of the human lung cancer cells that attaches readily to laminin. This variant line will serve as sentinel cells for further testing of anti-adhesive agents. We have postulated a consensus sequence using the positive sequences found in the screen and have found that this peptide (HYD1) is biologically active. A limited search of an electronic database has not shown that any currently available chemical compounds exist which would mimic the HYD1 peptide. Our current preliminary information suggests that the peptide will alter the sensitivity of the cells to the lethal effects of DNA damaging agents, such as ionizing radiation and chemotherapeutic agents. This research will lead to the development of anti-adhesive agents to prevent the persistent adhesion of human lung tumor cells within in the normal tissue and to modify the survival response of cells to damaging agents.

A. Jay Gandolfi, Ph.D.

University of Arizona
Award Amount FY 2000: $49,067

Role of Tobacco-Derived Cadmium in Prostate Disease

Precision-cut prostate slices offer a valuable new model for studying the prostate. This human slice system has advantages since it maintains the critical cell-cell interactions that are required for the proper functioning of this complex organ. In our initial studies, human prostate slices were maintained up to 3 days. However, due to the hormonal dependence for cell-cell interactions in prostate tissue, un-supplemented media was unable to maintain optimal prostate function. Thus, prostate slices were incubated for 1-3 days in a medium supplemented with hormones (pituitary extract, insulin, hydrocortisone, transferrin, and human epidermal growth factor). Human prostate slices incubated in hormone-supplemented media had enhanced prostate specific antigen and reduced cellular leakage of proteins. These results show that the supplementation of hormones, in particularly dihydrotestosterone, increases the viability and maintains cellular function of this human prostate model. This improved model will allow a better assessment of the impact of tobacco-derived cadmium on this target tissue.

Abstracts:

Schmelz M, Barrera J, Sallam K, Weyer C, Clark V, McDaniel K, Gandolfi AJ, Parrish AR, Weinstein R, Nagle RB. Human prostate organ culture as a model of adhesion molecule rearrangement. **Arizona Cancer Center Science Fair,** 1999.

Schmelz M, Sallam K, Parrish AR, Gandolfi AJ, Jones JCR, Nagle RB. Adhesion molecule rearrangement in human prostate organ culture. ASCB, December 1999.

Orozco J, Parrish AR, Schmelz M, Nagle RB, Gandolfi AJ. Optimization and characterization of precision-cut prostate slices as a tool for toxicological studies. Tox Sci 54:A1768, 2000.

A.A. Leslie Gunatilaka, Ph.D. University of Arizona

Award Amount FY 2000: $47,394

Mechanism-based Discovery of Novel Antitumor Agents for Plant

During the course of the second year of this project, 155 plant species were collected and extracted yielding 465 extracts. These extracts were subjected to two "in-house" mechanism-based anticancer bioassays. These bioassays detect compounds which have the ability to modify or cleave DNA, two mechanisms by which cell proliferation may be stopped, leading to suppression of growth of cancerous tissues. We have also initiated testing of some extracts in cancer cell line bioassays.

Of the 465 extracts tested, a total of 16 extracts (derived from 8 plant species) showed moderate potential anticancer activity in the above two mechanism-based bioassays. We have initiated bioassay-guided fractionation of one of these extracts, namely that of *Guardiola platyphylla* and continued fractionation studies we initiated last year on two plants, namely *Acourtia thurberi* and *Phoradendron juniperinum*. A novel isocedrane with moderate anticancer activity has been isolated and partially characterized from *Acourtia thurberi*. Currently we are directing most of our efforts to isolate and characterize potential anticancer compounds from these three plants based on their activity and previous reports. If these compounds turn out to be active against solid tumors such as colon and lung cancers, our results will have an impact on the elderly and tobacco-dependent portions of Arizona's population.

Evan M. Hersh, M.D. University of Arizona

Award Amount FY 2000: $150,000

Program Project to Develop Novel Gene Therapies for Tobacco-Related Cancers

The purpose of this program is to develop immuno-gene therapy for lung cancer via animal models, clinical trials and immunological studies. In Project I the clinical protocol has been initiated and 6 patients have been entered on the study. The treatment was well tolerated by all patients. Thus far 3 patients have gone off study because of progressive disease, 2 patients are active and 1 is just starting. In Project II the animal model has been fully developed. Transfection of 3LL lung cancer with HSP-65 has been successful. 3LL transfected with HSP-65 or the Null plasmid loses its tumorigenicity upon subcutaneous injection. Vaccination with transfected cells or Null plasmids does not induce tumor protection against parental tumor. Complimenting this work with lung cancer, we also showed that A375 melanoma and HEY ovarian cell tumors have prolonged survival when tumor bearing animals are inoculated with HSP-65 or the Null plasmid. In Project III we developed the immunological assay methods to characterize the patients receiving the gene therapy for lung cancer as well as the animal model subjects.

Douglas F. Lake, Ph.D.

University of Arizona
Award Amount FY 2000: $50,000

Development of a Peptide Therapy for Small Cell Lung Cancer

There is a paucity of reagents to diagnose and treat lung cancer. We are screening millions of compounds (peptides) with lung cancer cell lines. In this study lung cancer cells were co-incubated with combinatorial, bead-bound peptide libraries. Each bead has a single peptide species protruding from it so that a receptor or other protein on a lung cancer cell may bind it. The peptide is like a key, while the cell surface receptor is its cognate lock. Usually, only one key fits one lock. Lung cancer cells formed rosettes around individual beads suggesting a specific peptide-cell surface receptor interaction. Over 200 beads that were surrounded by lung cancer cells were selected from the library. Specificity was not confirmed, as colon cancer cells formed a rosette around the same beads as the lung cancer cells did. We are in the process of rescreening peptide beads and retesting them for binding to lung tumor cell lines. After reconfirmation, the peptides will be cleaved from the beads and tested for the ability to kill lung tumor cells.

Robin K. Pettit, Ph.D.

Arizona State University
Award Amount FY 2000: $150,000

Biological Research for Advancing New Anti-infective Drugs to Clinical Trials

Infectious disease is a leading cause of death in cancer patients. In 1998, 8,500 Arizona residents died of cancer or associated infectious disease. ADCRC funding has facilitated expansion of the Cancer Research Institute's (CRI) drug discovery program to include antimicrobials. In the past year, we used a microtiter format to screen natural products, semisynthetic and synthetic compounds for antibacterial and antifungal activities. The antimicrobial activities of the cribrostatins, a variety of combretastatins and montanastatin were reported. Preclinical *in vitro* evaluations and mechanism studies of dolastatin 10 (antifungal), the dolastatin 10 derivative Dov-Val-Dil-Dap-Phe-OMe (antifungal), 3β-acetoxy-17β-(L-prolyl)amino-5α-androstane (antibacterial) and 1-(3',4',5'-trimethoxyphenyl)-2-nitro-ethylene (antifungal) continued. *In vivo* toxicity and efficacy studies are in progress for the dolastatin 10 derivative Dov-Val-Dil-Dap-Phe-OMe, spongistatin 1 and 3β-acetoxy-17β-(L-prolyl)amino-5α-androstane. With ADCRC support, such promising compounds will proceed much more rapidly from the laboratory to thousands of Arizona patients with tobacco-related cancer and associated infectious disease.

Publications:

Pettit GR, Toki BE, Hearld DL, Boyd MR, Hamel E, Pettit RK, Chapuis JC. Antineoplastic agents 410. Asymmetric hydroxylation of *trans* combretastatin A-4. J Med Chem 42:1459-165, 1999.

Pettit GR, Tan R, Melody N, Kielty JM, Pettit RK, Herald DL. Tucker BE, Mallavia LP, Doubek DL, Nissen KA, Schmidt JM. Antineoplastic agents 409. Isolation and structure of montanastatin from a terrestrial actinomycete. **Bioorganic and Medicinal Chemistry** 7:895-899, 1999.

Ovechkina YY, Pettit RK, Cichacz ZA, Pettit GR, Oakley BR. Unusual antimicrotubule activity of the antifungal agent spongistatin 1. **Antimicrobial Agents and Chemotherapy** 43:1993-1999, 1999.

Pettit RK, Cage GD, Pettit GR, Liebman JA. Antimicrobial and cancer cell growth inhibitory Activities of 3β-acetoxy-17β3-(L-propyl) amino-5cα-androstane. **International Journal of Antimicrobial Agents** 15:299-304, 2000.

Pettit GR, Grealish MP, Hearld DL. Boyd MR, Hamel E, Pettit RK. Antineoplastic agents 443. Synthesis of the cancer cell growth inhibitor hydroxyphenstatin and its sodium diphosphate prodrug. **Journal of Medicinal Chemistry** 43:2731-2737, 2000

Abstracts:

Liebman JA, Pettit RK. Antibacterial activities of the steroidal peptide 3β-acetoxy-17β-(L-prolyl)amino-5α-androstane. **39th Interscience Conference on Antimicrobial Agents and Chemotherapy**, San Francisco, CA, 1999.

Crews LC, Pettit RK. *In vitro* antifungal activity of 1-(3',4',5'-trimethoxyphenyl)- 2-nitro-ethylene. **39th Interscience Conference on Antimicrobial Agents and Chemotherapy**, San Francisco, CA, 1999.

Joy J. Winzerling, Ph.D.

University of Arizona
Award Amount FY 2000: $50,000

The effects of Chemicals Found in Cigarette Smoke on Iron Metabolism of Lung Cancer Cells

Iron is an essential nutrient. The body makes proteins that can store and transport iron safely because free iron can provoke the formation of toxic free radicals. Both free radicals and iron are found in cigarette smoke and people exposed to smoke or iron dust often develop lung diseases. We are studying the effects of iron and smoke on lung cancer cells to determine what happens to lung cells exposed to airborne iron, and if we can reduce iron-related damage to the cells by administering compounds that bind iron. We have found that lungs cells have the same iron metabolism pathway as other cells, but they use this pathway differently depending on the iron concentrations to which they are exposed. We also have found that lung cells change the levels of messages for proteins involved in cell growth and death as a result of exposure to iron.

Publication:

Eisenstein RS, Iron regulatory proteins and the molecular control of mammalian iron metabolism. (Eds. McCormick, D.B., Bier, D.M. and Cousins, R.J.) Annual Review on Nutrition Volume 20:627-662, 2000.

Henry I. Yamamura, Ph.D.

University of Arizona
Award Amount FY 2000: $49,500

Adenylyl Cyclase Superactivation after Chronic Opioid Receptor Stimulation

Opiate drugs are the primary choice in the management of pain from various forms of cancer, including lung cancer resulting from smoking. Lung cancer has been responsible for 440 deaths per 1 million population in the period 1986-1990 in Arizona. We and others have found that chronic use of opiates causes tolerance or decreased responsiveness to the opiates and second messenger alterations such as adenylyl cyclase (AC) superactivation or AMP overshoot. Adenylyl cyclase superactivation has been proposed to play an important role in opiate tolerance, dependence and withdrawal.

The construction of recombinant cell lines stably expressing the human delta opioid receptor provides a useful tool to study the molecular mechanisms of adenylyl cyclase superactivation. We have shown previously that Chinese Hamster Ovary (CHO) cells expressing the human delta opioid receptor exhibit AC superactivation and cAMP overshoot in response to chronic SNC 80, a delta opioid agonist. Co-expression of the alpha-subunit of transducin-1 and the human delta opioid receptor blocks AC superactivation and cAMP overshoot. We hypothesize that the expression of alpha-transducin-1 scavenges free beta-gamma subunit of the G-proteins ($G\beta\gamma$) and that $G\beta\gamma$ subunits play an important role in opioid-mediated AC superactivation in CHO cells expressing the human delta opioid receptors.

Thomas P. Davis, Ph.D. 9909

University of Arizona

Award Amount FY 2000: $48,290

Nicotine Effects on Blood-Brain Barrier Integrity Function and Permeability

The blood-brain barrier (BBB) is a system of small capillaries in the brain that regulate the level of molecules entering and exiting the brain. Despite the importance of the BBB in maintaining the cerebral environment, remarkably little is known about the effects of nicotine on the BBB. Since smoking is a risk factor for stroke to Arizona residents, it is likely that nicotine may have an effect on permeability characteristics and the expression of tight junctional associated protein at the BBB. *In vitro* experiments have shown that both nicotine and cotinine together modulate the integrity of the BBB by directly opening the paracellular route of solute entry into the brain. We have also shown that acute nicotine treatment results in an opening of the BBB, shown by a statistically significant increase in [^{14}C]sucrose permeability. Additionally, *in vitro* exposure to both nicotine and cotinine and *in vitro* exposure to nicotine depleted the expression of tight junctional protein, ZO-1, in cerebral endothelial cells. These data provide evidence that nicotine alters BBB permeability, which may be due to changes in tight junctional protein expression of ZO-1.

Paul F. McDonagh, Ph.D.

University of Arizona
Award Amount FY 2000: $48,201

The Effects of Cigarette Smoke Exposure on the Leukocyte Contribution to Ischemia-Reperfusion Injury in the Heart

This research project investigates the effects of side-stream cigarette smoke (SSCS) exposure on the leukocyte contribution to ischemia-reperfusion injury in the heart. Two studies (study 3 and 4) have been the focus of the second year of this project. Study 3 investigated circulating neutrophil activity after mild exposure (1 hour/day for 3 days) to SSCS. The results of Study 3 identified no statistical significance in neutrophil activity between Sham-control and Exposure animals. Measurement of myeloperoxidase (MPO) activity in tissue revealed a significant increase in lung MPO activity (a marker of neutrophil accumulation in tissue) in the Exposure group ($P<0.08$). Heart MPO activity between groups was similar. In progress, Study 4 examines circulating neutrophil activity and accumulation in heart tissue after an induced myocardial ischemic event (heart attack). Preliminary results suggest an increase in neutrophil activity in the Exposure group early into reperfusion. In addition, survival after infarction maybe decreased in animals exposed to SSCS.

Abstract:

Mendoza SV, Gonzales RF, Hokama JY, Cohen Z, Davis-Gorman G , McDonagh PF. Mild exposure to side stream cigarette smoke does not significantly alter circulating neutrophil activity. **FASEB Journal** 14:A607 Abstract 449.21, 2000.

Raymond B. Nagle, M.D.

University of Arizona
Award Amount FY 2000: $116, 219

Improved Extracellular Matrix for Treatment of Tobacco Related Occlusive Vascular Disease

Aging and smoking remain major risk factors for the development of diseases of the blood vessels. Surgical treatment for damaged blood vessels remain limited to the use of devices that bypass the area of disease. Unfortunately, these devices do not perform optimally primarily because they are produced from materials that are not living. The results of the research performed as part of this research program provide evidence that a new type of artificial blood vessel may soon be available. Using a cell based treatment, the new vascular replacement device shows the ability to stimulate the formation of new blood vessels. Basic science research and preclinical animal studies have shown that these new devices exhibit improved function and may be able to replace diseased blood vessels. In the near future this research may provide an artificial blood vessel that stimulates the formation of a new living blood vessel in a patient which will remain functional for decades.

Duane Sherrill, Ph.D.

University of Arizona
Award Amount FY 2000: $49,903

Assessment of Patterns on Mendelian Inheritance of Addiction to Cigarettes

Our study objectives were to evaluate familial aggregation and mode of inheritance of nicotine addiction among smoking-dependent and non-dependent subjects in a population of Tucson families. For purposes of differentiating between dependent and non-dependent smokers for segregation analysis, the smoking-dependent phenotype was defined as current or past smoking of more than 20 cigarettes a day. We found no evidence of a major gene that controls this addiction phenotype. Our initial segregation analysis results suggest that an environmental model fits the data as well as the unrestricted model ($p>0.25$). It thus appears that parents and offspring have different distributions of the phenotype and that environmental factors account for the familial correlations observed. However, in our population there are significant gender and age differences in smoking habit. To correct for these and to obtain better resolution of the phenotype, we redefined smoking-dependence as "usual number of cigarettes smoked per day", adjusted for gender and age, and ran familial correlation and segregation analyses on the standardized residuals. In this analysis, we found significant correlation between mothers and daughters and between siblings (especially sisters). However, there was still no evidence of a major gene determining how many cigarettes/day are smoked on a usual basis in families (i.e., smoking-dependence).

