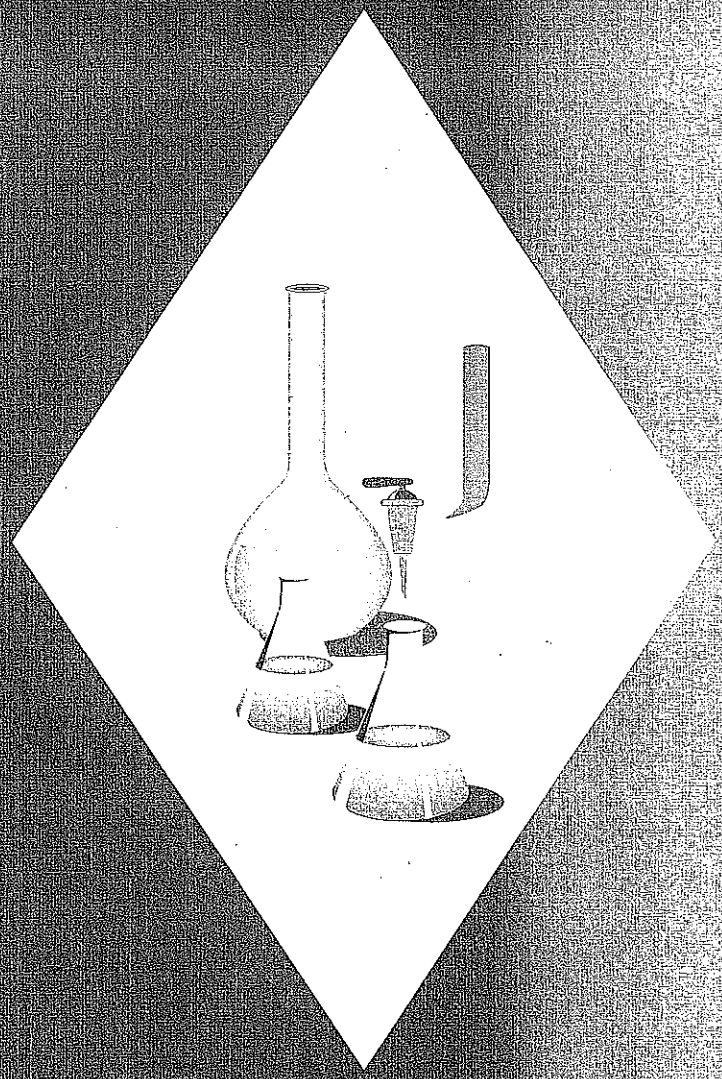
Arizona Disease Control Research Commission



1998 – 1999 Annual Report

January 2000

ARIZONA DISEASE CONTROL RESEARCH COMMISSION

ANNUAL REPORT
1998-99

Jane Dee Hull, Governor

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

COMMISSION MEMBERS

General Public
Lois Emden
Jose Cardenas, J.D.
Orme Lewis, Jr.

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

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

Staff
Executive Director: Dawn Schroeder, D.D.S., M.A.
Administrative Services Coordinator: Damika Brock
Fiscal Service Specialist II: Daniel Powell
Administrative Assistant II: Ismene Quintanilla

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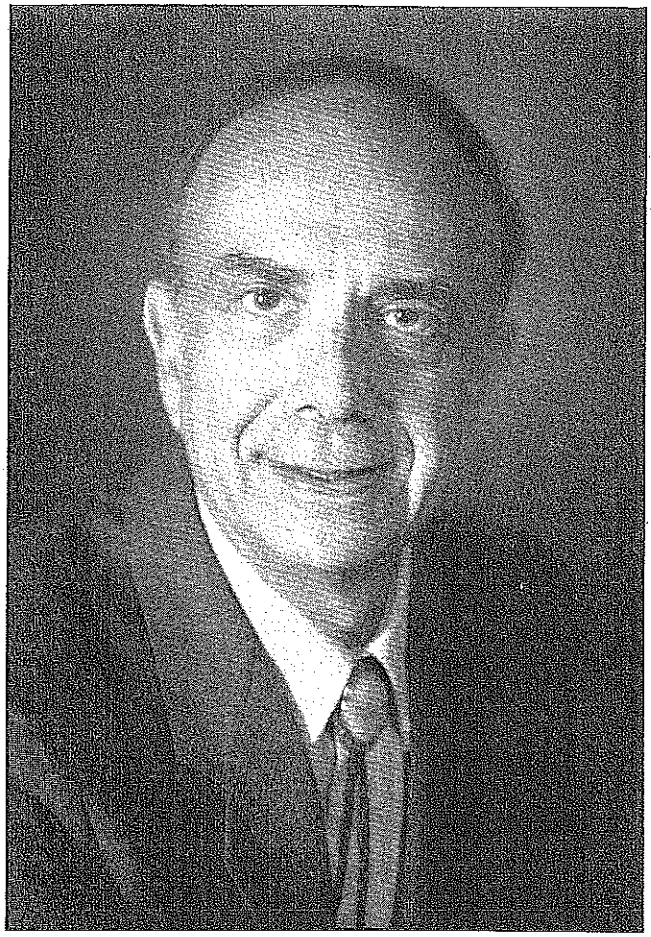
January 2000

In Memoriam
Sydney E. Salmon, M.D.
1936 -1999



This annual report is dedicated to the late Sydney E. Salmon, physician, healer, researcher, teacher and medical visionary *extra ordinaire*. Although the wider world will remember Syd for the renowned Arizona Cancer Center he created and also for the pioneering work he did in the field of human tumor cloning assay, we honor him here for something less visible but no less important. Syd Salmon was, more than anyone else, the Founding Father of the Arizona Disease Control Research Commission.

In a distant sense, the ADCRC started with an outbreak of aflatoxin in Arizona's milk supply back in the early 1980's. Since Governor Bruce Babbitt did not seem to appreciate the gravity of the crisis, Syd wrote the Governor a tart letter, lectured him on the carcinogenic properties of

aflatoxin and told him to visit the Medical School. As the rest of us always did, the Governor responded affirmatively to Syd and met with him for a short course on cancer in particular and medical research in general. When he had the Governor's ear, Syd told him that he should press for a new state commission, like the one in New York State, that would provide competitively-awarded funds for medical research in Arizona. Again, as the rest of us always did, the Governor responded affirmatively and, with the Governor's backing, Syd proceeded to muster political support by enlisting everyone he knew with clout and also by recruiting Bob Pettit, Director of the Arizona State University Cancer Research Institute.

In 1984, the Governor had a bill introduced into the Arizona Legislature which would, if passed, create the awkwardly named Arizona Disease Control Research Commission. It was then that Syd called the Arizona Cancer Society for its endorsement and spoke to me because, at the time, I was the Society's Chairman for Public Affairs. After the Cancer Society's officers were sweet-talked by the Governor and browbeaten by me, they grudgingly endorsed the bill and I enthusiastically joined Syd's crusade.

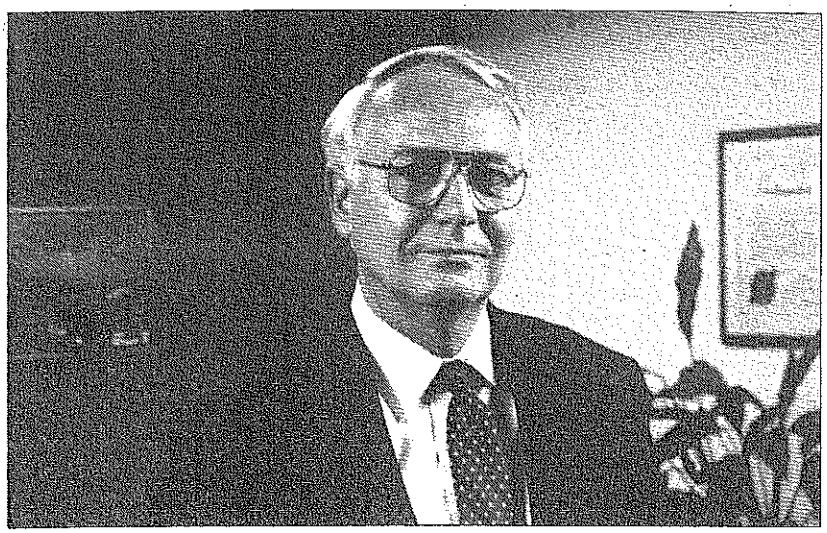
At first, things went smoothly and the bill sailed through the committees of both the House and the Senate. However, the proposed commission had no designated funding source and that, as it turned out, was a near-fatal problem. Since the legislators did not want to increase taxes or reallocate monies already called for, the bill was virtually dead on arrival when it reached the floor of the House of Representatives.

Again, Syd carried the day. This time it wasn't because of any political pressure he had generated but because, long before this legislative battle, he had been treating Bob Mills, a Phoenix lawyer who was dying with cancer. Mills was deeply grateful for Syd's care. Just before he died, Mills heard about the bill's fundless and pulseless condition in the House of Representatives and he asked his law firm's lobbyist, Jim Bush, to lean on Majority Leader Burton Barr for help. Barr, in turn, funded the commission with interest earnings on the Arizona delinquent tax fund. Because Syd had been a listening, caring, devoted bedside physician, the bill became law and the ADCRC was born.

Since then, the ADCRC has sponsored projects that have ranged in size from multi-year-multi-million-dollar investigations to three-month surveys costing as little as $1,000. It has promoted research into everything from heart disease to rattlesnake bites. In the process, the millions distributed by the ADCRC have attracted vastly more millions from out-of-state sources and, incidentally, have generated countless jobs in the state's scientific research sector.

The Oath of Hippocrates calls upon physicians to "lead your lives and practice your art in uprightness and honor." Our founder and friend not only fulfilled that Oath, he set a new standard for it. Thank you and Godspeed, Syd.

Peter D. Baird, Esq.



Message from the Chairman

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's Fiscal Year 1999 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 1999, the Commission's Sunset Year, the Legislature voted to continue the Commission until 2009. The Commission underwent financial and performance audits receiving favorable reviews from both the Auditor General and the General Accounting Office. The Sunset Review Committee, made up of members from both the House and Senate Health Committees, recommended the ten-year continuation.

The Commission continues to be involved in technology transfer and the patenting and licensing of discoveries funded with ADCRC monies. This year brought new challenges as several discoveries were evaluated for patent protection and future commercial use. The Commission works closely with a number of partners to obtain an unbiased evaluation of new technologies. The Commission filed its second patent application in FY 1999.

Four renowned cancer researchers from around the world evaluated the 17 proposals received in the Anti-cancer Drug Discovery Program. Using their input, the Commission selected 11 proposals for contract awards. Funds for major equipment totaling over $820,000 were provided to three of the projects. This program is forging new collaborations between the universities and between public and private institutions. The Commission is looking forward to reporting on the progress of this innovative program in FY 2000.

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 1998-99 are presented below.

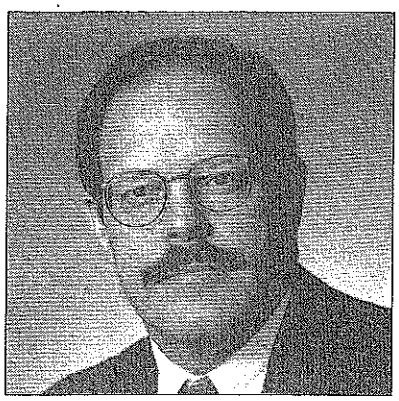
Henry Reeves, Ph.D., Chairman

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

Jose Cardenas, J.D.
Managing Partner, Law Firm of Lewis and Roca, Phoenix

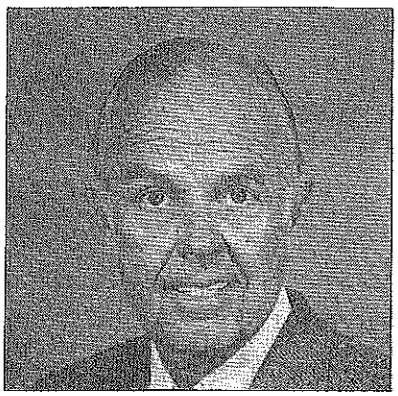


Commissioner Cardenas received a B.A. from the University of Nevada, Las Vegas in 1974 and a J.D. from Stanford University in 1977. He joined the firm of Lewis and Roca in 1978 where he practices primarily in the areas of commercial and civil litigation, intellectual property, and international law. He is a member of the Maricopa County, Arizona and American Bar Associations, the American Law Institute and the Hispanic National Bar Association. He served as president of the Los Abrogados Hispanic Bar Association from 1985 to 1988. Commissioner Cardenas is president of the American-Mexico Commission and is a member of the U.S. Delegation of the NAFTA Advisory Committee on Private Commercial Disputes. He serves as a member of the Minority Council Advisory Committee to Arizona State University President, Lattie Coor. Commissioner Cardenas was appointed to the Commission by Governor Symington in 1996. His term expired in May 1999.

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

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 will expire in May 2000.

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



Commissioner Lewis oversees commercial real estate interests and participates in environmentally sensitive businesses. He currently serves on the governing boards of the Arizona Historical Foundation, Arizona State University Foundation, Phoenix Children's Hospital, 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.

Medical Community

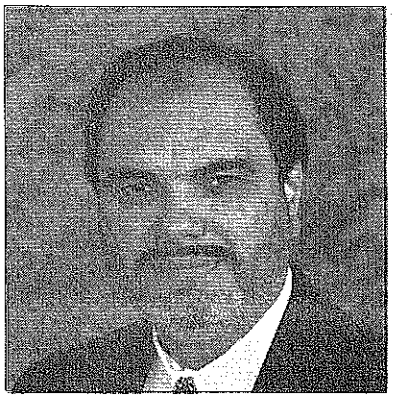
Patricia D. Moore, Dr.P.H., R.N.
Chair, Division of Community Health Nursing
Arizona State University

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

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

Commissioner Oakley received his A.B. from Washington University, St. Louis and an M.D. from the University of Missouri College of Medicine. He completed a rotating internship and four-year residency in General Surgery at St. Louis County Hospital. A member of the Arizona Medical Association for 38 years, Commissioner Oakley has also served as president and vice president. For 15 years, he was a preceptor professor for the University of Arizona College of Medicine. Commissioner Oakley has practiced general medicine and surgery in Prescott since 1962. Commissioner Oakley was appointed by Governor Symington in 1994 and reappointed in 1997. His term expires in May 2000.

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



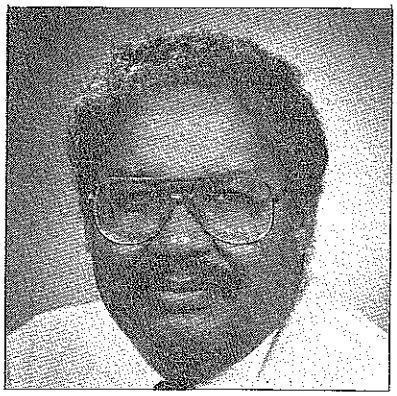
Commissioner Pereira received his B.S. in Chemistry from Georgia Tech in 1979. He graduated *Magna Cum Laude* from Emory University School of Medicine in 1983. After completing his Internal Medicine Residency at the same institution, he joined the staff of Mariposa Community Health Center as a Scholar 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 percent 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.

Scientific Research Community

T. Lon Owen, Ph.D.
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.
Associate Professor, Department of Radiology
University of Arizona



Commissioner Williams received his B.S. in Chemistry 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. His term expires in May 2000.

Commission Staff

Dawn 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 Brock
Administrative Services Coordinator

Ms. Brock joined the Commission staff in October of 1993. She accepted her current position in October of 1997. 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.

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 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.

Ismene Quintanilla
Clerk-Typist III

Mrs. Quintanilla came to the Commission in January 1998. 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 1998-99 Commission Activities

The Commission had 85 contracts with medical and health researchers in Arizona as of July 1998. Contract summaries are contained in Sections A-C. The section headings list the sources of funding and whether the project is in its first, second or third year of funding.

Abstracts for each project, outlining the progress made during the year, are included. Citations for scientific publications and abstracts arising out of the research are also listed. Lay summaries for new awards made in 1999 and scheduled to begin in FY 2000 can be found in Section E for the Tobacco-related Research Program and in Section G for the Anticancer Drug Discovery Program. These summaries provide an overview of the new research.

Approximately 1086 Requests for Proposals (RFPs) for 1998-99 awards were mailed to potential applicants in August 1997. The amount available for new tobacco-related research contract awards was approximately $1,900,000. The amount available for new anticancer drug discovery contract awards was approximately $7,000,000 over two years. In response to the RFPs, the Commission received 60 proposals in November of 1998 for the Tobacco-related Research Program and 17 proposals in December of 1998 for the Anticancer Drug Discovery Program. Sections D and F list the research proposals received in response to these RFPs.

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 184 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 22 award-winning research projects from among the applications submitted for the Tobacco-related Research Program.

Four internationally known researchers in anti-cancer drug discovery reviewed the proposals for this program and traveled to Arizona to individually interview each applicant. This team of experts also site-visited several of the research facilities in Phoenix and Tucson. In a Commission meeting held in March, the Commissioners received the panels written and oral reviews and awarded 11 research contracts. The Commissioners also made three awards totaling approximately $820,000 for the purchase of much needed major equipment for this research. During 1999-2000 the ADCRC will be managing 85 contracts.

SECTION A

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR ONE

Anne E. Cress, Ph.D.

University of Arizona
Award Amount FY 1999: $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 would prevent human lung tumor cells from adhesion to a protein called laminin in the lung. We have obtained pure peptide reagents which are present in mg amounts. We have discovered that the peptides are biologically active as determined by the human lung cell binding test. The peptides can inhibit human lung tumor cell adhesion to laminin. We have unexpectedly made two discoveries. One peptide promotes adhesion of the cells to laminin. This reagent may have the potential to promote laminin adhesion in other cell types, which 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 which attaches readily to laminin. This variant line will serve as sentinel cells for further testing of anti-adhesive 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 lung tissue.

A. Jay Gandolfi, Ph.D.

University of Arizona
Award Amount FY 1999: $49,134

Role of Tobacco-Derived Cadmium In Prostate Disease

Prostate cancer incidence and mortality has been linked with cigarette smoking and cadmium. In 1995, mortality due to prostate cancer in Arizona was in the top 40 percent of the United States. As there is no acceptable animal model of prostate cancer, we have recently developed a tissue slice model for human prostate. Slices made from regions of the prostate that are targets for disease have been incubated for long periods and found to retain their specific characteristics. Treatment of these slices with cadmium produces a toxicity in the region in which cadmium would be expected to produce its cancer. Human prostate slices represents a unique and highly relevant model to investigate tobacco-derived cadmium-induced prostate cancer.

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

Award Amount FY 1999: $49,962

Mechanism-based Discovery of Novel Antitumor Agents from Plants

During the course of the first year of this project, 166 plant species were collected and extracted yielding 498 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.

Of the 498 extracts tested, a total of 47 extracts (derived from 22 plant species) showed moderate potential anticancer activity in the above two bioassays. We have initiated bioassay-guided fractionation of 12 of these extracts. However, most of our efforts to isolate and characterize potential anticancer compounds were directed towards 2 plants (*Acourtia thurberi* and *Phoradendron juniperinum*) 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 more elderly and/or tobacco-dependent portions of Arizona's population since both of these cancers occur more often in our state.