Michael Berens, Ph.D.

St. Joseph's Hospital
Award Amount FY 2000: $49,387

Human Recombinant Receptor of Nicotine

Nicotine is a powerful tobacco substance that affects the brains and bodies of an estimated 25% of Arizonans, including adolescents. Nicotinic acetylcholine receptors (nAChR) are the molecular targets of nicotine action. nAChR play important roles in chemical signaling throughout the brain and body, and they usually respond to the natural chemical messenger, acetylcholine. When nAChR are briefly exposed to nicotine, they respond just as they do to acetylcholine. However, whereas acetylcholine is quickly eliminated from sites where it acts with nAChR, nicotine can act at those receptors for much longer, producing another spectrum of effects on the brain and body. Nevertheless, our understanding of how nicotine affects the human brain and body and contributes to habitual use of tobacco products is deficient.

In this project, powerful genetic engineering techniques have been used to introduce genes that code for human nAChR building blocks (subunits) in different combinations into human cell lines. Following our previous success in creating cells that make nAChR composed of only one kind of subunit, we have now succeeded in producing cells that make three different kinds of more complex nAChR, those containing α4 and β2 subunits, α4 and β4 subunits, or α4, β2 and α5 subunits. The genetically engineered α4β2 and α4β4 subtypes of nAChR have exceptionally strong interactions with nicotine. However, differences in these subtypes are clear when examining their interactions with nicotinic drugs such as cystisine, which is much more effective in stimulating α4β4-nAChR than α4β2-nAChR. Also, addition of α5 subunits to α4 and β2 subunits produces nAChR with diminished affinity for nicotine and many of its analogs. It has been necessary for us to prepare several of our own clones for nAChR subunits, but we are now ready to create cells that make nAChR composed in binary combinations of α2, α3, or α6 subunits with β2 or β4 subunits. nAChR composed of these subunits may mediate influences of nicotine on pleasure/reward, mood, and learning/memory. We also have updated many of our techniques for studies of nAChR, making them more amenable to high-throughput analyses. Our engineered cells represent valuable models for studies of acute or chronic nicotine action, particularly with relevance to development of nicotine dependence. Our studies continue to provide novel information and research tools of value to academic and industrial communities.

Allan L. Bieber, Ph.D.

Arizona State University
Award Amount FY 2000: $149,783

Composition of A Unique Receptor for Nicotine

The precise compositions, structures and functions of several neuronal forms of nicotine acetylcholine receptor (nAChR) have not been established. This deficiency compromises our understanding of their role in nicotine dependency and how nicotine affects functions of the nervous system that are controlled by nAChRs. Precise, sensitive techniques need to be developed for analyses of membrane associated protein complexes such as nAChR. One practical aim of this research project was achieved, namely to prepare a well-characterized subtypes of nAChR to different levels of purity and to use the materials as experimental standards for analyses by newly developed mass spectrometric (MS) techniques. Affinity mass spectrometric methods are rapid and sensitive and provide high resolution and great flexibility for samples at different levels of purification. Highly significant results were obtained with nAChR from *Torpedo californica* and α7-nAChRs derived from over expression in cultured cells. Molecular biological studies generated full length and truncated forms of nAChRs in cultured cells for use in additional structural studies by MS methods. The long-term goals of the project remain unchanged as we continue to explore compositions of less abundant forms of nAChRs from neuronal tissues and ancillary proteins that are closely associated with these important complexes. The methods and results from the first two years of the project have supplied us with tools to isolate and characterize some the less common forms of AChRs.

Joseph Grandpre, Ph.D.

University of Arizona
Award Amount FY 2000: $47,825

Diverted Youth: Testing a Tobacco Cessation Intervention for Adolescents

The second year of this research project designed to assess the relative efficacy of theoretically-based tobacco cessation counseling interventions for adolescents has been productive and continues what promises to be an important study. The program has been fully implemented for 16 months. Administrative personnel have been hired and trained. Adult and peer counselors have been hired and trained, and they have initiated counseling sessions with 153 clients (103 of whom have completed the program). Existing contacts with community resources such as schools, juvenile court officials, and probation officers have been maintained and new contacts have been established. Previous efforts to develop contacts in more remote areas of the state have been successful and these efforts continue. Program expansion and collaboration efforts also continue to be discussed with the Arizona Smokers' Helpline and the Tobacco Education and Prevention Program. Increasing enrollment and data analysis are the central aims for Year 2.

Daniel E. Shapiro, Ph.D.

University of Arizona
Award Amount FY 2000: $49,963

Physician-Patient Communication with Heavy Smokers: Comparing Motivational Interviewing with the Perspective Approach

Every year approximately 75% of ambivalent smokers in Arizona come in contact with a health professional. Unfortunately, while we have established guidelines for how professionals should communicate with resistant smokers, we have little data. This study compares smoking rates among smokers exposed to a prescriptive health professional who conducts a thorough but traditional authoritarian intervention in which smokers are urged to quit, with those exposed to a health professional using modified motivational interviewing, a technique that asks smokers to generate their own ambivalences about smoking and then emphasizes those back to the smoker. To date, we have recruited 190 smokers for this laboratory based study in its second year, and are ahead of schedule in recruitment. Preliminary results suggest that female smokers who receive a motivational interview from a health professional are more likely to significantly reduce their smoking behavior (as measured by biological verifications) than those receiving authoritarian prescriptive interventions. These results are preliminary. However, if these trends continue, we may be able to significantly reduce the number of female smokers in Arizona by teaching health professionals to use motivational interviewing rather than the prescriptive approach.

Neil M. Ampel, M.D.

University of Arizona
Award Amount FY 2000: $ 49,995

The Effect of Cigarette Smoking on Immune Function and Outcome in Male Patients with Active Coccidioidomycosis in Arizona

During the second year of this study examining differences in outcome and immune response to Valley Fever (coccidioidomycosis) between male smokers and non-smokers, we have studied a total of 83 subjects, of whom 59 were non-smokers and 24 were smokers. There were no significant differences between smokers and non-smokers with regard to race, ethnicity, underlying disease, type of coccidioidomycosis, age, or length of time with coccidioidomycosis. In addition, we have developed a technique whereby immune response to coccidioidomycosis can be assessed by examining blood from donors using flow cytometry. Analysis of groups with various forms of coccidioidomycosis using this method revealed that immune cells from those with active coccidioidomycosis produce significantly less interferon-gamma, a substance important in immune response, than cells from healthy, immune donors. This technique will be used to further define why those with active coccidioidomycosis fail to mount an appropriate immune response.

Publication:

Ampel NM, Christian L. Flow cytometric assessment of human peripheral blood mononuclear cells in response to a coccidioidal antigen. Med Mycol 37:123, 2000.

R. Clark Lantz, Ph.D.

University of Arizona
Award Amount FY 2000: $50,000

Environmental Tobacco Smoke Exposure in Developing Lung: Effects on Surfactant Metabolism

This application is investigating the effects of environmental tobacco smoke (ETS) on production of surfactant in developing postnatal lungs. Surfactant is a complex mixture of phospholipids and proteins that line the lungs and is essential for appropriate lung function. This year we have investigated mediators (transforming growth factor beta (TGF-β) and the antiinflammatory cytokine interleukin-10 (IL-10) that can affect the production of surfactant in neonatal lungs. We have determined the normal expression of these mediators and have been investigating the effects of ETS on their expression. We have identified the location and temporal expression of the three different forms of TGF-β in neonatal lungs. In addition, we have shown that exposure to cigarette smoke can lead to down regulation of IL-10 in the lung potentially leading to chronic inflammation. Since ETS exposure of young children can lead to severe lung problems, this research is important for the children of Arizona.

Abstracts:

Boyer TR, Lantz RC. Particulate and LPS exposure effects on stimulation of macrophage mediator expression. **Molecular Biology of The Cell** 10:2609, Suppl. S 1999.

Younis HS, Lantz RC. Localization of transforming growth factor -β1, -β2 and β3 protein expression during neonatal lung development **FASEB J** 14:A780, 2000.

Steven J. Barker, M.D.

University of Arizona
Award Amount FY 2000: $49,821

The Effects of Nicotine Patches on Maternal and Fetal Health

The Arizona Department of Health Services estimated that approximately 9000 women gave birth in Arizona during 1995 and 11% of those women voluntarily reported using tobacco products during pregnancy. Using tobacco products increases the risk of serious complications during pregnancy like miscarriage, still birth, and spontaneous abortion. One of the main constituents of tobacco products, nicotine, is suspected of causing some of these devastating outcomes. Many women try to quit smoking during pregnancy and may incorporate the nicotine patch into their stop smoking program. Using sheep as an animal model for human disease, we have demonstrated that nicotine blood levels attained in non-pregnant sheep wearing nicotine patches are comparable to those measured in humans wearing a nicotine patch. We have also documented that physiological changes do occur in non-pregnant sheep wearing nicotine patches. This data is an important first step in documenting any adverse effects that nicotine derived from nicotine patches may have during pregnancy.

We are currently finishing data collection in pregnant sheep and the lamb fetus. Results have not yet been evaluated as to confound the study. We will finish data collection in the pregnant ewes and lamb fetus by the middle of the current fiscal year. We are pleased to report that the study is progressing in a satisfactory manner.

Mark Brown, M.D.

University of Arizona
Award Amount FY 2000: $109,218

The Effect of Tobacco and Its Constituents on Placental Development Differentiation and Immunologic Function

This project seeks to determine the effects of smoking by pregnant women on the structure and function of their placenta. Since the placenta is the lifeline between a mother and her developing baby, changes in this organ may account for some of the complications seen in pregnancies of women who smoke. By collecting placentas after the birth of the infants and growing tissue and cells from them in the laboratory, we were able to show that maternal smoking alters the chemical signals used by tissues to communicate with each other in a way that makes it more difficult for the placenta to fight infection. There are also changes in the structural proteins made by the placentas of smoking mothers, changes which may impact the ability of the placenta to provide nutrients to the developing baby. Together, these effects of maternal smoking could contribute to the risks of complications of pregnancy, including low birth weight and prematurity.

Abstracts:

Miller HS, Gustafson M, Baradaran A, Saldana S, Brown M. II-4 production is increase in placentas obtained from pregnant smokers. J Soc Gynecol Investing 79 Suppl:286A, 2000.

Brown MA, Gustafson M, Saldana S, Miller H, Halonen M. Increased capacity of decidua from allergic women to produce II-4. Am J Respir Crit Care Med 161:A599, 2000.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY 2000: $127,675

Effects of Nicotine on the Development of Human T Cells

Although there have been some reports indicating that nicotine has deleterious effects on the immune system, little work has been done to determine the mechanisms of smoking-induced loss of immune function. During the first year of this project, we found that the addition of nicotine to an organ culture system that we have devised for the study of T cell development causes profound changes in mouse thymus-derived (T) cell production. We also found that nicotinic receptors are present on developing T cells, suggesting that nicotine binds to the cells and delivers signals to the cells that alters their development. In the second year of this study, we have characterized the nicotine receptors found in mouse and human thymus cells, as well as when they are expressed during development. We also found that nicotine can activate genes that are involved in the death of T cells, including T cells derived from the cord blood of infants born to women who were smokers. We will continue to use the organ culture method to 1) determine effects of nicotine and related ligands on development of human T cells, and 2) characterize the receptors that are responsible for the recognition of nicotine on different populations of T cells with the goal of developing inhibitors that will prevent the effects of nicotine on T cell development.

Joseph L. Graves, Jr., Ph.D.

Arizona State University West
Award Amount FY 2000: $50,000

The Impact of Nicotine on the Genetics of Aging: Exposure and Selection for Resistance

Long-term intergenerational impacts of nicotine exposure have not been well examined in humans. Correlation between specific disease susceptibilities, reproductive problems, and tobacco use has been established. Animal models have usually focused on chronic exposure impacts within a single generation. This study examines the impact of nicotine exposure on populations of *Drosophila melanogaster* that are genetically differentiated for components of life history (longevity and reproductive effort). The first year of the project established the existence of genetic variation for nicotine tolerance in *Drosophila* stocks and established the threshold toxicity levels for each stock. The longer-lived O stocks derived from selection for delayed reproduction showed significantly greater resistance to nicotine impacts on development. Selection for resistance to nicotine was begun in the second year of the project.

Mark L. Witten, Ph.D.

University of Arizona
Award Amount FY 2000: $126,598

Effects of Vitamin E Dietary Supplementation on Sidestream Cigarette Smoke-Induced Lung Injury

It was hypothesized that younger passive smokers may have an increased utilization rate of vitamin E in the lung, which is at especially high risk for oxidative damage. So, the effects of different doses of dietary vitamin E supplementation on sidestream cigarette smoke (SSCS) were studied in a neonatal mouse model. SSCS results in lung dysfunction in the developing lungs that is associated with changes of cytokine production and slow progress of body weight. Dietary intake of vitamin E (larger than 15-fold) supplementation may improve lung dysfunction of the SSCS-induced local host defense mechanisms. Moreover, vitamin E supplementation had a dose-response relationship for immunological viability. We concluded that the recommended dietary allowance (RDA) for vitamin E may not be adequate for the protection of children from environmental tobacco smoke.

Publications:

Wang S, Watson RR, Young S, Zhang Y, Bradshaw B, Witten M.L. Vitamin E supplementation improves lung function with systemic cytokine dysregulation. **The FASEB Journal** 13:A240, 1999.

Wang S, Young RS, Zhang J, Watson RR, Witten ML. Cytokine production of α- tocopherol supplementation in mice exposed to sidestream cigarette smoke. **The FASEB Journal** 14(4):A173, 2000.

SECTION C

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR THREE

Emmanual T. Akporiaye, Ph.D.

University of Arizona
Award Amount FY 2000: $50,000

Active Specific Immunotherapy of Breast Cancer by Inhibition of an Immunosuppressive Cytokine

We previously showed that antisense TGF-β gene therapy synergized with IFN-γ gene therapy to decrease the tumorigenicity of non-metastatic cancer cells. Here, we use a poorly immunogenic highly metastatic mammary carcinoma 4T1 to demonstrate that unlike B7.1 expression, antisense TGF-β and/or IFN-γ expression by these cells decreases their tumorigenicity. We show that the antitumor response mediated by T lymphocytes and associated with increased IFN-γ production by tumor draining lymph node cells. Additionally, when used as tumor vaccines, IFN-γ and/or TGF-β gene modified cell prolongs the survival of mice with residual metastatic disease. We also provide evidence that TGF-β enhances tumorigenicity by stimulating *in vivo* metastasis of tumor cells. These results lead us to conclude that TGF-β promotes tumor growth by suppressing host antitumor immune responses and stimulating metastasis. Therapeutic strategies that interfere with both processes should result in an improved antitumor effect that will result in a better clinical outcome.

Publication

McEarchern JA, Besselsen DG, Akporiaye ET. Interferon-γ antisense TGF-β transgenes synergize to enhance the immunogenicity of a murine mammary carcinoma. Cancer Immunol Immunother 48:63-70, 1999.

Sherry H. Chow, Ph.D.

University of Arizona
Award Amount FY 2000: $49,999

Pharmacokinetic Modeling of the Anabolites of Nucleoside Analogues

Zidovudine (AZT) is designed to mimic a naturally occurring compound necessary for DNA synthesis. AZT in itself is not active; it needs to be converted inside the cells by a series of enzymes to active products. This research program has studied the formation and elimination of the active AZT products in different animal tissues. The formation and elimination of the active AZT products were found to occur at different rates in different tissues. The elimination of the active AZT products occurred rapidly in the various tissues tested. Failing to maintain high levels of the active AZT products in pharmacologically relevant tissues could result in sub-optimal use of this important drug. Our findings could lead to the development of pharmacological agents capable of modulating the formation and elimination of the active AZT products and could potentially improve the therapeutic use of AZT not only in Arizona residents but also in other HIV infected individuals.