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

Award Amount FY 1999: $150,000

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

The purpose is to develop immuno-gene therapy for lung cancer via animal models, clinical trials and immunological studies. Lewis Lung Cancer (3LL) has been established, including tissue culture, transfection and growth curves. Transfecting agents including DMRIE/DOPE, DMRIE cholesterol, and others were studied. DMRIE/DOPE and superfect were optimal. We investigated transfection of 3LL and control of SW480 cells with HSP-65 and demonstrated successful transfection of HSP065 on growth of established human tumors in SCID mice (Hey and A375), showed a blank effect (antitumor activity of the plasmid backbone). We studied intratumoral HSP-65 gene transfer plus systemic IL-2 in the 3LL. No antitumor effect was seen because of rapid growth. An alternative strategy using pre-immunization with 3LL cells into sites primed with the GM-CSF gene is under development. For the clinicals, a protocol was written, reviewed and approved by the Human Subjects, Scientific Review and Biosafety Committees. The protocol was submitted and approved by the RAC of NIH and the FDA and an IND obtained. Research nurses and data managers have been trained. The protocol is activated and patients accrued.

Douglas F. Lake, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

Development of a Peptide Therapy for Small Cell Lung Cancer

We are utilizing a high throughput screening method called combinatorial chemistry to discover peptides that bind to and exert biological effects (death) on lung cancer cells. The power of this approach is that millions of peptides or compounds can be screened for binding in a single day. 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 cells surface receptor is its cognate lock. Usually, only one key fits one lock. After screening libraries with lung cancer cells, the cells rosette around individuals beads suggesting a specific peptide-cell surface receptor interaction. Specificity was confirmed, as breast cancer cells did not rosette the same beads that the lung cancer beads rosette. This project is continuing toward its goal of identifying peptides that induce lung tumor cells to stop growing or to die.

Robin K. Pettit, Ph.D.

Arizona State University
Award Amount FY 1999: $150,000

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

Infectious disease is a leading cause of death in cancer patients. Each year nearly 6,000 Arizona residents die of tobacco-related cancer or associated infectious disease. There is an urgent need to develop new anticancer and antimicrobial drugs, and ADCRC funding has facilitated expansion of the Cancer Research Institute's (CRI) drug discovery program to include antimicrobials. In the past year, we screened hundreds of CRI natural products, semisynthetic and synthetic compounds for antibacterial and antifungal activities. Approximately 10 percent of these compounds were antimicrobial. We have completed qualitative, quantitative and preclinical in vitro evaluations of four promising leads, dolastatin 10 (antifungal), spongistatin 1 (antifungal), 3β-acetoxy-17β-(L-prolyl)amino-5α-androstane (antibacterial) and 1-(3', 4', 5'-trimethoxphenyl)-2-nitro-ethylene (antifungal). In vivo toxicity and efficacy studies are in progress for dolastatin 10 and 3β-acetoxy-17β-(L-prolyl)amino-5α-androstane. Dolastatin 10 and 3β-acetoxy-17β-(L-prolyl)amino-5α-androstane appear to be nontoxic at relatively high doses. With ADCRC support, such promising compounds will proceed much more rapidly from the laboratory to thousands of Arizona patients with tobacco-related cancers and associated infectious disease.

Publications:

Petit RK, Pettit GR, and Hazen KC. Specific activities of dolastatin 10 and peptide derivatives against *Cryptococcus neoformans*. **Antimicrobial Agents and Chemotherapy** 42:2961-2965, 1998.

Pettit GR, Fkahive EJ, Boyd MR, Bai R, Hamel R, Pettit RK, Schmidt JM. Antineoplastic agents 360. Synthesis and cancer cell growth inhibitory studies of dolastatin 15 structural modifications. **Anti-cancer Drug Design** 13:47-66, 1998.

Pettit GR, Cichacz ZA, Tan R, Hoard MS, Melody N, Pettit RK. Antineoplastic agents 386. Isolation of sestertatins 1-3 from the marine sponge *Hyrtios erecta*. **J Natural Products** 61:13-16, 1998.

Pettit RK, McAllister SC, Pettit GR, Herald CL, Cichacz ZA. A broad spectrum antifungal from the marine sponge *Hyrtios*. **International J Antimicrobial Agents** 9:147-152, 1998.

Pettit GR, Toki B, Herald DL, Verdier-Pinard P, Boyd MR, Hamel E, Pettit RK. Antineoplastic agents 379. Synthesis of phenstatin phosphate. **J Medicinal Chemistry** 41:1688-1695, 1998.

Pettit GR, Srirangam JK, Barkoczy J, Williams MD, Boyd MR, Hamel E, Pettit RK, Hogan F, Bai R, Chapuis J, McAllister SC, Schmidt JM. Antineoplastic agents 365. Dolastatin 10 SAR probes. **Anti-Cancer Drug Design** 13:243-277, 1998.

Ovechkina YY, Pettit RK, Chichacz ZA, Pettit GR, Oakley BR. Unusual antimicrotubule activity of the antifungal agent spongistatin 1. **Antimicrobial Agents and Chemotherapy** In Press, 1999.

Joy J. Winzerling, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

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

We hypothesize that chemicals in cigarette smoke will alter iron metabolism in lung cancer cells. Further, we think that cigarette smoke will cause measurable changes in proteins involved in intracellular iron metabolism, and that these changes will promote free radical formation and lung damage. The proteins of interest include ferritin and the iron regulatory proteins (IRP1 and IRP2). In the past year, we have obtained and analyzed a lung cancer cell line (A549 cells). Cell viability is unaffected by increasing concentration of iron in the culture media administered as ferric ammonium citrate of 100 μM. We have obtained antibodies to the proteins and determined the amount of cells necessary to measure the ferritin subunits, IRP1 and IRP2. We also have determined the amount of cells required for measuring the activity of the IRPs by gel shift assay. We plan to assess the measurement of RNA by quantitative reverse transcriptase-polymerizing chain reaction (RT-PCR). Once all methods of measurement are tested, we will conduct the proposed studies.

Henry I. Yamamura, Ph.D.

University of Arizona
Award Amount FY 1999: $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. Chronic use of opiates causes tolerance or decreased responsiveness to the drug and adenylyl cyclase superactivation or cAMP overshoot. Adenylyl cyclase superactivation after chronic opiates is important in opiate tolerance, dependence and withdrawal. As a first step, we have identified the mRNAs for adenylyl cyclases VI and VII CHO cells and adenylyl cyclases VII and IX in B82 cells. These cells are often used as mammalian host cells for G-protein coupled receptors such as the opioid receptor.

Paul F. McDonagh, Ph.D.

University of Arizona
Award Amount FY 1999: $49,223

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

This research project aims to determine the effects of side-stream cigarette smoke exposure on the leukocyte contribution to ischemia-reperfusion in the heart. The studies in the first year aimed to determine if, and what duration of, side stream cigarette smoke exposure caused white cell activation, in-vivo. The functional properties of circulating leukocytes that were studied were: 1) expression of the neutrophil adhesion protein, CD11b, and 2) the neutrophil production of reactive oxygen species, ROS (oxygen derived free radicals). Two studies were performed in the first year. The first year study involved exposing rats to one hour of side-stream cigarette smoke per day for three weeks (each week, the schedule was five days of exposure and two days of no exposure). The second study involved one hour of cigarette smoke exposure per day for one month. Unexposed control groups were included in both studies. We found in both studies that exposure to SSCS caused an increase in the number of circulating neutrophils. Also, there was a trend in both studies for neutrophil CD11b to increase after a few days of exposure, but thereafter, Cd11b tended to return to baseline. Free radical production (ROS), fluctuated in the smoke exposure group with time. We suspect that cigarette smoke exposure is causing neutrophil activation leading to neutrophil sequestration in organs such as the heart and lungs. Therefore, we decided to develop an assay of neutrophil accumulation in tissue. Myeloperoxidase (MPO) is an enzyme produced specifically by neutrophils. The early assay results indicate that measured MPO activity is proportional to neutrophils. The technique will now be used to measure MPO in the heart and lungs of animals from Studies I and II.

Raymond B. Nagle, M.D., Ph.D.

University of Arizona
Award Amount FY 1999: $112,204

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 treatments for blood vessel occlusion remain limited to the use of devices which bypass the area of disease. Unfortunately these devices do not perform optimally primarily due to the production of these devices from materials which are non living. The results of the research performed as part of this research program provide evidence that a new type of artifical 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. 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 which stimulates the formation of a new living blood vessel in a patient which will remain functional for decades.

Thomas P. Davis, Ph.D.

University of Arizona
Award Amount FY 1999: $48,070

Nicotine Effect on Blood-Brain Barrier Integrity, Function and Permeability

Cigarette smoking and the application of nicotine patches have been associated with an increase risk for stroke. The blood-brain barrier (BBB) is a system of capillaries that separates the blood from the central nervous system. Despite the importance of the BBB in maintaining the cerebral environment, little is known about the effects of nicotine on the BBB. Since smoking is a risk factor for stroke, it is likely that nicotine may have an effect on the BBB. We have discovered that nicotine and cotinine treatment resulted in opening of a cell culture model of the BBB. Additionally, short exposures to nicotine in the rat caused an opening of the BBB. Longer doses of nicotine resulted in a return to normal BBB function. Additionally, we observed a decrease in a tight junctional protein, ZO-1, after nicotine and cotinine exposure. This protein is crucial for maintaining the "molecular gasket" between the blood and brain.

Publication:

Abbruscato TJ, Davis TP. Combination of hypoxia/aglycemia compromises *in vitro* blood-brain barrier integrity. J Pharmacol Exp Ther 289:668-675, 1999.

Duane Sherrill, Ph.D.

University of Arizona
Award Amount FY 1999: $49,942

Assessment of Patterns of Mendelian Inheritance of Addiction to Cigarettes

Our study objectives are to evaluate familial aggregation and mode of inheritance of nicotine addiction among smoking-dependent and non-smoking dependent subjects in a population of Tucson families. We found significant differences in prevalence and intensity of smoking in our population by sex and age. In preliminary analyses, the smoking-dependent phenotype was defined as a current or past smoker who regularly smoked more than 20 cigarettes daily. Thus far, we have found evidence of significant familial aggregation of smoking dependence between spouses, fathers and offspring, and siblings. There is no evidence, though, of a major gene and our results suggest that environmental factors may account for the familial correlations observed.

Two divergent approaches will be pursued in a attempt to obtain better resolution of the phenotype: 1) to test models of inheritance that incorporate age and sex-dependent effects; 2) to redefine the phenotype, adjusting for sex and age before testing the models.

Michael Berens, Ph.D.

Barrow Neurological Institute
Award Amount FY 1999: $49,052

Human Recombinant Receptors for Nicotine

Nicotine is a powerful tobacco substance that affects the brains and bodies of an estimated 25 percent 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 block (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 succeeded in producing cells that make nAChR composed of two kinds of subunit, specifically human α4 and β2 subunits. The α4β2 subtype of nAChR is thought to be the most abundant in the human brain and to have exceptionally strong interactions with nicotine. Human α4β2-nAChR made in our genetically engineered cells exhibit this strong interactions with nicotine. We also have progressed toward creating cells that make nAChR composed in binary combinations of α2, α3, or α4 subunits with β2 or β4 subunits. nAChR composed of these subunits may mediate influences of nicotine on pleasure/reward, mood, and learning/memory. Our engineered cells represent valuable models for studies of acute or chronic nicotine action, particularly with relevance to development of nicotine dependence.

Publications:

Lukas RJ. Cell lines as models for studies of nicotinic acetylcholine receptors. In *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities*. Americ SP and Brioni JD, Eds. Wiley-Liss, Inc., New York, pp. 81-97, 1999.

Lukas RJ, Changeuz J-P, Le Novere N, Albuquerque EX, Balfour DJK, Berg DK, Bertrand D, Chiappinelli VA, Clarke PBS, Collins AC, Dani JA, Grady SR, Keller KJ, Lindstrom JM, Marks MJ, Quik M, Taylor PW, Wonnacott S. International Union of Pharmacology. XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. Pharmacol Rev 51:397-401, 1999.

Peng J-H, Lucero L, Fryer J, Herl J, Leonard SS, Lukas RJ. Inducible, heterologous expression of human α7-neuronal nicotinic acetycholine receptors in a native nicotinic receptor-null human clonal lines. Brain Res 825:172-179, 1999.

Allan L. Bieber, Ph.D.

Arizona State University

Award Amount FY 1999: $149,504

Composition of a Unique Receptor for Nicotine

Nicotine dependence and habitual use of tobacco products are initially based on actions of nicotine at the diverse family of chemical signaling molecules called nicotinic acetylcholine receptors (nAChR). However, our understanding is deficient about the precise composition of several important nAChR subtypes and proteins that associate with them. This deficiency compromises our understanding about what happens during chronic nicotine exposure to nAChR and to the nervous system functions modulated by nAChR. In part, our limited understanding is attributable to a relative paucity of sophisticated techniques to analyze complex, cell-membrane-associated proteins such as nAChR. To achieve a necessary practical aim of this project, techniques have been adapted to prepare a well-characterized nAChR subtype at different levels of purity as an experimental standard. These preparations have then been analyzed using an unparalleled process of combined sensitivity, resolving power, and flexibility called matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS). Refinement of sample preparation has been achieved to facilitate MALDI-TOF-MS analyses of nAChR. In parallel, molecular biological studies have been used to engineer other nAChR subtypes in full-length and truncated forms for future MALDI-TOF-MS analyses. The long term goals of the project remain the same: we seek to establish the composition of less abundant, but important, nAChR subtypes found in the brain and their assembly partners. Work done in the first year of the project has set the stage for accomplishment of this goal using MALDI-TOF-MS analyses.

Dean Coonrod, M.D., MPH

Maricopa Medical Foundation
Award Amount FY 1999: $49,836

No Mas Cigarros-Smoking Cessation for Latinas

A survey tool was designed to collect baseline information on the population. To assure a cross section of acculturation levels, the survey was administered by experienced bilingual surveyors to 126 individuals from a variety of different sites. Each individual who completed a survey was given a grocery store coupon as an incentive to participate.

Results of an interim analysis is being used in development of the culturally appropriate smoking cessation behavioral intervention. With the assistance of consultants, Felipe Castro, Hector Balcazar and Curt Bay, development of the curriculum is in the final stages. Individuals in the community who are active in smoking cessation have also been enlisted as expert resources.

A health educator was hired to assist in the curriculum development and to run the behavioral intervention. She has attended the curriculum training for the American Lung Association curriculum and also the Arizona Cessation Training and Evaluation brief intervention training.

Scott Leischow, Ph.D.

University of Arizona
Award Amount FY 1999: $48,564

Diverted Youth: Testing a Tobacco Cessation Intervention for Adolescents

The first year of this research project designed to assess the relative efficacy of theoretically-based tobacco cessation counseling interventions for adolescents has been productive and has set the stage for what promises to be an important and timely study. Following formative research, program protocols, materials for clients, counselors, and program administration have been developed and implemented. All administrative personnel have been hired and trained. Both adult and peer counselors have been hired, trained and they have initiated counseling sessions with fifty clients (completing sessions with 22 clients by July 1, 1999). Contracts with community resources such as schools, juvenile court officials, and probation officers have been established and efforts to develop contacts in more remote areas of the state are currently under way. Potential program expansion and collaboration efforts are also being discussed with state tobacco prevention organizations. Increasing the scope of service is one goal for Year 2.

Daniel E. Shapiro, Ph.D.

University of Arizona
Award Amount FY 1999: $49,998

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

Every year approximately 75 percent of ambivalent smokers in Arizona come in contact with a health professional. 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 ambivalence about smoking and then emphasizes those back to the smoker. We have recruited 78 smokers and are slightly ahead of schedule in recruitment. Preliminary results, which will be presented at the Annual National American Psychological Association meeting in August, suggest that smokers who receive a motivational interview from a health professional are more likely to significantly reduce their smoking behavior (as measured by biological verification) than those receiving authoritarian prescriptive interventions. Interestingly, those receiving prescriptive advice are more likely to verbally express a plan to quit but are less likely to follow through. These results are preliminary. However, if these trends continue, we may be able to significantly reduce the number of 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 1999: $49,995

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

During the first year of this study examining differences in outcome and immune response to Valley Fever (coccidioidomycosis) between male smokers and non-smokers, a total of 44 subjects were entered. The proportion of subjects who were smokers was less than predicted. 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 Valley Fever. Moreover, there were no differences with regards to their skin-test reaction to Valley Fever, nor in their Valley Fever serum reaction, or in their severity of Valley Fever. Finally, a new test was developed to assess the immune response to Valley Fever which incorporates the properties of three different assays. A manuscript describing the results of this assay has been submitted for publication. To date, these preliminary results do not yet show a differences in response to Valley Fever between smokers and non-smokers, but results may change as the study continues.

Clark Lantz, Ph.D.

University of Arizona
Award Amount FY 1999: $49,998

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 lines the lungs and is essential for appropriate lung function. This year we have determined that ETS exposure leads to an increase in type II cells (cells responsible for production of surfactant). However, there is no change in the number or size of the organelles that store the surfactant prior to release. We have established an *in vitro* model that will allow us to study the effects of ETS while maintaining tissue architecture. We have begun determining the effects of ETS on lung tissue *in vitro*. Specific probes to measure the proteins found in the surfactant have been developed. Since ETS exposure of young children can lead to severe lung problems, this research is important for the children of Arizona.

Steven J. Barker, Ph.D.

University of Arizona
Award Amount FY 1999: $49,748

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 percent 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.

Mark Brown, M.D.

University of Arizona
Award Amount FY 1999: $125,567

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 infant had been born and growing tissue and cells from them in the laboratory, we were able to show that a small sample taken from anywhere in the placenta is representative of the placenta as a whole. We then studied signaling chemicals produced by the placenta used to coordinate activity between placental cells and blood cells. We were able to establish our ability to detect these chemicals, but as yet have not been able to collect enough samples from women who smoke to draw conclusions about any effects tobacco smoke might have on placenta structure and/or function.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY 1999: $122,269

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. 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. During the first year of this project, we have 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 have 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. We will continue to use the organ culture method to: 1) determine effects of nicotine and related ligands on development of T cells, including human T cells, and 2) characterize the receptors that are responsible for the recognition of nicotine on the developing 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 1999: $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 melaogaster* 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. The longer-lived O stocks derived from selection for delayed reproduction show significantly greater resistance to nicotine impacts on development. Threshold toxicity levels have been established that will allow selection in year 2 for greater nicotine resistance.

Mark L. Witten, Ph.D.

University of Arizona
Award Amount FY 1999: 132,752

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

Environmental tobacco smoke is a major risk factor for public health. Using a well-established murine model, we conducted the effects of vitamin E dietary supplementation on sidestream cigarette smoking, as measured by lung function, cytokines, and pathological evaluation. Eighty-eight C57BL/6 mice (half each gender, 7 months old) were randomly assigned to four groups: sham smoking (room air) with normal or 150-fold vitamin E diet, sidestream cigarette smoking with normal or 150-fold vitamin E diet. Vitamin E dietary supplementation significantly improved the sidestream cigarette smoking-induced increase of cytokine IL-1β, but IL-6. This study suggests that high intake of Vitamin E may improve dysregulation of the tobacco smoke-induced local host defense mechanisms through cytokine regulation.