William J. Grimes, Ph.D.

University of Arizona
Award Amount FY 2000: $50,000

HLA & p53: An *In Vitro* Study of Peptide Immunotherapy in Human Lung Cancer

Our goal has been to determine how different amino acids on immunogenic peptides determine binding to cloned *in vitro* expressed Class I MHC molecules. By screening a nonamer random peptide library for binding, we can determine which residue positions determine affinity. There are a number of known tumor antigens which can be processed to peptides that are displayed on Class I MHC molecules. Our studies allow us to modify residues on known cancer antigens to increase affinity, and make the antigens more likely to be recognized during an immune response to cancer. Our goal requires us to demonstrate that our designed peptide antigens are better bound by Class I MHC and that T cells that are immune to the tumor are better able to recognize the analogues. Our results show that our goal is achieved, and we will soon publish out results describing the designer tumor antigens and our methods for improving immune therapy of cancer.

Teh-Li Huo, M.D.

University of Arizona
Award Amount FY 2000: $50,000

Molecular Mechanism of Hormone-Regulated Calcium Transport in Kidney

We have successfully used an animal model to characterize abnormal calcium metabolism. Calbindin-D28k is an important calcium transport protein in the kidney. However, it is also present in the parathyroid gland; although its significance is unknown. We found that the absence of this protein in a special strain of mice (the calbindin -D28k gene knock out mice), the calcium metabolism is markedly altered. This finding has very important clinical implications. The immunosuppression drugs used in organ transplant patients, such as cyclosporin A and FK506, decrease the production of calbindin-D28k in many tissues and leads to high bone turn over and osteoporosis. This animal model can be used to study how to treat or prevent the osteoporosis induced by these drugs.

Douglas F. Lake, Ph.D.

University of Arizona
Award Amount FY 2000: $49,997

Genetic Immunization Against Mutant p53 for Lung Cancer

DNA immunization is a new technology that utilizes DNA, instead of protein, to induce an immune response. Upon immunization with DNA cells either have DNA directly deposited inside them or actively take up the DNA. This tricks the immune system into thinking that the cell containing the DNA is infected with a virus so the immune system will respond against it. Our group has been using DNA immunization to stimulate an immune response against mutant p53, an important gene product involved in many tobacco-related cancers. We developed a DNA vector encoding mutant p53 and used it to immunize mice against p53. Our results show that plasmid DNA gets inside immune cells and travels to immune organs. However, it only induced a weak immune response to mutant p53. In conclusion, since p53 is a "self-protein," stronger measures must be taken to break tolerance (our immune system is tolerant to self) to elicit potent immunity to tumors with mutant p53.

Lynn J. Manseau, Ph.D.

University of Arizona
Award Amount FY 2000: $49,600

Analysis of DrtGEF, A Regulator of RHO-Type GTPase Signaling

The formation and spread of tobacco induced lung cancer is regulated by molecular switches called RHO proteins. We have identified a gene in a fruit fly that encodes a RHO activator protein, DRTGEF. DRTGEF has a close homolog in humans; therefore, we expect that the analysis of its biological role will be relevant to understanding smoking induced carcinogenesis.

We have identified one of the proteins that work in DRTGEF in the cell, CDC42. Based on this finding we have generated molecular tools to investigate the consequence of perturbed DRTGEF/CDC42 signaling within the cell. We have found evidence that CDC42 works with DRTGEF within the fly. We have found some evidence that DRTGEF also works with SPIRE, a known actin binding protein. We produced an antibody against SPIRE that will be useful for future experiments.

Thomas P. Miller, M.D

University of Arizona
Award Amount FY 2000: 200,000

Developmental Treatment of Smoking-Related Cancers

This program (as funded) focuses on multidisciplinary clinical research evaluating new drugs and approaches to improve disease control and cure rates for patients with tobacco-related cancers. Head and neck and lung cancers, caused almost exclusively by tobacco and representing a significant percentage of new cases and deaths from cancer in Arizona each year, are targeted, along with other tobacco-related cancers (*e.g.*, pancreatic cancer). Benefitting from new knowledge from other Arizona Cancer Center (ACC) research initiatives, a series of clinical trials is planned. Of particular interest are strategies to overcome tumor resistance to drug or radiation therapy; drugs with novel mechanisms of action, including those interfering with tumor blood supply; improved drug delivery; and protection of normal tissues during therapy. Progress during year 3 includes significant strengthening of new therapeutics programs for these tumors at the ACC in Tucson and development of infrastructures for this work at the ACC in Scottsdale.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 2000: $450,000

Preclinical Development of New Anticancer Drugs Necessary to Improving Treatment of Tobacco Related Human Cancers

The Arizona Disease Control Research Commission's continued support for the Cancer Research Institute at Arizona State University has led, again, to significant progress in the development of new anticancer drugs for improving the treatment of human cancer patients. The United States National Cancer Institute (NCI) has continued the clinical evaluation of bryostatin 1 with 18 open/active human cancer clinical trials and 32 trials that are now closed, involving 136 clinical centers. Concerning the NCI's clinical evaluation of dolastatin 10, there are 3 open human clinical trials underway and 12 trials have closed. Over half of the trials are now phase II. Support from the Arizona Disease Control Research Commission (ADCRC) was essential for the development of both of theses new anticancer drugs to clinical trials, and the need for that assistance continues. We are pleased to report again that our combrestastatin A-4 prodrug, which was also developed with ADCRC support, continues to demonstrate that it is one of the most powerful cancer antiangiogenesis drugs known. The first 4 phase I human clinical trials continue to make progress and the results have been encouraging. Phase II trials are expected to begin within the year. Other significant advances include the continuation of the preclinical and clinical development of our auristatin PE discovery. All of the clinical trials involve tobacco etiology human cancer types. Finally, we continue to make progress with the preclinical development of our phenstatin, hydroxyphenstatin, and combretastatin A-1 prodrugs. In sum, support from the ADCRC has enabled us to make outstanding advances in the development of new anticancer drugs to improve treatment for tobacco related human cancers.

Publications:

Kamano Y, Kotake A, Hashima H, Hayakawa I, Hiraide H, Zhang HP, Kizu H, Komiyama K, Hayashi M, Pettit GR. Three new alkaloids, convolutamines f and g, and convolutamydine e, from the Floridian marine Bryozoan *Amathia convoluta*. Coil Czech Chem Commun 64:1147-1153, 1999.

Wall NR, Mohammad RM, Nabha SM, Pettit GR, Al-Katib AM. Modulation of clap-i by novel antitubulin agents when combined with bryostatin 1 results in increased apoptosis in the human early pre-b acute lymphoblastic leukemia cell line Reh. Biochem Biophys Res Commun 266:76-80, 1999.

Detivaud L, Pettit GR, Meijer L. Characterization of a novel CDK1-related kinase. Eur J Biol Chem 264:55-66, 1999.

Grosios K, Holwell SE, McGown AT, Pettit GR, Bibby MC. *In vivo* and *in vitro* evaluation of combretastatin a-4 and its sodium phosphate prodrug. **Brit J Cancer** 81:1318-1327, 1999.

Lorenzo PS, Bôgi K, Hughes KM, Beheshti M, Bhattacharyya D, Garfield SH, Pettit GR, Blumberg PM. Differential roles of the tandem ci domains of pkcó in the biphasic down-regulation induced by bryostatin 1. **Cancer Research** 59:6137-6144, 1999.

Mohammad RM, Limvarapuss C, Wall NR, Hamdy N, Beck FWJ, Pettit GR, Al-Katib A. A new tubulin polymerization inhibitor, auristatin pe, induces tumor regression in a human Waldenstrom's Macroglobulinemia xenograft model. **Mt J Oncology** 15:367-372, 1999.

Kamano Y, Kotake A, Nogawa T, Tozawa M, Pettit GR. Application of displacement thin-layer chromatography to toad-poison bufadienolides. **Planar Chromatography** 12:120-123, 1999.

Mohammad RM, Li Y, Mohamed AN, Pettit GR, Adsay V, Vaitkevicius VK, Al-Katib AM, Sarkar FH. Clonal preservation of human pancreatic cell line derived from primary pancreatic adenocarcinoma. **Pancreas** 19:353-361, 1999.

Kamano Y, Nogawa T, Kotake A, Tozawa M, Pettit GR. Separation of toad poison bufadienolides by hydrophobic gel. **J Liquid Chromatog** 22:2455-2465, 1999.

Pettit GR, Lippert JW III, Herald DL, Hamel E, Pettit RK. Antineoplastic agents 440. Asymmetric synthesis and evaluation of the combretastatin a-i sar probes (ls,2s) and (1r,2r)-l-2-dihydroxy-l-(2',3'-dihydroxy-4'-methoxy-phenyl)-2-(3',4',5'-tri-methoxy-phenyl)-ethane. **J Nat Prod** 63:969-974, 2000.

He Z, Eveihoch JL, Mohammad RM, Adsay NV, GR Pettit, Vaitkevicius VK, Sarkar FH. Magnetic resonance imaging to measure therapeutic response using an orthotopic model of human pancreatic cancer. **Pancreas** 21:69-76, 2000.

Lorenzo PS, Beheshti M, Pettit GR, Stone JC, Blumberg PM. The guanine nucleotide exchange factor rasgrp is a novel, high affinity target for diacyiglycerol and phorbol esters. **Molecular Pharmacology** 57:840-846, 2000.

Pettit GR, Knight JC, Collins JC, Herald DL, Boyd MR, Pettit RK. Antineoplastic agents 430. Isolation and structure of cribrostatins 3, 4, and 5 from the Republic of Maldives Cribrochalina *sp.* (*Porifera*). **Nat Prod** 63:793-798, 2000.

Grosios K, Loadman PM, Swaine D J, Pettit GR, Bibby MC. Combination chemotherapy with combretastatin a-4 phosphate and 5-fluorouracil in an experimental murine colon adenocarcinoma. **Anticancer Res** 20:229-234, 2000.

Kamano Y, Kotake A, Nogawa T, Hiraide H, Pettit GR, Herald CL. Separation of the bryostatin derivatives by high performance liquid chromatography. J Lig Chrom and Rel Technol 23:399-409, 2000.

Pettit GR, Toki BE, Xu J-P, Brune DC. Synthesis of the marine sponge cyclo-heptapeptide phakellistatin 5. J Nat Prod 63:22-28, 2000.

Pettit GR, Numata A, Cragg GM, Herald DL, Takada T, Iwamoto C, Riesen R, Schmidt JM, Doubek DL, Goswami A. Isolation and structure of schleicherastatins 1-7 and schleicheols 1-2 from the teak forest medicinal tree *Schleichera oleosa*. J. Nat. Prod 63:72-78, 2000.

Meijer L, Thunissen WH, White AW, Gamier M, Nikolic M, Tsai LH, Walter J, Cleverley KE, Salinas PC, Wu YZ, Biernat J, Mandelkow EM, Kim SH, Pettit GR. Inhibition of ciclin-dependent kinases, gsk-3 and ck1 by hymenialdisine, a marine sponge constituent. Chemistry and Biology 7:51-63, 2000.

Verdier-Pinard P, Kepler J, Pettit GR, Hamel E. Sustained intracellular retention of dolastatin 10 causes its potent antimitotic activity. Molecular Pharmacology 57:180-187, 2000.

Pettit GR, Lippert JW III, Boyd MR, Verdier-Pinard P, Hamel E. Antineoplastic agents 442. Synthesis and biological activities of dioxostatin. Anti-Cancer Drug Design accepted.

Pettit GR, Lippert JW III, Pettit RK. Antineoplastic agents 429. Synthesis of combretastatin a-1 and combretastatin b-1 prodrugs. Anticancer Drug Design accepted.

Pettit GR, Moser BR, Boyd MR, Schmidt JM, Pettit RK, Chapius J-C. Antineoplastic agents 460. Synthesis of combretastatin a-2 prodrugs. Anticancer Drug Design accepted.

Garth Powis, D. Phil

University of Arizona
Award Amount FY 2000: $150,000

Interdisciplinary Basic Science Program Project

This is an interdisciplinary project to study mechanisms of agents involved in the prevention of tobacco related cancers and how these diseases progress. There have been thirteen scientific publications. Project 1 has found that selenium, a dietary constituent that can protect against several forms of human cancer, can increase the activity of a group of enzymes called the thioredoxin reductases. A new thioredoxin reductase has been cloned and the mechanism by which selenium controls thioredoxin reductase activity has been established. Project 2 has found that several genes commonly mutated in human colon cancers affect polyamine and arachidonic acid metabolism. Two of theses genes may be mutated in colonic mucosal tissues of young smokers as a consequence of carcinogens in tobacco, thereby elevating their risk of developing colon cancer. Project 3 has established the mechanisms of the regulation of a group of proteins called matrix metalloproteinases (MMPs) in oral squamous cell carcinoma.

Publications:

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Gasdaska PY, Berggren MM, Berry MJ, Powis G. Cloning, sequencing and functional expression of a novel human thioredoxin reductase. FEBS Lettters 442:105-111, 1999.

Erdman SH, Ignatenko NA, Powell MB, Blohm K, Holubec H, Gerner EW. Alterations in polyamine metabolism in the *min* mouse model of gastrointestinal carcinogenesis. Carcinogenesis 20:1709-1713, 1999.

Powis G, Mustacich D. Thiroedoxin reductase. Biochem J 346:1-8, 2000.

Freemerman AJ, Powis G. A redox-inactive thioredoxin reduces growth and enhances apoptosis in WEH17.2 cells. Biochem Biophys Res Comm 274:136-141, 2000.

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Ignatenko NA, Besselsen D, Powell MB, Valle K, Blohm K, Guillen-Rodriguez J, Gerner EW. Chemoprevention of colon carcinogenesis by DFMO and sulindac in the *min* mouse. Proc AACR 41:494, 2000.

Meyskens Jr. FL, Gerner EW. Development of difluoromethylornithine (DFMO) as a chemopreventive agent. Clinical Cancer Research 5:945-951, 1999.

William R. Roeske, M.D.

University of Arizona

Award Amount FY 2000: $49,500

Regulation of the Human Delta Opioid Receptor

Smoking has been implicated in lung cancer, which is responsible for 44 deaths per 100,000 people in the period of 1986-90 in Arizona. Treatment of lung cancer has only a 13% five year survival. Lung cancer pain is generally controlled using opioid drugs. Effective opioid drugs such as morphine and methadone have a variety of undesirable side effects which include respiratory depression, constipation, nausea, addiction and withdrawal. A new class of opioid drugs known as the δ-opioid receptor drugs have recently been developed and studied in animal models. These drugs are effective in pain relief and have fewer side effects. However, these new drugs also show some evidence of drug tolerance in animal models. Our recent studies provide novel differences between δ-opioid receptor drugs that may be the basis for strategies for using SNC80 or DPDPE in the treatment of lung cancer pain without the induction of tolerance or side effects associated with the current opioid therapy. Results achieved for this year indicate that we have shown important differences between the two leading δ-opioid agonist, DPDPE and SNC80.

Publications:

Quock RM, Burkey TH, Varga E, Hosohata Y, Hosohata K, Cowell SM, Slate CA, Ehlert FJ, Roeske WR, Yamamura HI. The δ-opioid receptor: Molecular Pharmacology, signal transduction, and determination of drug efficacy. Pharmacol Rev in press, 2000.

Okura T, Cowell SM, Varga E, Burkey TH, Roeske WR, Hruby VJ, Yamamura HI. Differential down-regulation of the human δ-opioid receptor by SNC80 and {D-Pen^2, D-Pen ^{5}} enkephalin. Eur J Pharmacol 387:R11-R13, 2000.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY 2000: $37,402

Antitumor Agents Targeting Topoisomerase II

Work in this laboratory involves the rational design and synthesis of novel antitumor agents capable of inhibiting topoisomerase II. This enzyme catalyzes the relaxation of supercoiled DNA during cell division and, therefore, is a valuable target for cancer chemotherapy. The histological types of cancers targeted by the compounds developed in this laboratory include melanoma and non-small cell lung cancers. Both of these cancer types are common in residents of Arizona due to exposure to sun and the use of tobacco. Details of ongoing projects are outlined below:

- **Design of Topoisomerase II-DNA Crosslinkers.** These agents are designed to afford lesions that *cannot* be repaired by the cancer cell. Such repair results in resistance to the antitumor agent.

- **Iminoquinone-Based Topoisomerase II Inhibitors.** These novel topoisomerase inhibitors are undergoing *in vivo* trials at the National Cancer Institute.

- **DT-Diaphorase.** The inactivation of topoisomerase inhibitors by this enzyme is being studied with computer models. It is now possible to design inhibitors that cannot be enzymatically inactivated.

Publications:

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Suleman A, Skibo EB. Insights into the mechanism and substrate specificity of human DT-diaphorase through molecular modeling. **Biochemistry** In press.

Christopher P. Appleton, M.D.

Mayo Clinic Scottsdale

Award Amount FY 2000: $ 50,000

Experimental Determinants of Transmitral and Pulmonary Venous Flow: Further Relations to Left Ventricular Filling Pressure

In this third year of our three-year study, we have continued to obtain left atrial and left ventricular pressure-volume loops. These loops are a graphical representation of how the chambers of the heart function. Certain diseases change the properties of the heart, making it stiffer and preventing it from filling properly. Patients with this problem, experience shortness of breath when they exercise. We are interested in studying the degree of "stiffness" (compliance) and how changes in blood pressures affect other aspects of the heart function. We are concentrating on the left atrium (the boosting chamber) and the left ventricle (the pumping chamber), because it is changes in these heart chambers which results in heart failure symptoms due to abnormal filling. Besides being a significant disease of the elderly, unhealthy lifestyles (*e.g.*, smoking) may also adversely change the heart muscle. This work is important, as the population of Arizona ages. Understanding of how the heart works will improve the quality of care and reduce health care costs.

Ronald L. Heimark, Ph.D.

University of Arizona
Award Amount FY 2000: $132,713

Mechanisms of Vascular Dysfunction in Atherogenesis: Cell-Cell Interactions

Cardiovascular disease (CVD) remains the leading cause of death in the United States accounting for 1 of every 2.4 deaths. More than 50 percent of all deaths due to CVD result from coronary artery disease, which results from reduced blood supply by occlusion of arteries. The major risk factors of atherosclerosis, cigarette smoking, elevated blood cholesterol, and high blood pressure, contribute to endothelial injury. Injury to the endothelial lining of vessels promotes sticking of inflammatory cells, their migration into the smooth muscle layer of the vessel, and the release of cell signaling molecules (cytokines). Our central hypothesis was that intercellular junctional proteins are altered by the early events of atherosclerosis and, consequently, contribute to atherogenesis. Our results show changes in the expression and function of proteins in the adherens junctions (cadherins) and communicating junctions (connexins) between endothelial and smooth muscle cells by cytokines. Some of the mechanisms in smooth muscle cadherin and connexin function that we have elucidated in these studies play a role in cell survival and contribute to accumulation of cells and occlusion of arteries. These studies have provided molecular targets for subsequent drug discovery strategies toward treating atherosclerosis.

Publications:

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Lampe PD, TenBroek EM, Burt JM, Kurata WE, Johnson RG, Lau AF. Phosphorylation of connexin43 on serine368 by protein kinase c regulates gap junctional communication. J Cell Biol 14:1503-1512, 2000.

David K.Y. Lei

University of Arizona
Award Amount FY2000: $50,000

Modulation of Transcription by a Frequent Point Modulation in the Human Apolipoprotein A-I Gene Promoter

In the human apolipoprotein A-I (apo A-I) gene, the G to A substitution at -78 position with a frequency of 20% and C to T substitution at +83 position with a frequency of 6.1% have been found to be associated with increased plasma HDL levels. The promoter activities of apo AI.CAT reporter constructs, containing the two alleles of each point mutation, were determined by transient transfection as well as co-transfection with apolipoprotein regulatory protein (ARP-l) and hepatocyte nuclear factor (HNF4) in HepG2 cells. In the absence of the co-transfection, the transfection of -256A1.CAT with G to A substitution or C to T substitution appeared to increase the apo A-I promoter activity, but the increase was not significant. No marked changes of apo A-I promoter activity were observed in the transfection using the longer constructs -2500AI.CAT, with or without G to A or C to T substitutions. Gel shift assays revealed that G to A and C to T point mutations diminished the binding of certain nuclear factors to different DNA fragments around these point mutations of apo A-I gene promoter in HepG2 cells. Our southwestern blot analyses also confirmed that the interaction with a specific 97 kDa nuclear factor was mostly abolished by using the DNA fragments with G to A (-78) or C to T (+83) substitution. The present data suggest that the reduced binding of nuclear factors to the A allele (-78) or the T allele (+83) may contribute to an increase in apo A-I gene expression, which may subsequently result in the elevated plasma HDL levels reported in human population studies.

Edward Castaneda, Ph.D.

Arizona State University
Award Amount FY 2000: $50,000

Long-Term Effects of Nicotine - New Molecular Mechanisms

Fibroblast growth factor 2 (FGF-2) is a protein found in the central nervous system in most mammals. It is thought to play a role in promoting cell growth during development of the nervous system and in maintaining the health of nerves in the adult nervous system. In cancer, it has been shown that FGF-2 is no longer regulated normally. We have shown that nicotine up regulates FGF-2 gene expression and this abnormal FGF-2 expression may be in part responsible for increase in cancer associated with smoking. Our research is aimed at understanding the mechanism by which nicotine regulates FGF-2 expression in cells derived from the nervous system. We have found a part of the human FGF-2 gene by which nicotine regulates its expression. Our experiments have been focused on finding other proteins that may also be deregulated by nicotine exposure and thus may be implicated in smoking related cancers.

Richard R. Vaillancourt, Ph.D.

University of Arizona
Award Amount FY 2000: $49,183

Intracellular Signal Transduction Pathways Activated by Nicotine

Drugs of abuse, including nicotine, cocaine and opiates, stimulate the production of dopamine in the brain. This stimulation of dopamine production results in addictive behavior. The characterization of reward pathways, as well as pathways implicated in addiction, has provided clues to many aspects of substance abuse including craving and reward. Our lab is interested in tyrosine hydroxylase (TH), the rate-limiting enzyme involved in the production of dopamine. We have discovered a novel interaction between tyrosine hydroxylase and PITSLRE p110, a protein kinase that has been implicated in mRNA production through the activation of RNA polymerase. Although TH and PITSLRE p110 physically interact, we have found that TH is not phosphorylated by PITSLRE p110 as had been hypothesized. Thus, further studies will focus on whether phosphorylated TH activates PITSLRE p110. We propose that the association between these proteins may be important for nicotine mediated dopamine pathways in the brain.

Li-Wen Lai, Ph.D.

University of Arizona

Award Amount FY 2000: $50,000

Enhancement of Non-Viral Gene Transfer in the Lung

The ultimate goal of this project is to develop a non-viral gene therapy for pulmonary diseases. To enhance gene delivery and prolong gene expression, we tested the effect of polycations (positively charged molecules) on liposome-mediated gene transfer in lung epithelial cell line. We identified polylysine as an efficient enhancer for liposome-mediated gene transfer *in vitro*. The new compounds were tested on the CAII deficient mice. An increase of gene expression was observed when the therapeutic gene was delivered with polylysine-liposome formulation compared with liposome only formulation. The gene expression persists for 3-4 weeks after the gene therapy as measured by PCR, Northern blot analysis and Western blot analysis. To assess the side effects of gene therapy, we measured the anti-CAII antibody titers and BUN levels in mice after gene therapy. Our data suggested that the new compounds increased the *in vivo* gene transfer without a significant increase of toxicity. The information obtained from this study will be valuable for guiding the future development of gene therapy for clinical trial in a variety of pulmonary diseases.

Patricia B. Hoyer, Ph.D.

University of Arizona
Award Amount FY 2000: $49,999

Mechanisms of Ovarian Follicular Cell Death Initiated by Ploycyclic Aromatic Hydrocarbons

Loss of ovarian small follicles results in menopause in women. Women who smoke cigarettes are known to enter early menopause. Our studies evaluated mechanisms by which three polycyclic aromatic hydrocarbons (BaP, DMBA, 3-MC) contained in cigarette smoke destroy ovarian follicles in rodents. We compared the relative potencies of these PAH's by determining and calculating an ovotoxic index (OI) for each. Our most noteworthy finding was that repeated exposure of mice to low doses of the PAH's was much more damaging than single high-dose exposure effects. This raises concerns that studies using high-dose exposure to ovotoxicants cannot accurately predict potential effects in women who are chronically exposed to low doses (habitual smokers). Because of the large population of elderly women in Arizona, there is an increased concern about health problems associated with menopause. The results of our studies help in estimating the health risks in women who have been life-long smokers.

Abstracts:

Borman SB, Christian PJ, Sipes IG, Hoyer PB. Ovotoxic species differences between rats and mice with 3-mehtylcholanthrene and 7, 12-dimethylbenz[a]anthracene. The Toxicologist 48:1822, 1999.

Borman SB, Christian PJ, Sipes IG, Hoyer PB. Ovotoxic index: a method to predict differences in susceptibility of mice and rats to environmental ovarian toxicants. Biol Reprod Suppl. I 60:349, 1999.

Borman SB, Christian PJ, Sipes IG, Hoyer PB. Relative ovotoxicity of polycyclic aromatic hydrocarbons in mice and rats. Abstract #7, 17th Annual Meeting, Mountain West Society of Toxicolog Breckenridgey, CO, Winner: First Place, Poster Presentations, 1999.

Publications:

Borman SB, Christian PJ, Sipes IG, Hoyer PB. Ovotoxicity in female Fischer rats and B6 mice induces by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol Appl Pharmacol 167:191-198, 2000.

Borman SM, Devine PJ, Hoyer PB. Ovotoxic index. Relative assessment of environmental chemicals. Center for Toxicology News Letter University of Arizona, Spring/Summer 1999.

Paula D. Johnson, D.V.M., M.S.

University of Arizona

Award Amount FY 2000: $49,940

Cardiac Teratogenicity: The Combined Effects of Exposure to Trichloroethylene and Cigarette Smoke in the Pregnant Sprague-Dawley Rat

This research evaluates the combined effects of inhalation of second hand cigarette smoke (SSCS), trichloroethylene (TCE), and imbibed TCE on the developing fetal rat heart. Treatment of pregnant rats with combinations of SSCS and TCE via inhalation exposure has been successful. All treatments are complete and hearts are in the process of evaluation. To maintain anonymity, fetal hearts are blinded, by code, to the dissector. Thus, hearts are continuously evaluated, but conclusions cannot be drawn until completion.

Because of the known harm that results from SSCS inhalation and the effects of TCE as a cardiac teratogen in the fetal rat model, it is suspected that the combination will be deleterious to the developing heart. As Arizonan's, not just to those in areas of contamination, this is of extreme importance because of the potential exposure to TCE from drinking water and from inhalation of water vapor from evaporative coolers, showering and cooking.

Pamela J. Kling, M.D.

University of Arizona

Award Amount FY 2000: $47,647

The Effect of Smoking on Oxidants Erythropoietin and Growth Factor in Human Milk

Pregnant smokers give birth to infants with increased prematurity and a greater risk of mortality. Yearly, 8500 (12.3% of) pregnant women giving birth in Arizona smoke, with low birthweight almost 3 times more common in women who smoke. Premature low birthweight infants experience more complications than term infants including anemia and decreased antioxidant status. Ill premature infants with anemia must be exposed to additional oxidants, such as transfusions and supplemental oxygen. The body normally balances the toxic and beneficial effects of oxygen-induced free radicals, but this balance is impaired in premature infants. Mothers who smoke also commonly feed infants human milk. Human milk composition is altered with smoking, and its intrinsic antioxidant capacity may also be impaired. Human milk is so beneficial to infant nutrition, smoking is not prohibitive to breast feeding. We set out to study the antioxidants in milk of both smokers and nonsmokers and the effect of smoking and breast feeding on infant antioxidant status in premature infants.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY 2000: $150,000

Analysis of Autoantibodies to T-cell Receptors in Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease characterized by chronic systemic inflammation predominantly 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. 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. We have found that individuals suffering from RA tend to have increased levels of autoantibodies directed against the recognition molecules on their own thymus derived lymphocytes (T cells). T cells are a major cell type mediating immune and autoimmune reactions. These autoantibodies are predominantly of the immune macroglobulin (IgM) class and react with the combining site region of the T-cell receptor (TCR). 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 present a distinct disease-related population that uses different immunoglobulin variable region genes. Our analysis of these novel antibodies 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 with smoking causing a more rapid progression of lung disease with significant increases in clinical intervention.

During the past year we completed the characterization of heavy and light variable gene usage in monoclonal human autoantibodies derived from RA patients and published the results. We have also completed the initial characterization of these molecules for recognition of TCRs, intact cells and defined synthetic epitopes. Recognition characteristics of the individual antibodies were analyzed, establishing that some antibodies were remarkably specific in their capacity to recognize defined antigenic determinants epitopes of TCR variable domains; whereas, others showed "epitope recognition promiscuity" in binding to distinct epitopes that show no identity in amino acid sequence. Studies to determine functional properties of the RA-derived monoclonal autoantibodies *in vitro* were initiated. We derived monoclonal IgG autoantibodies to TCRs epitopes and to markers of the senescent cell antigen. We developed gene constructs enabling us to produce by genetic engineering methods recombinant VH/VL single chain molecules modeling the combining sites of one of our specific monoclonal autoantibodies as well as one of the more highly epitope promiscuous examples.

These studies provide a firm molecular basis for understanding the specificity of the autoantibodies to TCRs arising in autoimmunity and also to assess their functions in immunomodulation and possible pathogenesis.

Robey IF, Schluter SM, Yocum DE, Marchalonis JJ. Production and characterization of monoclonal IgM auto antibodies specific to the T-cell receptor. **Journal of Protein Chemistry** 19:9-21, 2000.

Marchalonis JJ, Robey IF, Schluter SF, Yocum DE. Epitope promiscuity of human monoclonal autoantibodies to T-cell receptor-combining the site determinants. **Appl Biochem & Biotech** 83:31-52, 2000.

Judith B. Ulreich, Ph.D.

University of Arizona
Award Amount FY 2000: $93,705

Arizona Liver Transplantation Research: Optimizing Organ Replacement in Tobacco-Related Liver Diseases

In Arizona in 1999 and 2000, there were 15-18% fewer liver transplants than in 1998 because of the severe organ shortage. We have shown the efficacy of a chemical, dimethyl sulfoxide (DMSO), in preventing damage to livers where the blood has stopped flowing (ischemia) whether it is given prior to or following ischemia. Addition of DMSO to cold preservation solutions did not improve tissue viability unless DMSO was also given prior to ischemia. Static preservation of livers proved superior to pump perfusion. DMSO was more effective in protecting donor livers than were other agents reported in the literature. Information provided by this research should eventually be used to provide Arizonans with a greater supply of donor livers for transplantation The results of the study should also apply to transplantation of other organs such as kidneys and conditions of cardiac arrest or shock where there is compromised flow of blood.

Merrie Brucks, Ph.D.