Publication:

Wang S, Watson RR, Young S, Zhang Y, Bradshaw B, Witten ML. Vitamin E supplementation improves lung function with systemic cytokine dysregulation. **FASEB J** 13:A240.

Michael Burgoon, Ph.D.

University of Arizona
Award Amount FY 1999: $132,288

The Smoke Free Work Place: Overcoming Resistance and Implementing Changes in Smoking Behavior

This first year of a project designed to assess the process by which a large health organization can best become smoke free has been full of challenges. Initial barriers such as accessing employee data, budget cuts, and major organizational changes were plentiful, but we have managed to implement some innovative research. Broadening the scope of services to more general health concerns, we have piqued employee interest and may even have a positive impact on tobacco habits in an indirect fashion.

Monthly activities have attracted the participation of a few hundred employees and have also made the issue of employee wellness very salient among the 3,200 employees. Data gathered this year will help other large organizations in their efforts to become smoke free while overcoming substantial resistance from tobacco users. Efforts on this project have also facilitated the preparation of a NIH proposal that will significantly benefit Arizona citizens if funded.

Mary H. Burleson, Ph.D.

Arizona State University West
Award Amount FY 1999: $49,970

Stress and Smoking: The Effects of Daily Events, Gender, and Nicotine Dependence on Smoking and Smoking Urges

In the current study we examined how stressful events and psychological distress relate to cigarette smoking and urges to smoke. In addition, we explored the potential moderating role of both gender and nicotine dependence in the stress-smoking relationship. The study design called for 15 participants in each of 4 groups: female nicotine dependent, male nicotine dependent, female non-dependent, and male non-dependent for a total of 60 participants. The study required participants to complete data sheets four times a day for 14 days resulting in 56 repeated observations per person, 2600 observations for the entire sample. At each assessment participants completed a checklist of minor stressful events and rated the psychological distress associated with the most stressful event from that assessment interval. Participants recorded cigarettes smoked and urges to smoke, times he or she wanted to smoke but did not smoke, for each assessment interval. Here we report preliminary findings based on a sample of 47 adult smokers (n=15 female dependent, n=12 male dependent, n=15 female non-dependent and n=5 male non-dependent).

We tested a total of 12 multilevel models specifying either stressor count or distress rating as the Level-1, within person, predictor and either gender or nicotine dependence status, yes or no, as the Level-2, macro-level predictor with the dependent variable being cigarettes smoked, urges to smoke or a composite of cigarettes smoked and urges.

Results for cigarettes smoked were mixed. While stressor count, number of stressful events, was not a significant Level-1 predictor of smoking behavior, subjective distress ratings were significantly and positively related t smoking ($ps<.05$). However, in the model specifying distress as the Level-1 predictor and gender as the Level-2 predictor, the main effect of distress was qualified by a distress by gender interaction. in all four models specifying urges to smoke as the dependent variable, the Level-1 predictors, stressor count and distress, were significant positive predictors of urges ($ps<.001$). We found no interactions in the urges models. Stressors and distress were both significant predictors of the composite, sum, of cigarettes smoked and urges ($ps<.01$). However, gender moderated the effects of stressors and distress ($ps<.05$) for interaction effects where it was the Level-2 predictor.

SECTION B

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR TWO

Emmanuel T. Akporiaye, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

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

TGF-β is an immunosuppressive molecule produced by tumor cells that enables them to escape from immune cell-mediated destruction. Previously, we demonstrated that insertion of an antisense copy of the TGF-β (αsT) and the IFN-γ genes into non-metastatic EMT6 murine mammary carcinoma cells generates an anti-tumor response more potent than that by EMT6 cells expressing either gene alone. Theses data suggest that down-regulation of TGF-β-mediated immunosuppression combined with IFN-γ-mediated immune activation is a useful strategy for treating cancer. In this study, we also demonstrate that inserting either gene alone in metastatic mammary carcinoma cells (4T1) reduces their ability to form primary tumors and to spread to distant organs. Studies are ongoing to determine the effect of simultaneous expression of αsT, IFN-γ, and B7.1 by 4T1 cells on metastatic spread and the treatment residual disease.

Publication:

McEarchern JA, Besselsen DG, Akporiaye ET. Interferon-γ and 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 1999: $49,999

Pharmacokinetic Modeling of the Anabolites of Nucleoside Analogues

This research program has performed studies to develop a physiologically based pharmacokinetic model to help describe and predict the time course of zidovudine (AZT) levels in different target tissues. The model was first developed and validated using data collected from the mouse and was subsequently scaled up to protect human situations. The model predicted the presence of subtherapeutic concentrations of AZT in the brain and lymph node tissues following a single oral intake of a 200 mg dose. Since the brain and lymph nodes are important targets in treating HIV infection, our findings bear therapeutic implications applicable not only to Arizona residents but also to other HIV infected individuals.

Publication:

Chow H-H, Brookshier G, Li P. Tissue disposition of zidovudine and its phosphorylated metabolites in zidovudine-treated healthy and retrovirus infected mice. **Pharmaceutical Research**, 15:139-144, 1998.

William J. Grimes, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

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

Our goal for the project is to study how peptide antigens are recognized by the immune system, and how modified peptides could be used in cancer therapy. In the first two years of our project, we have developed new methods for determining how cell proteins that regulate an immune response specifically bind peptide antigens from cancer cells. We have been successful in determining how a number of different alleles or version of these histocompatibility proteins specifically recognize antigens, and the rules which allows us to predict which peptides should have the highest affinity and thus be potential antigens for therapy. We have also shown that peptides with altered amino acids that should make them stable upon patient injection can still be potential antigens, and would be predicted to produce a stronger immune response. In a separate project, we have prepared cell lines from humans that can be cultured in our laboratory, and show specific immune recognition of peptide antigens. We now plan to combine the two systems to directly determine efficacy of designed peptide drugs as new anticancer agents.

Publication:

Smith MH, Nuara AA, Egen JG, Sirjani DB, Lam KS, Grimes WJ. Baculoviral expressed HLA class heavy chains used to screen a synthetic peptide library for allele-specific peptide binding motifs. **Molecular Immunology** 35:1033, 1998.

Teh-Li Huo, M.D., Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

Molecular Mechanism of Hormone-regulated Calcium Transport in Kidney

The goal of this research project is to find out the basic mechanisms of abnormal calcium metabolism in hypercalcemia, osteoporosis, and kidney stone disease, focusing on the study of kidney calcium transport. In the past two years, we were in the progress of discovering the most important calcium transporter that is responsible for the kidney calcium reabsorption. We have successfully used the advanced scientific techniques to "fish out" several candidates of the novel calcium transporters. Currently we are in the process of identifying the complete sequences of these candidates. Besides this, we are also using a specific strain of mouse, which has calcium leak in the urine, to further characterize the kidney calcium transport mechanism. The results from this research will have significant impact on the development of new drugs, which can be used to treat hypercalcemia, osteoporosis and kidney stone disease more effectively.

Publication

Gagnan-Brunette M, Huo TL, Yeh C-C, Christakos S. Effect of calbindin-D28k on sodium transport by the luminal membrane of the rabbit nephron. **Mol Cell Endoc** 152:161-8, 1999.

Douglas F. Lake, Ph.D.

University of Arizona
Award Amount FY 1999: $49,883

Genetic Immunization Against Mutant p53 for Lung Cancer

Genetic immunization is a new technology that utilizes DNA instead of protein to induce an immune response. Transfer of DNA encoding a gene of interest inside a cell results in production of the gene product, protein. 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 suggest that DNA immunization elicits immunity against both mutant and wild type p53. An immune response against wild type p53 is not necessarily bad because tumor cells over-express p53, while the half-life of wild type p53 in the nucleus of normal cells is approximately 8 minutes. In conclusion, our results show that immunization with p53 DNA elicits a general activation of the immune system, something that must be explored further as we optimize expression of p53 *in vivo*.

Lynn J. Manseau, Ph.D.

University of Arizona
Award Amount FY 1999: $48,491

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 the 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 with 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 are screening for flies in which the DRTGEF/CDC42 signaling within the cell. We are screening for flies in which the DRTGEF is eliminated or altered so that we can determine how this affects the fly. Also, it is important to know where DRTGEF functions in the fruit fly, so we are in the process of raising antibody against DRTGEF and are fusing DRTGEF to a protein that glows.

Thomas P. Miller, M.D.

University of Arizona
Award Amount FY 1999: $200,000

Developmental Treatment of Smoking-related Cancers: A Program Project

This program (as founded) focuses on multidisciplinary clinical research to evaluate new drugs and approaches which may ultimately lead to improved treatment and higher cure rates for patients with tobacco-related cancers. Head and neck and lung cancers, tumors caused almost exclusively by tobacco, as well as other tobacco-related cancers (e.g., pancreas cancer) representing a significant percentage of both new cases and deaths from cancer in Arizona each year, are targeted. Benefitting from new knowledge from other Arizona Cancer Center research initiatives, a series of clinical trials is planned. Of particular interest are: 1) strategies to overcome tumor resistance to drug or radiation therapy; 2) drugs with novel mechanisms of action, including those interfering with tumor blood supply; 3) improved drug delivery; and 4) protection of normal tissue during therapy. Progress during year two includes initiation of two new clinical studies in lung cancer, one new study in head and neck cancer and the development of two new studies of lung cancer and head and neck cancer.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 1999: $450,000

Preclinical Development of New Anticancer Drugs Necessary to Improving Treatment of Tobacco-related Human Cancer

The Arizona Disease Control Research Commission support for the Arizona State University Cancer Research Institute has continued to lead to outstanding progress in the development of new anticancer drugs for improving human cancer treatment. The U.S. National Cancer Institute has now expanded the clinical evaluation of bryostatin 1 to over forty human cancer clinical trials involving 159 clinical investigations in 136 clinical centers. In addition, nine phase II clinical trials of bryostatin 1 continue in Canada and another four in England. The initial phase II human clinical trials of dolastatin 10 through the U.S. National Cancer Institute are underway and will be expanded to over twenty. Both of these new anticancer drugs required ADCRC assistance for their development to human clinical trials and that requirement continues essentially daily. Another very exciting development based on ADCRC support was the confirmation that our Combretastatin A-4 prodrug is one of the most powerful tumor antiangiogenesis drugs now known. The first four human cancer clinical trials began last November, and current results have stimulated preparation for phase II trials. Other important advances include the continued preclinical and clinical development of our auristatin PE discovery. All of these clinical trials include tobacco etiology human cancer types. In addition, we have recently discovered and have under preclinical development the very useful phenstatin, diphenstatin and Combretastatin A-1 prodrugs. In summary, the ADCRC support has again led to outstanding research progress.

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Khan P, McGown AT, Dawson MJ, Jayson G, Prendiville JA, Pettit GR, and Crowther D. High-performance liquid chromatographic assay for the novel antitumor drug, bryostatin-1, incorporating a serum extraction technique. **J Chromatography B** 709:113-117, 1998.

Pettit GR, Tan R, Xu J-P, Ichihara Y, Williams MD, Boyd MR. Antineoplastic agents 398. Isolation and structural elucidation of cephalostatins 18 and 19. **J Nat Prod** 61:955-958, 1998.

Kamano Y, Yoshida M, Zhang H-P, Pettit GR, Kosuge N, Kawamura M. Effect of Antineoplastic macrolide bryostatin 10 on steroidogenesis in the primary cultured bovine adrenocortical cells. **Jikeikai Med J** 45:1-9, 1998.

Wender PA, DeBrabander J, Harran PG, Jiminez J-M, Koehler MFT, Lippa B, Park C-M, Siedenbiedel C. Analogs of bryostatin 1. **Proc Natl Acad Sci** 95:6624-6629, 1998.

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Pettit GR, Cichacz ZA, Tan R, Herald DL, Melody N, Hoard MS, Doubeck L,. Hooper JNA. The isolation and structure of a scalarane-type sesterterpene from the Indian Ocean Porifera *Hyrtios erecta*. **Coll Czech Chem Commun** 63:1671-1677, 1998.

Wender PA, Brabander JD, Harran PG, Hinkle KW, Lippa B, and Pettit GR. Synthesis and biological evaluation of fully synthetic bryostatin analogues. **Tetrahedron Lett** 39:8625, 1998.

Kamano Y, Kotake A, Hashima H, Inoue M, Morita H, Takeya K, Itokawa H, Nandachi N, Segawa N, Yukita A, Saitou K, Katsuyama M, Pettit GR. Structure-cytotoxic activity relationship for the toad poison bufadienolides. BioMed Chem Lett 6:1103-1115, 1998.

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Garth Powis, Ph.D.

University of Arizona
Award Amount FY 1999: $150,000

Interdisciplinary Basic Science Program Project

Project 1 has cloned a new form of thioredoxin reductase and has studied mechanisms by which the chemopreventive agent selenium regulates the activity of this class of enzymes. Inhibiting thioredoxin reductase has been shown to inhibit downstream target such as ribonucleotide reductase and transcription factors to inhibit cancer cell growth. *Project* 2 has found that several commonly mutated genes in human colon cancers, including the adenomatous polyposis coli (APC) tumor suppressor gene, affect gene expression of polyamine synthesis. APC is thought to be mutated in colonic mucosal tissues of especially young smokers as a consequence of carcinogens in tobacco. Inhibitors of polyamine synthesis suppress intestinal carcinogenesis in mice. *Project* 3 has found that co-culture of dermal fibroblasts with oral squamous carcinoma cells leads to increased matrilysin expression and this effect can be blocked by calcium chelation suggesting involvement of integrin receptors. Antisense matrilysin expression did not consistently down regulate matrilysin expression.

Scott K. Reaves, Ph.D

University of Arizona
Award Amount FY 1999: $50,000

Regulation of Human Tumor Suppressor p53 Gene Expression by Zinc Status

p53 is a tumor suppressor gene that is thought to be involved in more than half of all human tumors. One of the major functions of p53 is its ability to regulate a protective and vital process known as programmed cell death or apoptosis. This process is crucial in preventing cancer or tumor development. Because both zinc deficiency and zinc supplementation have been shown to influence specific aspects of apoptosis, we are examining how zinc status affects the p53 gene. We established a zinc-deficient model in a human liver cell line and then measured p53 mRNA and protein levels. In the zinc-deficient cells, p53 mRNA was increased but p53 protein remained unchanged. These findings suggest that the expression of the p53 gene may be sensitive to cellular zinc levels. We hope that our work will eventually help in establishing optimal dietary zinc levels that may reduce the risk of certain types of cancer.

William R. Roeske, M.D.

University of Arizona
Award Amount FY 1999: $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 percent 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. Objectives achieved this year indicate that we can identify unique drug profiles in our human receptor models that will enable us to address these important concerns.

Publication:

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, 1999.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY 1999: $46,570

Anti-tumor Agents Targeting Topoisomerase II

The goal of the ADCRC-funded research was the development of new inhibitors of the enzyme topsosomerase II, which plays a role in unpacking the chromosomal DNA for replication. A related project was the development of reductive alkylating agents of DNA. Like topoisomerase II inhibitors, these compounds interfere with the normal function of DNA in the rapidly dividing cancer cell. These agents have the advantage of activation by the cancer cell, in other words the cancer cell commits suicide by converting the drug to the toxic form. These agents are effective against lung cancer and are among the most active under study at the National Cancer Institute.

Finally, a comprehensive study of the reaction of known antitumor agents with DNA was carried out in order to assist in the development of more effective antitumor agents.

Christopher P. Appleton, M.D.

Mayo Clinic, Scottsdale

Award Amount FY 1999: $50,000

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

In this second year of our three-year study, we have obtained left atrial and left ventricular pressure-volume loops. These loops are graphical representations of how the chambers of the heart function. Certain diseases change the properties of the heart, making it more stiff 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 changes in these heart chambers can result in heart failure due to abnormal filling. Besides being a significant disease of the elderly, unhealthy life styles (*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 patient health care costs.

Ronald L. Heimark, Ph.D.

University of Arizona
Award Amount FY 1999: $132,713

Mechanism of Vascular Dysfunction in Atherogenesis: Cell-Cell Interactions

Atherosclerosis is a multifactorial disease of smooth muscle proliferation resulting in occlusion of the major elastic arteries. There are multiple risk factors in the non-smoking population, and smoking is synergistic and is associated with a four-fold increase in risk of cardiovascular disease. Despite our increased understanding of the cellular events that lead to atherosclerotic lesion development, the precise mechanisms by which risk factors contribute to atherosclerosis are unclear. The risk factors include hypertension, hyperlipidemia, and diabetes which contribute to dysfunction of the endothelial cell lining of blood vessels. A consequence of this endothelial dysfunction is increased permeability of blood components, including lipoproteins and monocytes. Our results show cytokines released by blood cells alter the barrier function of endothelium by disrupting junctional organization and mRNA expression of cell-cell adhesion molecules. The infiltrating lipoproteins, cytokines and growth factors released from blood cells stimulate the characteristic proliferation of the smooth muscle cells (SMCs) in vascular occlusive disease. We have identified significant cell-cell communication differences in quiescent and proliferating SMCs. Moreover, we found changes in adhesive molecules mediating cell-cell interactions, which maintains smooth muscle cells in a non-proliferative state. Our studies suggest mechanisms for endothelial dysfunction and smooth muscle growth regulation during the formation of an atherosclerotic plaque.

David C. Bloom, Ph.D.

Arizona State University
Award Amount FY 1999: $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 increases in cancers associated with smoking. Our research is pointed towards 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. To date we have identified two proteins present within a human brain library that interact with elements of the FGF-2 promoter and are capable of activation.

Richard R. Vaillancourt, Ph.D.

University of Arizona
Award Amount FY 1999: $48,954

Intracellular Signal Transduction Pathways Activated by Nicotine

It is well established that drugs of abuse, including nicotine, cocaine and opiates, stimulate the production of the neurotransmitter dopamine in the brain. To the user this stimulation by dopamine production translates into addictive behavior. Therefore, the characterization of reward pathways, as well as pathways implicated in addiction will provide clues to many aspects of substance abuse, including craving and reward. Our lab is interested in tyrosine hydroxylase, the rate-limiting enzyme involved in the production of the neurotransmitter dopamine. We have discovered a novel interaction between tyrosine hydroxylase and a protein that has been implicated in mRNA production by activation of RNA polymerase. Further studies will determine whether this interaction is important for nicotine addiction. At this time, we predict that the association may be important for nicotine mediated dopamine pathways in the brain.

Paul Enright, M.D.

University of Arizona
Award Amount FY 1999: $49,766

Spirometry for the Detection of High Risk Smokers in Southern Arizona Primary Care MD Offices

We formed an alliance with Tobacco Freeways to provide smoking cessation counseling and follow-up of the adult cigarette smokers who participate in our study. We successfully recruited 50 primary care physicians in the Tucson area who agreed to participate in our study. We visited all of their offices, explained the study, obtained their consent and left study form packets. For the 25 physicians randomized to the spirometry intervention study group, we scheduled a meeting with their staff, and then taught them how to perform spirometry tests and complete the smoking questionnaire. We completed a MS Access forms entry database, contact management software (to efficiently call the patients 6 months after study entry), and software to rapidly check and report the quality of the spirometry tests. All of the study physicians are now referring their patients who smoke to the local Tobacco Freeways smoking cessation classes. The PCPs with spirometers are now testing their smoking patients for early emphysema (COPD), which may prompt them to quit smoking and halt progression of the disease.