University of Arizona
Award Amount FY 2000: $50,000

The Cumulative Impact of Tobacco Advertising on Young Children's Socialization to Pro-Tobacco Attitudes and Behaviors

Data collection has been completed. Our goal is to determine which children understand tobacco use and tobacco users, what the understand and when they understand it. As of June 30, 2000, members of our team have completed individual interviews with 364 children and received surveys from 228 parents or guardians, an 87.4% response rate. Data from the interviews include children's reactions to tobacco advertising, comments about tobacco users and non-tobacco users, and ratings of statements about tobacco use, collected from children as they matured over a 3 year time period. Data from the parental surveys include measures of household tobacco use and parental style. Quantitative (*i.e.*, numerical) and qualitative (*i.e.*, text-based) data from all three years has been transformed into appropriate formats for statistical and meaning-driven, interpretive analyses. Preliminary analysis tasks have also been completed (*e.g.*, interview data has been qualitatively coded for basic content and analytical variables like grade and gender of interviewee, and survey data has been checked for invalid entries). The assessment of research hypotheses has begun. Because of on-going lawsuits, the results of analyses will be released first to ADCRC, and then to scholarly outlets when all analysis is complete. It is expected that analysis will be completed by the end of the calendar year.

Theodore M. Dembroski, Ph.D.

University of Arizona
Award Amount FY 2000: $74,115

Biobehavioral Risk Profile of Smokers

A new intervention designed to enhance smoking cessation among young Arizona smokers', average age–21, showed that both males and females were significantly ($p<.01$) more likely to agree to cessation efforts (73%) if they were made aware of their own blood pressure (BP) and heart rate (HR) reactions induced by smoking during a health education presentation. Health education alone and a control group showed only 55% and 38% agreement, respectively. Knowledge of one's own dramatic cardiovascular reactions to smoking helps overcome the "it won't happen to me" belief and other individual differences associated with resistance to quit smoking efforts. Only one of several measures of hostility (anger-out) was associated with refusal rates ($p<.04$), and none of the measures of psychological distress were significantly related to smoking status. Results suggest that adding personalized physiological debriefing to stop-smoking education programs in an effective strategy.

SECTION D

PROPOSALS RECEIVED

TOBACCO-RELATED RESEARCH

FY 2000

Ahmad	University of Arizona	Effect of Tobacco Smoking on HIV-1 Pathogenesis and Disease Progression in Mothers and Infants Following Perinatal Transmission	$50000 50000 50000
Ahmad	University of Arizona	Effect of Smoking on the Molecular Mechanisms of AIDS Pathogenesis	$150000 150000 150000
Akporiaye	University of Arizona	Role of Tumor-Derived Transforming Growth Factor Beta (TGF-B) in Metastatic Lung Cancer	$50000 50000 50000
Bearss	University of Arizona	Development of a Transgenic Mouse Model of Pancreas Cancer	$49007 43439 0
Berg	University of Arizona	Optimal Treatment of Prolonged Ventricular Fibrillation: CPR First *vs.* Defibrillation First	$50000 50000 50000
Bloom	University of Arizona	Lung Function Decline and Airway Remodeling in Asthma	$148679 149434 146603
Burgoon	University of Arizona	Adolescent Emotion and Anti-Smoking Message Effects	$130778 141455 115244
Camhi	University of Arizona	The Function of Heme Oxygensase-1 (HO-1) in Lung Cancer	$49441 49496 49421
Chen	University of Arizona	Molecular Mechanisms of Oxidant and Nicotine Induced Cardiac Toxicity	$50000 50000 50000
Collins	University of Arizona	Characterization of the Effect of Nicotine on the Lung Sodium-Phosphate Transporter (NaPi-IIb)	$49500 49500 49500

Consroe	University of Arizona	Molecular Mechanism of Cannabinoid Action at the Human DB1 Receptor	$49522 49522 49522
Dallas	University of Arizona	Visual Human Based Validation of Right Ventricular Measurements	$48650 49531 49126
Davis	University of Arizona	Nicotine Exacerbates Stroke Damage to Brain Microvasculature	$149919 149919 149919
DeLuca	University of Arizona	Nicotine Effects on Human Stem Cell Differentiation *In Vitro*	$50000 50000 50000
Dieckmann	University of Arizona	Peroxisome Assembly: The Importance of Organelle Integrity for Proper Lipid Metabolism	$50000 50000 50000
Einspahr	University of Arizona	COX-2 Expression in Colorectal Adenomas Smoking History and Adenoma Recurrence	$148345 149675 134202
Eisenberg	University of Arizona	The Social Context and Dynamics of Smoking Cessation, Cessation Maintenance and Relapse During the Pregnancy & Post Partum Period	$96498 94230 64459
Flink	University of Arizona	Integrin Regulation of the Cardiomyocyte Cell Cycle	$50000 50000 50000
French	University of Arizona	Nicotine Dependence and Dopamine Neurons: Electrophysiological and Molecular Studies	$128093 124046 127288
Friedman	University of Arizona	Mycobacterium Tuberculosis Genes Involved in Survival within Macrophages	$50000 50000 50000

Funk	University of Arizona	Investigation of a Novel Neuroprotective Agent in Stroke	$110567 105135 103698
Garver	University of Arizona	The Effect of Nicotine on Caveolin-1 Function and Metabolism of Intracellular Cholesterol	$48380 48685 49282
Gerner	University of Arizona	Interdisciplinary Basic Science Program in Colon Carcinogenesis	$150000 150000 150000
Gervay-Hague	University of Arizona	The Synthesis of Chemotherapeutics Targeting Small Cell Lung Cancer	$48305 49710 49960
Hall	University of Arizona	The Role of Repair Deficiency Mutations in Breast Cancer	$50000 49954 48800
Harris	University of Arizona	Development of High Level Gene Expression Systems for Gene Therapy	$49995 49995 49995
Heuser	Phoenix Heart Center	Thermal Evaluation of Arteriosclerotic Plaque: A First Step in Predicting Risk of Heart Attack	$50000 50000 0
Holberg	University of Arizona	Identification and Functional Characterization of Genetic Polymorphisms Associated with Increased Susceptibility to Chronic Obstructive Pulmonary Disease Due to Cigarette Smoking	$149128 144376 148270
Hurley	University of Arizona	Drug Targeting of G-Quadruplexes as a Way to Reestablish the Normal Death Program in Cancer Cells	$143194 144871 144871
Johnson	St. Joseph's Hospital	Receptor Genetics and Nicotine Addiction	$137605 143684 148673

Jones	University of Arizona	Heart Health for Arizona's Deaf Communities	$49500 0 0
Joyce	Sun Health Res. Inst.	Smoking and Parkinson's Disease: Neuro-protectant or Artifact	$150000 150000 150000
Lopez	Phoenix Heart Center	Evaluating Unique Treatments for Tobacco-Related Peripheral Arterial Disease	$50000 50000 0
Lukas	St. Joseph's Hospital	Stoke, the Blood Brain Barrier, Nicotine and Nicotinic Acetylcholine Receptors	$373527 363570 370268
Lukas	St. Joseph's Hospital	Behavioral Cellular Molecular and Genetic Factors Influencing Nicotine Dependence	$499727 496242 499607
Macia	Arizona State University	A Study of the Effect of Controlled Oxygen Delivery on Retinopathy of Prematurity	$49948 49921 49970
Marchalonis	University of Arizona	Analysis of Autoantibodies to T-Cell Receptors in Rheumatoid Arthritis	$150000 150000 150000
Marshall	Health Serv. Adv. Group Inc.	Examining the Impact of Asthma on Arizona Patients	$150000 150000 0
Miller	University of Arizona	Smoking Cessation in Pregnancy: A Comparison of Counseling and Sustained-Related Bupropion	$144245 148515 136531
Miller	University of Arizona	The Effectiveness of Counseling and Bupropion Hydrochloride in Prevention of Postpartum Smoking Recidivism	$135045 138137 133872

Miller	University of Arizona	The Immunologic and Genetic Consequence of Tobacco Smoke Exposure During Pregnancy	$145457 149534 148783
Moffitt	Maricopa County DPH	The Effects of Tobacco Use on Tuberculosis Morbidity	$31486 29946 30398
Nakazato	University of Arizona	The Crisis in Liver Transplantation in Arizona: Non-heart-beating Donor Organs as a Solution in Tobacco-related Liver Disease	$149283 146505 149571
Price	University of Arizona	Synergistic Mutagenicity of Aflatoxin and Tobacco Product Extracts	$44700 42662 0
Regan	University of Arizona	FP Prostanoid Receptor Isoforms in Human Heart Disease	$49015 49621 49902
Roeske	University of Arizona	Agonist Specific Regulation of the Human Delta Opioid Receptor	$49500 49500 49500
Sadrzadeh	University of Arizona	Tobacco Smoke Activates Neutrophils and Increases Oxidative Damage in Maternal and Fetal Blood	$148031 146284 138148
Schmidt	Arizona State University	*In Vivo* Efficacy Evaluation of New Anticancer Drugs	$118204 130587 135525
Selmin	University of Arizona	gp91-phox as a Biomarker of Exposure to Tobacco Smoke	$49914 48793 49352
Sherrill	University of Arizona	Skin Test Reactivity to Common Aero-allergens as a Risk Factor for Smoking-related Respiratory Diseases	$45479 47298 49190

Skibo	Arizona State University	Development of New Antitumor Agents	$106949 136350 138299
Sparks	Sun Health Research Institute	The Effect of Nicotine in an Animal Model of Both Cardiovascular Disease and Alzheimer's Disease	$420648 393551 405160
St. John	University of Arizona	Nicotine Receptors in the Developing Spinal Cord	$138128 143510 149705
Turzillo	University of Arizona	Steroid Production and the Oxidative Stress Response in Ovarian Follicular Cells: Effect of Nicotine and Cotinine	$50000 50000 50000
Vaillancourt	University of Arizona	Altered Protein Activity and Expression Due to Nicotine Withdrawal	$49246 48971 49464
Wang	University of Arizona	Role of Pulmonary C-Fibers in Pathogenesis of Sidestream Cigarette Smoke	$61860 64335 66911
Wu	St. Joseph's Hospital	Nicotine and alpha 7-Nicotinic Acetylcholine Receptors	$149494 149589 149863
Xia	University of Arizona	Mechanisms of Cadmium Embryotoxicity: Using Bovine *In Vitro* Fertilization and Embryo Culture System as a Model	$49927 49886 49931

SECTION E

NEW CONTRACT AWARDS

TOBACCO-RELATED RESEARCH

BEGINNING IN FY 2000

David Bearss, Ph.D.

University of Arizona
Award Amount FY 01: $49,017

Development of a Transgenic Mouse Model of Pancreas Cancer

Some of the leading basic science and clinical researchers in the field of pancreas cancer research met at a recent Pancreas Cancer Think Tank meeting held on September 17-19, 1999 in Park City, Utah. The purpose of this meeting was to identify the most crucial needs in the field of pancreas cancer research and to come up with a plan to overcome the various obstacles facing the field. One of the overwhelming consensus deficiencies that was identified by this group was the need for better animal models of pancreas cancer. Pancreatic cancer is the fifth leading cause of adult cancer mortality in the United States with 29,000 deaths expected this year. Close to 90% of patients diagnosed with pancreas cancer will die within the first year following diagnosis. The deadliness of this disease has encouraged a search for risk factors to identify high-risk individuals. The most consistent risk factor that has been identified is cigarette smoking; heavy smokers have an increased incidence more than ten times greater than nonsmokers. Two of the most important challenges for treating pancreas cancer are the difficulty in early diagnosis and a lack of agents having activity in advanced disease. Because of the late diagnosis, the understanding of the molecular events involved in the onset and progression of pancreas cancer are not well understood. Animal models of pancreas cancer are needed to better understand the disease and to help in the development of new therapies focused on the prevention and cure of pancreas cancer. The existing pancreas cancer animal models involve either chemical carcinogen induced tumors in hamsters or transplantable human tumor cell line into immuno-compromised mice. Both of these models have serious limitations with respect to replicating human disease. With the advent of techniques to produce genetically engineered cancer-prone mice, the possibility of creating a mouse model that more closely resembles human pancreas cancer has become feasible. This proposal is focused on the development and characterization of genetically engineered mice that will develop pancreas cancer. The generation of this model will represent a significant step forward in the understanding of the process of pancreas tumor formation and will provide a model for the preclinical evaluation of agents designed to prevent or treat pancreas cancer.

The **goal** is to develop and characterize transgenic mouse models of adenocarcinoma of the pancreas that appropriately reproduce the human disease condition. The **hypothesis** is that expression of different tumor promoting oncogenes, specifically in the pancreas of mice, will lead to the induction of adenocarcinoma of the pancreas in these mice. The **objectives** of the project are to 1) produce transgenic mice that will specifically express the *K-ras* and *Her-2/neu* oncogenes in the mouse pancreas, 2) characterize tumors arising in the transgenic mice, 3) breed the *K-ras* and *Her-2/neu* transgenic mice to mice deficient in tumor suppressor gene p53, and 4) evaluate the effects of loss of p53 function on tumor properties. It is anticipated that these transgenic mice will represent models of pancreas cancer that closely recapitulate the human condition and will allow for the development of novel therapies for the prevention and cure of pancreas cancer.

Robert Berg, M.D.

University of Arizona
Award Amount FY 01: $50,000

Optimal Treatment of Prolonged Ventricular Fibrillation: CPR First *vs.* Defibrillation First

Cigarette smoking and other forms of tobacco use have been well established to cause atherosclerosis (hardening and degeneration of the arteries) and ventricular fibrillation (sudden cardiac arrest). In other words, hardening of the arteries and sudden cardiac arrest are tobacco-related diseases. Nearly one half of tobacco-related deaths each year are due to cardiovascular diseases, and most of these cardiovascular deaths are due to sudden cardiac arrest and hardening of the coronary arteries. Cardiovascular disease remains the number one cause of disease-related death in Arizona as well as in the entire nation. Approximately one million Americans and 10,000 Arizonans die each year from cardiovascular disease. One-third of these deaths are due to sudden cardiac arrest.

The American Heart Association recommends immediate defibrillation for sudden cardiac arrest (ventricular fibrillation). Although defibrillation is very effective for cardiac arrests of brief duration, it is less effective for prolonged cardiac arrest. The vast majority of cardiac arrests occur out of hospitals and, therefore, are prolonged before defibrillation is available. If a different approach to prolonged cardiac arrests is more effective, it could save thousands of Arizonans' lives from this tobacco-related disease.

The goal of this research is to improve treatment for patients with prolonged sudden cardiac arrest due to tobacco use. The principal objective of this project is to determine whether CPR prior to defibrillation (CPR First) for two minutes will improve outcome compared to immediate defibrillation in swine models of pre-hospital cardiac arrest. We will perform these studies in a situation that stimulates typical cardiac arrest out of a hospital, and typical paramedic response times. We will evaluate 1) the initial responses to defibrillation, 2) 24-hour survival, and 3) brain function of the 24-hour survivors.

In the second and third years of the study we will induce coronary artery obstruction with a steel plug prior to cardiac arrest to mimic the additional real-life situation of both tobacco-related sudden cardiac arrests and coronary artery disease (tobacco-related sudden cardiac arrest can occur with or without coronary artery disease). Our CPR research laboratory at The University of Arizona is the only institution in the world that has studied sudden cardiac arrest in this realistic model.

The specific hypotheses are that CPR first will result in improved rates of 1) successful initial resuscitation, 2) 24-hour survival, and 3) 24-hour survival with normal brain function, compared to the standard therapy of defibrillation first.

Qin Mary Chen, Ph.D.

University of Arizona
Award Amount FY 01: $50,000

Molecular Mechanisms of Oxidant and Nicotine Induced Cardiac Toxicity

Arizona is among the states that have the highest population of tobacco smokers. Tobacco smoking is an important risk factor for heart diseases. Smoking produces chemicals that contribute to oxidative stress that damages living tissues and contributes to heart diseases. Heart failure is a significant contributor to mortality and to the decline of the quality of life in smokers and passive smokers. Heart enlargement is a key element in the pathogenesis of heart failure. Identifying the molecules that control heart enlargement within heart cells is extremely important in prevention and treatment of heart failure. We have experimental evidence indicating that oxidants are an important cause of heart enlargement. Using isolated rat heart cells, we can find out the genes and proteins within heart cells that actually control the process of enlargement. Nicotine, a constituent of tobacco and a medication agent for smoking cessation, can add stress to the heart. Nicotine intake worsens heart injury. It is not known how nicotine works in heart cells. We propose to study in detail the effect of nicotine on the genes and enzymes that are turned on by oxidants.