John Hall, Ph.D.

University of Arizona
Award Amount FY 1999: $59,711

When Gains Go Up In Smoke: Explaining Adolescents' Negative Reactions to Smoking Prevention Campaigns

This study set out to examine why the gains made in teaching young children to avoid tobacco seem to disappear when these same children enter adolescence. Research focused on the different advertising techniques most typically used by tobacco companies to promote their product and by health agencies to prevent tobacco use. The results of this study indicate that the techniques employed by tobacco companies result in more compliance than techniques used by anti-tobacco groups. Specifically, messages that explicitly tell teenagers not to smoke or use tobacco may backfire and actually increase tobacco use and experimentation. However, allowing young people some freedom in choosing whether to use tobacco may result in more overall compliance to anti-smoking messages. Based on results of this effort, additional funding has been acquired from the National Institute on Drug Abuse and a major proposal is under review at the National Cancer Institute.

Li-Wen Lai, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

Enhancement of Non-Viral Gene Transfer in the Lung

Our goal is to develop a non-viral gene therapy for pulmonary diseases. We have previously tested the effect of polycations (positively charged molecules) on liposome-mediated gene transfer in lung epithelial cells. We have successfully identified polylysine as an efficient enhancer for liposome-mediated gene transfer *in vitro*. Based on the in vitro data, we studied the new compounds 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 three weeks after the gene therapy as measured by PCR, Northern blot analysis, and Western blot analysis. We have also developed an ELISA assay to measure the anti-CAII antibodies in mice after gene therapy. This technology enables us to assess the side effect of gene therapy. The results from the ELISA assay showed that very low level of anti-CAII antibodies titer was detected in the animals after gene therapy. 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.

Michael D. Lebowitz, Ph.D.

University of Arizona
Award Amount FY 1999: $112,647

Evaluation of the Effects of Smoking on the Development of Chronic Pulmonary and Cardiovascular Diseases in Arizona

This study is a longitudinal follow-up of a community population studied since 1972 whose objectives have been to evaluate cardiopulmonary morbidity and mortality. The current objectives are to determine the risk factors for such diseases. Final morbidity and mortality follow-up to do so is still underway.

Recent findings to date include the following:

- Further significant predictors of respiratory and cardiac deaths were smoking, prior disease pulmonary and cardiovascular disease (including hypertension) and initial lung function, for pulmonary, cardiovascular, lung cancer and total mortality.
- There is an impact of physical activity (in addition to smoking) on pulmonary and cardiovascular disease onset (incidence). There is a close relationship of physical activity with lung function, and with pulmonary disease (diagnoses and symptoms). The latter relationship is different in females, where it appears protective, especially over age 65.

Publication:

Sherrill DL, Enright PL, Kaltenborn WT, Lebowitz MD. Predictors of longitudinal change in diffusing capacity over 8 years. Am J Respir Crit Care Med In press, 1999.

Patricia B. Hoyer, Ph.D.

University of Arizona
Award Amount FY 1999: $49,907

Mechanisms of Ovarian Follicular Cell Death Initiated by Polycyclic Aromatic Hydrocarbons

Loss of ovarian small follicles can cause ovarian failure (menopause is women). Menopause is associated with a variety of health risks. Therefore, early onset of menopause provides longer exposure to these risks. Age at menopause is significantly accelerated in women who smoke cigarettes (or are exposed to second hand smoke). The goal of our studies is to evaluate mechanisms by which three polycyclic aromatic hydrocarbons (BaP, DMBA, 3-MC) contained in cigarette smoke cause significant loss of ovarian follicles in rats and mice. To date we have determined the lowest effective dose, and the earliest day of repeated dosing in which impending ovarian damage is caused by these chemicals. Unlike previous studies using single high-dose exposures of laboratory animals to these ovotoxic chemicals, our method of exposure more closely mimics the chronic low-dose exposures of women smokers. By this approach, we have determined that in general, mice are more susceptible than rats to these chemicals, and their rank order of ovotoxicity is 3-MC>DMBA>BaP. 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 will help determine the extent of ovarian damage that might be predicted from exposure to each of these chemicals in women who have been life-long smokers.

Publications:

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

Diawara MM, Chaves KJ, Hoyer PB, Williams DE, Dorsch J, Kulkosky P, Franklin MR. A novel group of ovarian toxicants: The psoralens. **J Biochem Mol Toxicol** 13:195-203, 1999.

Kao S, Sipes IG, Hoyer PB. Early effects of ovotoxicity induced by 4-vinylcylcohexene diepoxide in rats and mice. **Reprod Toxicol** 13:67-75, 1999.

Borman SM, Van DePol BJ, Kao S, Thompson KE, Sipes IG, Hoyer PB. A single dose of the ovotoxicant, 4-vinylcylcohexene diepoxide, protects against atresia in rat small ovarian follicles. **Toxicol Appl Pharmacol** In press, Date undetermined.

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

University of Arizona
Award Amount FY 1999: $49,530

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. Additional final year exposure to TCE drinking water will also occur. To maintain anonymity, fetal hearts are blinded, by code, to the dissector. Thus, hearts are continuously evaluated, but conclusions can not 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. This is of extreme importance to Arizonan's, not just to those in areas of contamination. The potential for exposure to TCE comes not only from drinking water, but also from inhalation of water vapor emitted by evaporative coolers, showering and cooking.

Paul St. John, Ph.D.

University of Arizona
Award Amount FY 1999: $125,646

Nicotine Receptors in the Spinal Cord

Work completed in this collaborative project confirms that diverse nAChR subtypes are expressed in the mammalian spinal cord. New findings indicate that there actually is more diversity in spinal cord nAChR subtypes than there is in the supra-spinal brain. Furthermore, findings from this work indicate that nAChR subtypes may have diverse cellular and subcellular locations within the spinal cord, and suggests constraints on their functional roles in the adult spinal cord. These findings suggest that nicotinic ligands can be developed to selectively affect some of these spinal nAChR subtypes, as they are expressed on unique subsets of spinal neurons, perhaps to beneficially alter processing of pain and other sensory messages and the control of movement. These results are particularly relevant to the health and welfare of the over 1 million Arizonans who regularly use tobacco products. nAChR subtypes are the principal biological targets of nicotine from tobacco.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY 1999: $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 percent of Americans with a female to male ratio 3:1. The incidence of the disease is increased to approximately 5 percent in certain tribes of native Americans, and the prevalence in the Tucson area is approximately 5 percent due to the influx of individuals suffering from the disease and the high percentage of native Americans in the area. 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. These autoantibodies are predominantly of the immune macroglobulin (IgM) class and react with the combining site region of the T-cell receptor. The autoantibodies against self Tcrs probably play a role in immune regulation, and the elevation of these antibodies in RA patients may indicate a dysfunction of normal immunological mechanisms. The central question to be addressed is whether these antibodies are essentially the same ones expressed in low levels by healthy individuals in immunoregulation, or whether they present a distinct disease-related population that uses different immunoglobulin variable region genes. The antibodies were detected using a novel synthetic immunopeptide approach developed here, and their presence offers new approaches for diagnosis and potential therapy for this prevalent and crippling autoimmune disease. Adverse lung complications occur in approximately 20 percent of rheumatoid arthritis patients with smoking causing a more rapid progression of lung disease with significant increases in clinical intervention. Since a strong correlation has been found between levels of rheumatoid factor and smoking in males, it will be necessary for us to correlate levels of anti-T-cell receptor and gene usage with sex and smoking status in our patient population.

Publications:

Marchalonis JJ, Garza A, Lake DF, Landsperger WJ, Susal C. Recognition of defined epitopes by affinity-purified anti-immunoglobulin Fab autoantibodies isolated from HIV-infected humans. **J Mol Recognition** 12:169-176, 1999.

Lake DF, Salgaller ML, van der Bruggen P, Bernstein RM, Marchalonis JJ. Construction and binding analysis of recombinant scTcrs derived from tumor infiltrating lymphocytes and a CTL clone directed against MAGE-1. **Intl Immunol** 11:745-751, 1999.

Marchalonis JJ, Robey I, Schluter SF, Yocum DE. Epitope promiscuity of human monoclonal autoantibodies to T-cell receptor combining site determinants. Appl Biochem and Biotech Accepted for publication, 1999.

Judith B. Ulreich, Ph.D. University of Arizona

Award Amount FY 1999: $96,652

Arizona Liver Transplantation Research: Optimizing Organ Replacement in Tobacco-related Liver Disease

Many potential transplant recipients die waiting for livers. Use of organs from non-heart-beating donors (NHBD) might reduce the organ shortage. However, periods when blood is not flowing in NHBD produce damage that can lead to graft failure in recipients post-transplantation. Rats were pretreated with DMSO prior to clamping off the livers. After an hour, the liver as unclamped. The blood vessels in the liver of the living animal were observed using high resolution microscopic methods. The numbers of white cells adhering to the walls, swollen cells lining the vessels and vessels containing blood flow were counted. Creating ischemia by cutting off the flow of blood to the organ resulted i damage to cells and reduced blood flow through the organ. Pre-treatment with DMSO prevented these adverse events from occurring in the ischemic lobe. This suggests that pretreatment with DMSO would prevent tissue damage, thus providing additional organs suitable for transplantation.

Publications:

Ulreich JB, Maveddat M, Boles JL, Yeung KK, Zavala JL, Chaves RA, Nakazato PZ. Dimethyl sulfoxide protects kidneys from damage caused by warm ischemia in non-heart-beating donor f344 rats. The Toxicologist 42:375, 1998.