The goal of our research is to find the critical molecules inside heart cells that control heart enlargement or heart failure. These molecules are switches or wires that link tobacco smoking to heart diseases. These molecules can be specific genes or proteins that control heartbeat or heart growth. Using rat heart cells in culture as an experimental model system, we will identify the genes and proteins that are affected by oxidants from smoke. We will study the relationship between oxidative stress, nicotinic stress and heart cell enlargement by looking into changes in the activity or level of these switch or wire molecules.

James F. Collins, Ph.D.

University of Arizona
Award Amount FY 01: 49,500

Characterization of the Effect of Nicotine on the Lung Sodium-Phosphate Transporter (NaPi-IIb)

Smoking is well known to effect lung physiology and to have adverse effects on surfactant production by the alveolar type II cells in the lung epithelium. Our recent data has shown high expression of a sodium-phosphate (Na/Pi) transporter in the human lung. This transporter functions to bring phosphate in to the lung cells, and it is known that these cells have very high needs for phosphate for production of surfactant (which is a protective, mucous coating that lines the lungs). It is also known that surfactant production in the lung lining is decreased in smokers and that this clearly has adverse effects on the physiology and the function of the lung. We therefore surmise that nicotine and smoking must cause changes in expression of this abundant sodium-phosphate transporter in the lung. Therefore, this problem seems extremely relevant to the State of Arizona as 21.2% of the adult population and 200,000 youths are known to be smokers here, and pulmonary related diseases directly related to smoking cost the taxpayers of the state a tremendous amount of dollars. Therefore, we believe that the investigation of the expression and regulation of this sodium-phosphate transporter in the human lung could lead to developments in treatment and advances in therapy for individuals who have damaged the lining of their lungs by smoking.

We propose to test the hypothesis that expression and regulation of sodium-phosphate transporter type II (NaPi-IIb) in the lung epithelium of smokers is altered by exposure to nicotine. Our first specific aim will be to determine the level of regulation of this transporter in type II alveolar cells by nicotine. Our second specific aim will be to look at short-term regulation of this transporter in the human, alveolar type II cells by nicotine by observing protein movement and modification within the cells after exposure to nicotine. The third specific aim will be to observe long-term effects of nicotine on the sodium-phosphate transporter in the lung by looking at regulation of the NPT gene in these cells. These studies will be expedited by the fact that we have previously identified potential regulatory regions of this human gene that likely mediate this response to chronic exposure to nicotine. Overall, these studies should allow us to determine precisely how the expression of this transporter is effected by exposure to the high levels of nicotine in cigarette smoke and its role in surfactant production in the lungs of smokers.

Paul Consroe, Ph.D.

University of Arizona
Award Amount FY 01: $49,522

Molecular Mechanism of Cannabinoid Action at the Human CB_1 Receptor

Smoking has been implicated in several forms of cancer including that of the lung. This has significant health consequences for the residents of Arizona as a 1990 survey indicated approximately 25% of the state population smoked (Marcus et al., Public Health Reports 109: 125). Lung cancer takes a terrible toll on Arizonans. It was responsible for 44 deaths per 100,00 population in the period 1986-1990 (Miller et al., NIH Pub. No. 93-2789). Standard cancer treatment often involves the use of radiation and chemotherapy. These treatments often induce severe nausea, weight loss and pain in patients, at times severe enough to require the termination of therapy. Cannabinoid drugs, such as Δ^9- tetrahydrocannabinol (Δ^9-THC), the major psychoactive drug in marijuana, have been demonstrated to reduce chemotherapy-induced nausea, reduce weight loss and relieve pain in cancer patients. Two cannabinoids, nabilone and dronabinol (Δ^9-THC), have been approved by the Food and Drug Administration as antinausea agents. Unfortunately, these drugs also have disturbing side effects including dizziness and memory loss. The work outlined below examines cannabinoid-stimulated intracellular signaling pathways that may be targeted to separate medically important effects of cannabinoids from unwanted side effects.

The effects of cannabinoid drugs on the central nervous system are mediated by a protein on the nerve cell surface known as the cannabinoid CB_1 receptor. This receptor activates a family of GTP binding (G) proteins that modulate cannabinoid effects in nerve cells. We have previously demonstrated that different cannabinoid drugs do not activate G proteins to the same extent. It is not yet clear whether drugs with less activity (partial agonists) stimulate all of the G proteins capable of interacting with the cannabinoid CB_1 receptor to a lesser degree than maximally active drugs (full agonists) or whether partial agonists activate only a subset of the G protein family members (G protein subtypes). We hypothesize that partial cannabinoid agonists activate only some of the G proteins capable of interacting with the cannabinoid CB_1 receptor. We will thus determine if structurally distinct cannabinoid drugs differentially activate G protein subtypes. As evidence suggests that G protein subtypes have some selectivity as to functional pathway activated, development of cannabinoid drugs that selectively activate certain G proteins may provide a means to separate medically useful drug effects from side effects.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY 01: $50,000

Nicotine Effects on Human Stem Cell Differentiation *In Vitro*

Although there have been some reports indicating that nicotine has deleterious effects on the immune system, little work has been done to determine the mechanisms of smoking-induced loss of immune function. This is especially true of the potential effects on the developing immune system of the fetus. A clear understanding of how tobacco products cause the loss of immune function would go a long way towards developing effective counter-measures to prevent immune system dysfunction. Indeed, since the active addicting component of tobacco is nicotine, and new drugs currently being developed for the treatment of depression and pain are derived from substances that interact with the same cellular components that react with nicotine, information derived from a study that targets nicotine action on the immune system will be crucial to assure that the drugs do not cause inhibition of immune function.

The purpose of the proposed research is to evaluate the effects of nicotine on the developing B cells, the cells which eventually produce antibodies in response to immune stimulation. We have developed an *in vitro* fetal liver organ culture (FLOC) system that mimics the growth and differentiation of both human and murine B cells. The system apparently also supports the production of monocytes and the hematopoietic stem cells (HSCs) which are the progenitors of all blood cells in the body. We have found that the addition of nicotine to this system causes profound changes in the number of all of these cell types in the FLOC system. We will use the *in vitro* organ culture method to determine effects of nicotine and related ligands on development of human HSCs, B cells and monocytes. Using information that we are gathering in a parallel study on human T cell development, we also hope to determine if acetylcholine receptors (AChR) for nicotine are present on HSCs. This information should allow us to ascertain how nicotine can manifest its effects on the development of HSCs. It should also provide insight as to how nicotine exposure can alter the function of blood cells before birth, and how these effects can be prevented.

Edward French, Ph.D.

University of Arizona
Award Amount FY 01: $128,093

Nicotine Dependence and Dopamine Neurons: Electrophysiological and Molecular Studies

Cigarette smoking is associated with coronary heart disease, stroke, ulcers, increased respiratory infections, cancer, emphysema, and increased risk of premature or low birth weight infants. Like addiction to heroin and cocaine, nicotine dependence is a chronic, relapsing disorder. In fact, some researchers suggest that dependence on nicotine is actually greater than that of other drugs. Notably, one out of three people who try cigarettes becomes dependent. A most recent statistic also found that over the past ten years that teenage smoking increased 50%. Thus, the health care and other economic costs attributed to the 25% of the population of Arizona who are hooked on tobacco are enormous. Nicotine addictive properties, like those of other drugs of abuse, have now been linked to the activation of pleasure or reward pathways in the brain. It is the stimulation of a specific population of brain dopamine neurons that likely begins the response that leads to feelings of pleasure and reinforces the repeated use of nicotine containing products. Thus, by understanding the cellular responses and processes by which nicotine stimulates the dopamine reward system, we may be able to devise new therapeutic strategies to treat nicotine dependence. This proposal will focus on identifying the sites and mechanism of action by which nicotine turns on dopamine nerve cells to produce its pleasurable effects.

The general objective of the proposed studies is to characterize, through electrophysiological and molecular biological techniques, the effects of acute and long-term exposure to nicotine on the activity of those brain dopamine and non-dopamine neurons mediating both nature and drug-induced reward. The individual experiments proposed below will test the general hypothesis that nicotine alters dopamine neuronal activity both directly and indirectly through stimulation of nicotinic acetylcholine receptors (nAChR), and that these two processes involve nicotinic receptors with unique subunit composition (s) and pharmacological properties. In addition, long-term exposure to nicotine may alter differentially the response of the various subtypes to nicotine, the possible cause for tolerance and craving associated with the chronic use of tobacco. This project is a collaborative effort melding together electrophysiological, pharmacological and molecular studies of dopamine neurons in rat brain. In addition, behavioral measurements will also be used in those experiments aimed at determining the electrophysiological and molecular biological consequences of long-term exposure to nicotine.

Janet L. Funk, M.D.

University of Arizona
Award Amount FY 01: $110,567

Investigation of a Novel Neuroprotective Agent in Stroke

Tobacco use is a major risk factor for the development of stroke and heart disease, diseases that account for one-third of all deaths is the state of Arizona. In addition to contributing significantly to mortality, stroke and ischemic heart disease cause disabilities that place a great burden on those who suffer from these disorders, as well as on their families and community. Survivors of stroke with neurologic impairment, including paralysis, have devastating disabilities. Neurologic defects, including memory disorders, are occurring with increasing frequency in those suffering from ischemic heart disease as our ability to resuscitate victims of cardiac arrest improves. In both settings, a lack of oxygen triggers a local reaction in the brain resulting in the death of nerve cells that do not regenerate. As research studies begin to unravel the step-by-step process leading to nerve death, it has become apparent that the process can be interrupted. A window of opportunity for treatment exists after blood flow has stopped when the loss of many of these neurons can be prevented. Clinical trials using synthetic drugs which target different steps along the cell death pathway have met with some success in limiting nerve cell loss. However, serious side effects have hampered the routine use of most drugs studied to date. Attention has turned to determining whether any of the body's own protective mechanisms, unleashed in the brain in response to a lack of oxygen, can be harnessed and used to prevent additional brain injury. Treatment trials are already underway in humans to determine whether, as has been seen in animals, treatment with increased amounts of certain neuroprotective proteins already expressed locally in injured brain can prevent further loss of nerve cells after stroke.

Recent experiments in our laboratories have shown that a lack of oxygen to brain during stroke triggers an increased production of parathyroid hormone-related protein (PTHrP). PTHrP is a peptide hormone that has already been administered safely to humans for treatment of non-neurologic disorders. PTHrP has been shown by other laboratories to prevent the nerve death that occurs in response to certain neurotoxic drugs or during aging— processes that stimulate cell death pathways that are similar to those initiated in stroke. We hypothesized that, in response to a lack of oxygen, local increases of PTHrP occur in brain areas where neurons are at risk of dying in order to protect against or limit cell death. If this hypothesis is true, administration of PTHrP to stroke or cardiac arrest victims could help to prevent additional nerve cell death, and thereby, decrease permanent neurologic damage such as paralysis or memory loss. To test these hypothesis, the following research objectives are proposed: 1)using animal models of unilateral stroke and of ischemic brain injury that occurs with cardiac arrest, determine exactly when and where PTHrP is expressed in the brain and 2) compare the areas of PTHrP release with when and where nerve death occurs. This information will be used to design experiments wherein inhibitors of PTHrP will be administered directly into the brains of animals with stroke or ischemic injury in order to test its efficacy as a protective treatment which could eventually be used in humans.

Eugene W. Gerner, Ph.D.

University of Arizona
Award Amount FY 01: $150,000

Interdisciplinary Basic Science Program in Colon Carcinogenesis

Colon cancer is the second leading cause of cancer deaths in Arizona and the United States. Since cancer is rapidly becoming the leading cause of all deaths in our society, colon cancer is a significant public health problem facing the citizens of Arizona. Colon cancer is one of several cancers associated with tobacco use, although the precise mechanism by which smoking influences colon cancer incidence is unknown. Efforts to reduce smoking and other forms of tobacco use are essential to limit the incidence of all smoking-related cancers. However, it is also crucial to understand the basic mechanisms leading to the development and spread of these cancers. Information on the molecular and cellular mechanisms of colon cancer and the role of tobacco components in these processes are necessary to develop effective strategies for preventing and treating this and other tobacco-related diseases. The Arizona Cancer Center is internationally recognized for its research on basic mechanisms of colon cancer causation and chemoprevention of colon cancer. This proposed research involves faculty from several departments on the University of Arizona campus, bringing together strengths in genetically altered rodent models of colon cancer and the molecular biology and biochemistry of colon cancer development.

The goal of this program is to conduct interdisciplinary basic research into the mechanisms of colon cancer development and to determine the role of tobacco-specific carcinogens in this disease process. An important consequence of this work will be the identification of novel strategies for prevention of colon cancer, the second leading cause of cancer deaths in our society. The hypothesis to be tested in this research is that tobacco-specific carcinogens interact with specific genetic risk factors to increase the likelihood of colon cancer incidence. This interaction could occur in colon epithelial cells (cells which become cancerous) or in endothelial cells (cells lining blood vessels). The objectives of the program are as follows:

1. Determine if cancer-causing components of tobacco smoke interact with genetic factors to increase the risk of colon cancer development.

2. Document the effects of cancer causing components of tobacco on processes leading to colon cancer formation in genetically altered rodent models of this disease.

3. Identify effective methods of preventing colon cancer in individuals with increased risk of this disease.

Laurence H. Hurley, Ph.D.

University of Arizona
Award Amount FY 01: $143,194

Drug Targeting of G-Quadruplexes as a Way to Reestablish the Normal Death Program in Cancer Cells

Tobacco related cancers are a common cause of death in Arizona. In particular, cancers associated with the lung and gastrointestinal tract (*e.g.*, colon and stomach) have been causally related to tobacco use. For many of these cancers there is presently no effective therapy, and there is an urgent need to develop effective treatment. Even where treatment is initially effective, resistance to drug treatment quickly develops, and the patient suffers relapse and dies of the cancer. We have identified a means to potentially prevent the emergence of resistance to chemotherapy and make cells more sensitive to drug treatment. In this proposed research, we will evaluate the usefulness of this approach in human cancer cells and use animal model systems to test the potential in treatment of human disease. The Arizona Cancer Center is at the forefront of basic research and its translation into clinically useful treatments. This research proposal is an interdisciplinary program of basic science involving drug design and development and potential clinical application using animal model systems. If successful, this research would provide significant new opportunities to help treat cancer patients in Arizona.

The overall goal of this research is to reestablish the normal death program in cancer cells which is lost during the progression of the disease which then leads to drug resistance. This is one of a number of new research programs at the Arizona Cancer Center in which novel approaches to drug design and development are being established alongside other well-established prevention and treatment programs. The three objectives of the program are as follows:

1. to study the mechanisms for controlling expression of cellular factors which can effect the normal death program in cancer cells,
2. to identify new drug molecules which inhibit the expression of cellular factors which prevent turning on the death program, and
3. to establish animal model systems to evaluate these drugs for treatment of patients with cancer.

Elaine Jones, Ph.D.

University of Arizona
Award Amount FY 01: $49,500

Heart Health for Arizona's Deaf Communities

Cardiovascular disease (CVD) is the leading cause of death in Arizona and in the United States. Nationwide, CVD accounts for 35% of deaths of persons over 25 years old and for over 30% of deaths in Arizona. Cardiac risk factors which people can change include smoking, hypertension, diabetes, sedentary lifestyle, obesity, elevated LDL, and the lack of stress management. There have been many efforts to persuade specific groups to improve their heart-health behaviors. None has targeted multi-ethnic deaf communities represent a unique intersection of people with physical disabilities and ethnic minority groups. Deaf individuals carry the same genetic risk for CVD as the general population but may be more vulnerable to CVD because they have limited access to health related information due to communication barriers, lower income and less education than the general population. In addition, 20-35% of deaf persons have a secondary disability, and at least one third are from ethnic minority groups. When communication barriers, low income, limited education, and secondary disabilities combine with membership in an ethnic minority group, the risk of CVD must soar. Existing heart-health programs are highly unlikely to reach deaf community members. The proposed one-year project is the first phase in creating a sustainable heart-health program in Arizona's multi-ethnic deaf communities.

Our vision (goal) is that multi-ethnic deaf communities across the nation will have the capacity for sustained heart health promotion programs that address the physical, sociocultural and ethnic dimensions of deaf adults and their families. Our specific aim is to develop and pilot test a multi-level community-based heart-health program to decrease modifiable cardiovascular risk factors in Arizona's multi-ethnic deaf communities. The multi-level heart-health program will include classroom teaching, home activities, and deaf community events. The program will be implemented by deaf adults who are trained as health educators and community health workers, will education others about modifiable CVD risk factors through classes on strategies to encourage health-behavior change. Our objectives for this first phase are to 1) conduct a more extensive assessment of health concerns and CVD risk status in the deaf community; 2) expand our community coalition to the Phoenix metropolitan area; 3) complete preparations for field testing the heart-health family program by a) selecting and translating written questionnaires and program materials into videotaped, sign language formats, b) training a deaf heart-health teacher and a deaf community health worker, c) pilot testing the heart-health program, and 4) developing a detailed evaluation plan for Phase II, field testing. No hypotheses will be tested in this first phase of the project, though we will conduct a focused project evaluation.

Ronald J. Lukas, Ph.D.

Barrow Neurological Institute
Award Amount FY 01: $373,051

Stroke, the Blood Brain Barrier, Nicotine, and Nicotinic Acetylcholine Receptors

Stroke is the leading cause of serious disability and the third leading cause of death in Arizona and nationally. Tobacco use is associated with an increased risk of stroke. However, precise roles of tobacco constituents (such as nicotine) and/or metabolites in stroke remain obscure. Moreover, more information is needed about whether and how stroke severity, steps in the progression of damage following stroke, stroke treatment, and recovery from stroke are influenced by tobacco use. The blood brain barrier (BBB) is a system of small capillaries in the brain that maintains and protects the brain and its unique fluid environment, the cerebrospinal fluid (CSF). Normally, the BBB restricts exchange from the blood to the brain and CSF. However, function of the BBB may be compromised in stroke, contributing to increased brain damage. Remarkably, little is known about effects of tobacco use and nicotine on the BBB. For example, nicotine exposure can increase fluid build-up in the brain and can deplete factors that help dissolve blood clots, but the bases for these effects have been unknown. Nicotine has powerful effects on nerve cells in the brain due to actions at its targets, nicotinic acetylcholine receptors (nAChR). However, recent evidence suggests that nAChR are also found on cell types that help create the BBB. Thus, there is good reason to think that tobacco use and nicotine exposure can have direct effects on the BBB and that these effects can contribute to stroke.

The broad goal of this program of clinical and basic research is to establish effects of tobacco use and nicotine exposure on incidence, severity, progression and treatment of stroke; on recovery from stroke; and on the BBB. The broad thesis of the research program is that tobacco use and nicotine exposure affects the structure and transport characteristic of the BBB and that these effects have deleterious consequences during stroke. PROJECT 1 tests the hypotheses that nicotine exposure alters BBB transport and has additive negative effects with stroke on the BBB by altering BBB structure and by altering protective responses to stroke. PROJECT 2 tests the hypothesis that tobacco use increases the severity of stroke and compromises recovery by enhancing the "vasogenic"stage of post-stroke brain damage. PROJECT 3 tests the hypotheses that targets for nicotine action, nAChR, are found on microvascular endothelial cells, peri-vascular astrocytes, and/or peri-vascular neuronal structures that contract the vascular bed. PROJECT 4 will test the hypothesis that microvascular endothelial cells make nAChR and will establish the properties, including nicotine sensitivity, of those cells and their receptors. Ultimately and collectively, these studies seek to define mechanisms involved in effects of nicotine and tobacco use on stroke and on the BBB. Aside from facilitating design of new strategies to prevent and treat stroke, insight might also be gained into how to regulate function of the BBB, particularly to facilitate therapeutic drug access to the brain.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY 01: $150,000

Analysis of Autoantibodies to T-Cell Receptors in Rheumatoid Arthritis

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are classic autoimmune disease associated with elevated levels of autoantibodies. Both are prevalent in the State of Arizona, but RA has an abnormally high incidence of approximately %5 in the Tucson area due to the influx of individuals suffering from the disease and the high percentage of Native Americans. High levels of RA-associated autoantibodies termed rheumatoid factors (RFs) correlate with poor long term prognosis, and a correlation between levels of these autoantibodies and smoking has been documented in males. Furthermore, smoking causes a more rapid progression of RA associated lung disease and significant increases in the need for clinical intervention. Under previous support of the ADCRC, we found that RA patients have significantly elevated of autoantibodies directed against recognition molecules of their own thymus derived lymphocytes (T cell receptor). We have generated monoclonal autoantibodies from RA patients and determined that these are novel recognition molecules that have the potential to modulate the T cell arm of the immune system. Autoantibodies of RA patients are usually of the IgM, the immune macroglobulin class, whereas those of SLE patients tend to be of the IgG or major serum immunoglobulin class. The ability to generate specific monoclonal molecules gives us the unique opportunity to 1)determine the role genes have in generating these two autoimmune diseases, 2) study their biological functions in interaction with T cells, and 3) determine their function in modulating the immune response. It is essential to determine whether these disease-related molecules represent populations correlated with the rheumatic diseases or whether these are essentially the same ones expressed in low levels by healthy individuals in immunomodulation. Our combined molecular and genetic approach offers new possibilities for diagnosis and potential therapy.

We hypothesize that the initially occurring autoantibodies to autologous TCR that occur in RA patients arise from an over-expression of naturally occurring immunomodulatory IgM autoantibodies. However, with the course of progressive disease, later autoantibodies may be qualitatively distinct from the initial autoantibodies due to selection and recruitment. SLE, the predominant anti-T-cell receptor autoantibodies are of the IgG isotype, and it must be determined whether the specificities and gene usage of these are exactly same or even related to those expressed by the original IgM molecules. Our major specific aims are as follows:

1. Continue and refine our analysis of monoclonal IgM autoantibodies to TCR from RA patients by considering at least three more patients and comparing the properties of the new molecules with those of our existing database and those to be generated from SLE patients and normal individuals.

2. Generate monoclonal IgG autoantibodies from individuals with SLE. We have established the feasibility of this in preliminary studies but propose to analyze the monoclonals from five different individuals. SLE derived molecules are of considerable interest for possible relevance to the pathogenesis of disease because it has been already established that monoclonal IgG autoantibodies of SLE patients directed against DNA can cause disease.
3. Analyze interactions of monoclonal anti-TCR autoantibodies with intact T cells and their effects upon antigen specific and immunoregulatory T cell functions under defined *in vitro* cell culture conditions.

Hugh Miller, M.D.

University of Arizona
Award Amount FY 01: $123,600

The Effectiveness of Counseling and Bupropion Hydrochloride in Prevention of Postpartum Smoking Recidivism

Approximately 22 million (22.6%) women in the United States (CDC, 1997) and 20.6% of women in Arizona use cigarettes (CDC, 1997). The prevalence of cigarette smoking among pregnant women in the United State overall is 21.5% (US DHHS, 1997), and 8.5% in Arizona according to the 1997 Arizona Health Status and Vital Statistics report. Health problems in infants and children associated with maternal smoking include higher rates of a) sudden infant death syndrome, b) asthma, c) respiratory infections, d) otitis media, e) decreased scores on achievement tests, and f) behavioral problems. Many of these conditions are potentiated by continued neonatal and childhood exposure to cigarette smoke. During pregnancy 20-41% of women who smoke, spontaneously quit. A review of studies concerning smoking cessation counseling programs designed for pregnant women indicated that additional cessation rates of 10-43% have been achieved using various interventions. Unfortunately, 70-87% of women who quit smoking during pregnancy relapse by the end of the first year post delivery. Although short term benefits of smoking cessation during pregnancy are realized, any potential long term benefits of childbearing women and their families are lost as the result of postpartum relapse. Reinitiating cigarette smoking in the post delivery period places the woman, her child and other members of her household at direct risk for a multitude of health problems. Few studies have focused on postpartum relapse prevention with specific attention directed towards increased maternal stress, depressive symptoms and misperceptions about the effect of environment tobacco smoke (ETS) exposure that surround the immediate postpartum period. Evidence suggests that women are at particularly high risk for immediate postpartum relapse resulting in ETS exposure for their infants and that counseling alone produces only minimal long term results. Given the high incidence of relapse among women and the subsequent serious health consequences of this problem, research directed appropriate interventions to reduce postpartum recidivism is essential.

This proposed research focuses on postpartum relapse prevention with specific attention directed to women who have successfully stopped smoking during pregnancy. Our first goal is to determine the effectiveness of an intervention designed to maintain long term post delivery abstinence among women who have successfully quit smoking during pregnancy. Our second goal is to identify factors associated with smoking relapse or abstinence among women receiving a relapse prevention intervention as compared to women who receive usual postpartum care and follow up.

Objectives of this study are to 1) determine effectiveness of a post delivery relapse prevention counseling cessation, to reduce postpartum smoking relapse and promote long term abstinence from cigarette smoking, 2) determine which variables (personal, demographic, and psycho-social) are associated with postpartum relapse, 3) determine which variables (personal, demographic, and psychosocial) are associated with continued abstinence in the first postpartum year, and 4) determine which variables (personal, demographic, and psychosocial) are predictive of postpartum smoking relapse or abstinence among women who receive either usual postpartum care, or a postpartum relapse prevention intervention consisting of counseling and the availability of a sustained release Bupropion (Zyban), or placebo.

This research will assist in better understanding the issues underlying postpartum relapse to aid in the development and design of smoking relapse prevention programs for postpartum women. The ultimate goal of this project is to develop effective and appropriate ways to assist women in maintaining life long abstinence from cigarette smoking to promote the heath initiatives embraced by local, Arizona and federal health policy. Significant relapse prevention will promote optimal health among women of reproductive age, as well as their children and families.

John W. Regan, Ph.D., M.D.

University of Arizona
Award Amount FY 01: $49,051

FP Prostanoid Receptor Isoforms in Human Heart Disease

Heart disease is the number one killer of Americans, with smoking, age, obesity, and diabetes being significant risk factors. Because the state of Arizona is one of the top retirement regions in the country, a large proportion of the Arizona population is at risk. In addition, Arizona has a large population of native Americans for whom diabetes is an immense problem. Smoking increases the detrimental effects of other risk factors on the development of heart disease; consequently, there is a great interest in the health care community in the prevention and cure of heart disease. Practical research geared towards identifying a means to intervene at specific sites in the affected cells may prove critical for the development of life saving drugs.

The increase in prostaglandins found in heart disease, for example $PGF_{2\alpha}$, has been directly linked to cardiac hypertrophy following hypertension, contributing to cardiac failure and cell death. $PGF_{2\alpha}$ binds to a receptor located in the cell membrane, called the FP receptor, activating an intracellular second messenger pathway via a G-protein to produce its physiological effects. Smoking also leads to the generation of prostaglandin-like substances by free radicals, some of which have been shown to bind to the FP receptor and activate the second messenger pathways (Kunapuli *et. al.* JBC 97, 98). Intervention at the receptor itself or within the cell signaling pathways could potentially block the harmful effects of prostaglandins, creating a powerful new therapy for treating heart disease.

The goal of the project is to identify the forms of prostaglandin receptors present in the human heart activated by $PGF_{2\alpha}$, and determine what proteins and second messenger pathways are involved. Our lab has extensive experience investigating prostaglandin receptors, including several key publications. Recently we have discovered a novel variant of the FP receptor in sheep ovary and have been characterizing the differences between this isoform and the previously cloned receptor. Because each isoform produces different effects in the cell, the relative abundance and location of specific isoforms will have an impact on the pathophysiology of disease attributed to activation of the receptor. Comparing the findings from our studies on differences in calcium levels between the two isoforms of the FP receptors and reports in the literature on altered calcium levels in heart tissue, we suspect that the novel FP isoform also exists in human heart. Thus we hypothesize that the detrimental effect of $PGF_{2\alpha}$ in coronary disease is mediated by multiple FP receptor isoforms in human heart. To test this hypothesis we will carry out the following Specific Aims:

1. Identify the FP receptor isoforms present in cardiac tissue by screening a human heart cDNA library using RACE PCR.

2. Identify proteins that interact with FP receptor(s) using the yeast two-hybrid system.
    a) Use full length FP receptor(s) as bait to screen a human heart cDNA library.
    b) Use a constitutively active form of the FP receptor(s) as bait to screen the same library.
3. Determine the second messenger pathways involved in $PGF_{2\alpha}$ stimulation of FP receptors, and the localization of FP receptor isoforms.

Jean M. Schmidt, Ph.D.

Arizona State University
Award Amount FY 01: $118,204

In Vivo Efficacy Evaluation of New Anticancer Drugs

The need for improved cancer chemotherapy is acute, since smoking-related cancers continue to plague Arizonans. Families of cancer patients contact the ASU Cancer Research Institute frequently, asking for information on new anticancer drugs, and they are desperate. The most recent statistical data available from 1995 (Arizona Department of Health Services) estimate that 18,000 Arizonans per year can be expected to be treated for invasive cancers. The American Cancer Society estimates that 1 in 2 males, and 1 in 3 females will develop cancer in their lifetimes, and a high proportion of these cancers are related to effects of smoking tobacco. The Arizona State University Cancer Research Institute is dedicated to new anticancer drug discovery, and bringing our drug discoveries to clinical trials, where benefits to patients can be seen, turns out to be a long, slow process. The focus of this proposal is to provide *in vivo* xenograft data on several new anticancer drugs which will speed up their preclinical development. Arizonans cannot be dependent only on the very slow-moving National Cancer Institute drug development program. We need to move forward with anticancer drug development with our state resources and facilities, because it can be done here. We have the expertise to do it.

The overall goal of this proposal is to facilitate the transfer of newly developed anticancer drugs to clinical application in the fight against tobacco-related cancers. To do so, anticancer drugs must be evaluated in relevant *in vivo* tests, which we propose to do here in severe combined immunodeficient (SCID) mouse xenografts of tobacco-related human tumors. The *in vivo* data are of great importance in deciding whether a drug should proceed to further preclinical development steps (toxicology, pharmacology, and formulation). Without *in vivo* evaluations new drugs, that have been discovered in the ASU Cancer Research Institute (CRI) and have shown very potent and promising results in inhibiting several kinds of human tumor cells, would not proceed to preclinical development. We propose to evaluate six of the ASU CRI's most promising new anticancer drugs in this *in vivo* study. The SCID mice to be used here, with engrafted human tumors, are an excellent and sensitive experimental system for this type of *in vivo* study. The results will be used in furthering the preclinical and clinical development of the successful drugs, in conjunction with oncologists at clinical research facilities, through collaborations with the Arizona Cancer Center in Tucson, Arizona and elsewhere.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY 01: $106,949

Development of New Antitumor Agents

The ideal cancer drug would be a magic bullet able to kill only cancer cells. A drug that targets only lung cancer cells would not affect normal cells resulting in less toxicity for the lung cancer patient. The side effects of cancer drugs are well known and include nausea, vomiting, loss of hair, *etc.* Our approach to solving this problem has been to exploit the enzyme DT-diaphorase in our drug designs. Cancer cells can possess high or low concentrations of this enzyme, which can be employed in the design of specific antitumor agents as follows. A cancer possessing high levels of DT-diaphorase can be targeted by a drug activated by this enzyme. On the other hand, a cancer possessing a low level of DT-diaphorase can be targeted by a drug inactivated by this enzyme. The levels of cancer diaphorases are known in many cases; and thus, the appropriate drug can be chosen for treatment. In the proposed project, we will develop drugs both activated and inactivated by DT-diaphorase targeting lung cancer as well as melanoma. In preliminary work we have designed melanoma and non-small-cell lung cancer specific agents, both of which could be important to the people of Arizona.

Drugs Activated by DT-diaphorase. ADCRC and NIH funded work in this laboratory has been involved with the design of a new class of DNA cleaving agent based on the pyrrolo [1,2-a] benzimidazole (PBI) ring system. These agents are designed to alkylate the phosphate backbone of DNA upon DT-diaphorase-mediated reduction resulting in a hydrolytically-labile phosphotriester. The cytotoxic event is the hydrolysis of this ester resulting in DNA cleavage. We have developed a new series of indole and cyclopent(b)indole analogues showing excellent *in vivo* activity as well as cancer selectivity. In this proposal we will continue to develop these active analogues by doing the following: 1)analogue synthesis using combinatorial methods, 2) DNA alkylation studies and mechanistic studies of cytotoxicity, and 3) measurement of DT-diaphorase substrate activity and computer-modeling of new drug structures into the enzyme active site.

Drugs Inactivated by DT-diaphorase. ADCRC, NIH, and American Cancer Society funded work in this laboratory has also been involved with the design of topoisomerase II inhibitors based on the 6-acetamidopyrrolo [1,2-a] benzimidazole (APBI) system. The APBIs are inactivated by 2-electron reduction and, therefore, show a strong inverse correlation with DT-diaphorase levels (the highest inverse correlation of 20,000 compounds in the National cancer Institutes' archives). The unchanged (oxidized) APBI, rather than the reduced form, appears to act as an inhibitor of the first step of topoisomerase II - mediated relaxation of supercoiled DNA. Topoisomerase II is an enzyme involved in the relaxation of DNA so that cell replication can proceed.

These findings prompted a study of structural variants of the APBI ring system, the benzodiimidazole and dipyrroloimidazobenzimidazole quinone system. In this proposal we will

continue to develop these active analogues by doing the following: 1) analogue synthesis using combinatorial methods, 2) DNA intercalation and topoisomerase II inhibition studies, and 3) measurement of DT-diaphorase substrate activity and computer-modeling of new drug structures into the enzyme active site.

Adele Turzillo, Ph.D.

University of Arizona
Award Amount FY 01: $50,000

Steroid Production and The Oxidative Stress Response in Ovarian Follicular Cells: Effect of Nicotine and Cotinine

Infertility affects approximately 1 of 6 couples in the United States. In 2/3 of infertile couples (10% of all couples), the cause of infertility is attributable to the female. In Arizona, there are approximately 1 million women of childbearing age; thus it is estimated that 100,000 of these women will experience (or have already experienced) difficulty trying to conceive a child. One lifestyle choice that is clearly associated with infertility in women is cigarette smoking. In Arizona, 25-30% of women of childbearing age smoke. In addition, 10-18% of women in this age group smoked in the past or are exposed to secondhand smoke. Of particular concern are teenage smokers. Because the effects of tobacco use on female fertility can persist even after smoking cessation, teenage women who smoke (or are exposed to secondhand smoke) may jeopardize their future ability to reproduce. Cigarette smoke contains free radicals that can lead to oxidative stress in a number of tissues throughout the body. This oxidative stress causes irreversible damage to cells and eventual cell death. Since the toxic components of cigarette smoke are transported through the bloodstream, every tissue in the body including the ovary is potentially susceptible to the deleterious effects of free radicals from cigarette smoke. The first step in the establishment of pregnancy is the development of a healthy ovarian follicle that produces hormones and nurtures the egg. There is little information available regarding potential mechanisms by which the toxic components of cigarette smoke affect the ovarian follicle. It is possible that oxidative stress caused by the toxic components of cigarette smoke leads to impaired function and eventual death of ovarian cells, resulting in sub-optimal ovarian function and infertility. The experiments described in this proposal are designed to address the relationships among smoking, oxidative stress, and function of ovarian cells.

The majority of previous studies demonstrating an association between impaired ovarian function and cigarette smoking are descriptive in nature. To fully understand the relationship between smoking and ovarian function, it is imperative to study the fundamental molecular and cellular consequences of exposure to constituents of cigarette smoke in ovarian cells. The goal of the research in this proposal is to determine how constituents of cigarette smoke influence the function of ovarian follicular cells. We hypothesize that components of cigarette smoke cause

oxidative stress in ovarian cells. This oxidative stress has the potential to cause death of ovarian cells, thus interfering with normal estrogen production and egg development. To address this hypothesis, we will develop an experimental model that utilizes bovine ovarian cells. A bovine model was chosen because many aspects of follicular development and function in the cow are strikingly similar to those in the human. Our objectives are to 1) determine the effects of nicotine and its primary metabolite, cotinine, on hormone production by ovarian cells; 2) characterize molecular and cellular parameters of oxidative stress and cell death in ovarian cells; and 3) determine whether nicotine and cotinine induce oxidative stress and death of ovarian cells. The findings of this study will enhance our understanding of the mechanisms underlying the negative effects of smoking on fertility in women. This information will be valuable not only to basic scientists but also to health care professionals that provide reproductive care to women of childbearing age in Arizona and throughout the country.

SECTION F

ANTI-CANCER DRUG DISCOVERY RESEARCH

YEAR ONE

FY 2000

A.A. Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY 2000: $249,949

Discovery of Novel Anticancer Drugs from Rhizosphere Microflora of Desert Plants: An Unexploited Source of Bioactive Natural Products

During the first year of the project, roots of 82 species of desert plants representing 67 genera and 34 families have been sampled. We have isolated and stored 15,424 bacteria and 1,604 fungi to create a Library of Desert Plant Rhizosphere Microorganisms. Of these, 471 fungi and 365 bacteria have been cultured and 805 extracts prepared for screening in a panel of three cell lines representing breast, lung, and central nervous system cancers. Extracts derived from 29 fungi and 2 bacteria inhibited the growth of at least one cancer cell line by >90%. They were selected for detailed investigation. Bioassay-guided fractionation of 11 fungal extracts was initiated. From a partially identified *Aspergillus* species we have isolated and characterized one new and two known microbial metabolites exhibiting significant anticancer activity. If these compounds turn out to be active against solid tumors such as breast, prostate, colon and lung cancers, our results will have an impact on the more elderly and/or tobacco-dependent portions of Arizona's population since these cancers are prevalent in our state.

Emmanuel Katsanis, M.D.

University of Arizona
Award Amount FY 2000: $230,603

Improvement of Anticancer Immune Responses Generated by Chaperone Protein Associated Tumor Peptides

Our laboratory has developed protocols to isolate heat shock proteins (HSPs) or chaperone proteins from tumors. We and others have shown that HSPs carry with them tumor specific proteins or antigens. The conventional way of isolating HSP-antigens is very labor intensive and not practical for future clinical use. Therefore, we have developed a novel rapid method for isolating HSP-antigens from tumors. We have demonstrated that the purified HSP-antigens, when used as vaccines, can induce potent and specific immunity against tumors in mice. Our studies provide evidence that HSPs can be easily and efficiently isolated by this technique and used effectively to immunize against disseminated malignancies.

Graner MW, Raymond A, Romney DA, He L, Whitesell L, Katsanis E. Immunoprotective activities of multiple chaperone proteins isolated from murine B cell leukemia/lymphoma. Clinical Cancer Research 6:909-915, 2000.

Graner MW, Raymond A, Akporiaye E, Katsanis E. Tumor-derived multiple chaperone enrichment by free solution-isoelectric focusing yields potent anti-tumor vaccines. Cancer Immunology Immunotherapy In press.

David K. King, M.D.

Good Samaritan Medical Center
Award Amount FY 2000: $88,503

Clinical Trials Network

Greater Phoenix CCOP has been awarded a contract by the ADCRC for clinical research management of patients undergoing treatment with cancer drugs developed through other ADCRC contracted organizations. However, no drugs were tested in clinical trials by the Greater Phoenix CCOP during the period from 7/01/99 to 6/30/00. No funds were dispersed to the Greater Phoenix CCOP during this time period.

Eugene A. Mash, Jr., Ph.D.

University of Arizona
Award Amount FY 2000: $249,874

Rational Design of Thioredoxin Active Anticancer Drugs

We are studying a human protein called *thioredoxin* that is overproduced in lung, colon, and other human tumors. Inhibition of thioredoxin, either through mutation or by the addition of chemical inhibitors, reduces and in some cases eliminates the cancer-causing effects. Thioredoxin is a promising target for anticancer drug therapy. We are developing specific chemical inhibitors of thioredoxin for possible use as anticancer drugs. More than thirty compounds related to a lead compound identified from the National Cancer Institute database have been synthesized and tested for thioredoxin inhibitory activity. Two promising drug candidates have emerged from this collection. These compounds are more active and less toxic that the lead compound and are presently being tested in a living mouse model system. We are also studying the mechanism of action of these compounds so that we may produce even more selective and potent drug candidates.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 2000: $749,982

Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs

The overall goals and objectives of this project have been sharply focused on vigorously expediting the transition from discovery of a new anticancer drug through the preclinical development states to initiation of Phase I human cancer clinical trials. Given appropriate resources, there is ample opportunity and rationale for considerably shortening the preclinical development phases, and ADCRC support has allowed us to reduce the overall process following discovery. Furthermore, the rapid availability of the research results at each stage has encouraged the NCI to enter the development process at critical stages to assist us in advancing to the next stage. Indeed, the ADCRC and NCI resources are now being aimed at certain of our anticancer drug candidates in a relay-type fashion that is greatly accelerating the preclinical processes.

Pettit GR, Grealish MP, Herald DL, Boyd MR, Hamel E , Pettit RK. Antineoplastic agents 443. Synthesis of the cancer cell growth inhibitor hydroxyphenstatin and its sodium diphosphate prodrug. **J Med Chem** 43:2731-2373, 2000.

Idso SB, Kimball BA, Pettit III GR, Garner LC, Pettit GR, Backhaus RA. Effects of atmospheric CO_2 enrichment on the growth and development of *Hymenocallis littoralis* (Amaryllidaceae) and the concentrations of several antineoplastic and antiviral constituents of its bulbs. **Amer J Botany** 87:769-773, 2000.

Beutler JA, Mccall KL, Herbert K, Herald DL, Pettit GR, Johnson T, Shoemaker RH, Boyd MR, Novel cytotoxic diterpenes from *Casearia arborea* (Flacourtiaceae). **J Nat Prod** 63:657-661, 2000.

Pettit GR, Ducki S, Orr B. Antineoplastic agents 443. Synthesis of pancratistatin prodrugs. **Anti-Cancer Drug Desig,** accepted.

Pettit GR, Melody N, Herald DL. Antineoplastic agents 450. Total synthesis of (+) -pancratistatin with (+) -narciclasine as relay. **J Org Chem** accepted.

Pettit GR, Lippert III JW, Herald DL. A Pinacol Rearrangement/Oxidation Synthetic Route to Diphenstatin. **J Med Chem** In press.

Robin L. Polt, Ph.D.

University of Arizona
Award Amount FY 2000: $52,325

Synthesis and Methodology for New Glycosyltransferase Inhibitors with Potential Anti-Metastatic Activity

Exterior surfaces of cells are covered with carbohydrates, which control many aspects of cell growth and function. Selective inhibitors of certain cell-surface carbohydrates (glycosphingolipids or GSL's) have been synthesized using new chemical methods developed in this laboratory, and tested using insect nerve cells based on the tobacco horn worm, *Manduca sexta*. Progress has been made both in the synthesis of the inhibitor drugs called PDMP's and in the insect nerve cell assays. We have isolated GSL's from insects and are in the process of determining their chemical structures. Insect nerve cells that have been exposed to minute quantities of PDMP show slower axon development, presumably because their GSL expression has been altered. Similar studies at the Arizona Cancer Center with human cancer cells have shown that PDMP's block metastasis in a test tube assay. We hope to use the simpler insect assay system to develop more effective PDMP molecules for clinical use.

Publication:

Polt RL. Amino Acid Derivatives, A Practical Approach, Chapter 9, *pp.*, 101-114. Barrett, GC. Ed., Oxford University Press, Oxford, U.K. 1999.

Garth Powis, D. Phil

University of Arizona
Award Amount FY 2000: $750,000

Arizona Cancer Center Novel Anticancer Drug Discovery and Development

The year's work has been marked by several exciting developments. Using as a starting material, a chemical found in the creosote bush indigenous to Arizona, we have synthesized a large number of related compounds and identified three that have particularly interesting properties. One has antitumor activity against a number of human tumors growing in mice and a patent has been prepared for this compound. Two other compounds protect mice against UV light-induced skin cancer and will be developed as possible protective agents for use in subjects at high risk of developing skin cancer. Also, cutting edge drug discovery technologies have been developed that will allow more efficient cancer drug discovery. They include robotic screens for detecting anticancer drug activity in thousands of compounds and DNA microarray gene chip technology that allows the effects of potential drugs on several thousand human genes in a cell to be detected in a single experiment.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY 2000: $155,990

Preclinical Development of the PBIs

At the outset of this contract, we had in hand a series of compounds called the PBIs that has a good possibility of seeing use in the clinic. One of the specific aims of the contract was to design more active analogues using our rational approach. In carrying out this specific aim over the past year, we were able to develop computer models that greatly assisted in anticancer drug design. In addition, simple chemical and biochemical test were developed that could predict the efficacy of a drug even before animal studies were carried out. The histological types of cancers targeted by the compounds developed in this laboratory include melanoma and ovarian cancers. Both of these cancer types are common in residents of Arizona. Ongoing projects are outlined below.

Specific areas of endeavor in the past year include:

- Synthesis of Cyclopent[b]indole and Indole Libraries, Identification of Leads. A large library of compounds were prepared to test the rational approach.
- DNA Alkylation and Cleavage Studies. Documentation of how the drug attaches to DNA.
- ^{13}C-NMR Studies of DNA Alkylation. A new and rapid means of determining how the drug attaches to DNA.
- Molecular Modeling. Computer models are used to predict / rationalize interactions with DNA.
- Semiquinone pKa Measurements. These measurements predict the metabolic fate of the antitumor agent.

Craigo WA, LeSueur BW, Skibo EB. Design of highly active analogues of the pyrrolo [1,2-a]benzimidazole antitumor agents. **J Med Chem** 42:3324-3333, 1999.

Xing CG, Wu P, Skibo EB, Dorr RT. Design of cancer specific antitumor agents based on aziridlinylcyclopent[b]indoloquinones **J Med Chem** 43:457-466, 2000.

Ouyang A, Skibo EB. The iminium ion chemistry of mitosense dna alkylating agents. Enriched ^{13}C-NMR studies. **Biochemistry** 39:5817-5830, 2000.

Xing C, Skibo ES. Sigmatropic reactions of the aziridinyl semiquinone species. Why aziridinyl benzoquinones are metabolically more stable than aziridinyl indoloquinones. **Biochemistry** In press.

Huang X, Suleman A, Skibo E B. Rational design of pyrrolo[1,2-a]benzimidazole based antitumor agents targeting the DNA major groove. **Bioor Chem** In press.

Luke Whitesell, M.D.

University of Arizona
Award Amount FY 2000: $226,456

Development of Antisense Oligonucleoptides as Chemotherapeutic Agents for Intratumoral Administration

A gene implicated in the malignant behavior of many cancers is the type 1 insulin-like growth factor receptor (*IGF-IR*). Overproduction of this receptor allows cancer cells to escape death and drives them to grow excessively. During the first year of ADCRC support, we synthesized a library of small DNA pieces (oligos) which were exactly opposite or antisense to 100 different sites within the *IGF-IR* gene. Unfortunately, none of them selectively decreased the receptor level in tumor cells. We tried several different chemical modifications of the oligos to improve their stability and specificity, but these materials also proved ineffective. As a result, we adopted a new strategy which makes use of compounds that do not decrease the level of receptor but, instead, block its ability to function in cells. We have already found significant antitumor effects with the compound AG1024. We expect that this research will provide important information on how to use this new class of anticancer agents; and hopefully, it will identify a specific compound for eventual clinical trial in cancer patients.

SECTION G

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