Ulreich JB, Patel M, Maveddat M, Boles JL, Nakazato PZ. Dimethyl sulfoxide protects organs from loss of viability induced by warm ischemia. Toxicology Letters 95:191, 1998.

Ulreich JB, Levy MA, Boles JL, Hayden CW, Johnson PW, Whitehead JJ, Nakazato PZ. Viability and cyp2e1 content of ischemic liver are maintained at pre-ischemic levels by dimethyl sulfoxide. Proceedings of the 16th Annual Meeting of the Mountain West SOT 1998.

Ulreich JB, Boles JL, Levy MA, Roy R, Whitehead JJ, Johnson PW, Andreoni KA, Nakazato PZ. Cytochrome P450 2E1 Content of ischemic liver in maintained at pre-ischemic levels by dimethyl sulfoxide (DMSO). The Toxicologist 48:408, 1999.

Merrie Brucks, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

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

Substantial progress has been made toward determining which children understand what about tobacco use and tobacco users and when they understand it. As of June 30, 1999, members of our research team have completed individual interviews with 261 children and received surveys from 228 parents/guardians (an 87.4 percent 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. 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 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.

Evelyn Cesarotti, Ph.D.

Arizona State University
Award Amount FY 1999: $63,821

Interventions to Decrease Second-Hand Smoke Exposure in School Children with Asthma

Current research indicates that exposure to cigarette smoking is a major cause of new cases of asthma in children as well as contributes to emergency room visits, hospitalizations, and school absences. The research goal was to implement a program of interventions to increase knowledge and self-care management in children with asthma and their parents through education and counseling. A total of 326 children participated in the two-year study. The research showed that 40 percent of parents who completed questionnaires reported that their children were exposed to smoking. However, most children who received the education and/or counseling programs did have significant increases in knowledge and self-care, fewer school absences and less emergency room visits than those who did not receive the programs. Of the 76 parents of children with asthma who participated in the follow-up evaluation, 45 percent of those who smoked did make positive changes in smoking behaviors such as smoking less, smoking outside, or stopping smoking.

Theodore M. Dembroski, Ph.D.

University of Arizona
Award Amount FY 1999: $74,808

Biobehavioral Risk Profile of Smokers

A major objective of this research is to use a young adult smoker's own cigarette-induced blood pressure (BP) and heart rate (HR) reactions to enhance a health education effort to promote smoking cessation. Young Arizona smokers (19 and 20-year-olds), were actually shown how their own BP and HR dramatically increased after they smoked a cigarette. The smokers then reviewed health education materials showing how their cardiovascular systems were being damaged by the exaggerated increases. Young adults who were exposed to this combined approach were more likely to agree to quit smoking for 24 hours on their own and then enter a smoking cessation project (76 percent) than those who received only cardiovascular health education alone (56 percent). We believe that the knowledge of one's own cardiovascular reactions to smoking helps overcome the "it won't happen to me" belief that is so prevalent, especially among younger smokers.

SECTION C

CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR THREE

David S. Alberts, M.D.

University of Arizona
Award Amount FY 1999: $109,519

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

In Year 3 we completed the administration and data entry of the smoking assessment questionnaire. All available baseline polyp tissue materials have been reviewed, and the Ki-ras analyses for 735 baseline tissue samples have been completed. This number included all available samples. In addition, per QA/QC protocol, 190 K-ras analyses were repeated in the Colorado laboratory and 200 were repeated in the Johns Hopkins laboratory. This process found that there was a 70-80 percent correlation in the intra and inter-laboratory analyses for Ki-ras. The results revealed that smoking was not associated with Ki-ras mutations found in the baseline adenomas. In addition, the preliminary analysis of the smoking questionnaire and cotinine data found that 92 percent of subjects were able to report the correct smoking status across three different smoking assessment tools. The importance of the research to the residents of Arizona is a clearer and better understanding of the exposures and biomarkers associated with adenomas and colorectal cancer. This understanding will lead to more effective primary prevention and chemoprevention efforts.

Publication:

Martinez ME, Maltzman T, Marshall JR, Einspahr J, Reid ME, Sampliner R, Ahnen DJ, Hamilton SR, Alberts DS. Risk factors for Ki-ras protooncogene mutation in sporadic colorectal adenomas. **Cancer Research** In press, 1999.

Abstracts:

Martinez ME, Maltzman T, Marshall JR, Einspahr J, Reid ME, Guillen-Rodriguez, van Leeuwen B, Ahnen DJ, Alberts DS. Association between cigarette smoking and K-ras mutation in colorectal adenomas. SER, Houston, March 1999.

Martinez ME, Maltzman T, Marshall JR, Einspahr J, Reid ME, Sampliner R, Maltzman T, Ahnen D, Ambrosone CB, Thompson P, Kadlubar FF, Alberts DS. Cigarette smoking, N-acetyltransferase 2, and Ki-ras mutations in adenomatous polyps. AACR #1609, Philadelphia, April 1999.

Maltzman T, Knoll K, Martinez ME, Byers T, Marshall JR, Reid ME, Einspahr J, Hart N, Bhattacharyya AB, Bogert C, Sampliner R, Alberts DS, Ahnen D. Association of Ki-ras and poly characteristics. AACR #279, Philadelphia, April 1999.

Paul Consroe, Ph.D.

University of Arizona
Award Amount FY 1999: $49,522

Antiemetic Drug Development for Cancer Treatment

Cannabinoid agonists, like the prescription drug Marinol® (=THC of marijuana), stimulate brain cannabinoid (=CB1) receptors to produce desirable (antivomiting and analgesia), and undesirable (e.g., amnesia), effects. We discovered that two new cannabinoid drugs, AM630 and SR141716A, have very unique effects. AM630 and SR141716A each blocked the binding of cannabinoid agonists to CB1 receptors in mouse and guinea pig brains, and human CB1 receptor cells. However when given alone, AM630 and SR141716A each stimulated the CB1 receptor, but they produced effects (on CB1 receptor proteins) that were opposite to the effect of the cannabinoid agonists given alone. Perhaps these drugs have only the good effects, *i.e.*, similar to marijuana (like preventing vomiting and pain) and opposite to marijuana (like improving memory). Mouse, guinea pig and human CB1 receptors are virtually identical, and thus our findings may lead to important new therapeutic drugs without the side effects of Marinol®.

Publications:

Consroe P. Brain cannabinoid systems as targets for the therapy of neurological disorders. Neurobiol Dis 5: 534-551, 1998.

Consroe P. Clinical and experimental reports of marijuana and cannabinoids in spastic disorders. In, *Marijuana for Medicine*, Nahas GG, Sutin KM, Agurell S. Eds. Humana Press, Totowa, NJ, pp. 611-617, 1999.

Abstracts:

Landsman RS, Consroe P, Makriyannis A, Deng H, Roeske WR, Yamamura HI. The effects of cannabinoid agonists and inverse agonists at the human cannabinoid CB1 receptor stably expressed in CHO cells. Soc Neurosci 24:1245, 1998.

Burkey TH, Waite SL, Landsman RS, Consroe P, Roeske WR, Yamamura HI. Agonist induced CAMP overshoot in human cannabinoid CB1 receptor transfected Chinese hamster ovary cells. Soc Neurosci 24: 612, 1998.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

Organ Culture Approaches for Transplantation of Human Stem Cells

During the first year of this project, we determined the best means to purify the progenitors of T and B cells that must be replaced in transplants of patients undergoing intensive chemotherapy for smoking-induced lung cancer. Last year, we used this method to show that the T cells derived from murine fetal liver donors appear to be qualitatively different from those obtained from the adult bone marrow with respect to the types of T cells that they produce. This year we have found that fetal liver and bone marrow derived precursors may have different subpopulations of precursors that produce T cells with different kinetics and different efficiencies. This information will be useful in determining the best sources for replacing immune cell precursors lost during cancer chemotherapy. We also found that human B cells can be produced best if their precursors are treated sequentially with the appropriate cytokines.

Our organ culture methods allow for a tremendous expansion of cells with the phenotype of the progenitors of all hematopoietic cells. Currently, bone marrow transplantation is the only effective therapy for lung cancer patients, but the low frequency successful "takes" for these procedures and the limited amount of donor material are major difficulties in treatment. The recent data suggest that we may be able to expand the number of progenitors outside of the body so that there will be more donor material to perform transplants. We have also begun gene therapy studies with the aim of providing a means of inserting genes into the progenitor cells that will aid in rapid growth of these cells in transplant recipients. The development of a reliable means of inserting these genes will greatly aid in rapid reconstitution of T cell responses in transplant patients.

Jacquelyn Gervay, Ph.D.

University of Arizona
Award Amount FY 1999: $29,700

The Synthesis of C-glycoside Sulfones as Potential Cancer Therapeutics

We have developed unique methods for the synthesis of a new class of compounds having the general structure sugar-CH_2-SO_2CH_2-X that are designed to mimic natural substrates having the general structure sugar-O-PO_2-O-X. The synthetic design allows both the sugar and "X" to be easily substituted to make a number of important analog readily accessible. We were able to prepare a potential inhibitor of fucosyl transferase, which is an enzyme that directly correlates with metastatic potential of tumors. This compound is being screened in a fucosyl transferase assay. If inhibition is observed, this potential drug candidate will be tested using an *in vitro* screening model for solid tumor selective inhibitor, and finally subjected to an anti-metastatic assay.

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY 1999: $127,299

Effects of Smoking on Persistent HPV Infection Among Reproductive Aged Women

Over one million cases of cervical dyplasia, the precursor lesion to cervical cancer, are diagnosed each year in the U.S. Reproductive age women (ages 18-35) are at the greatest risk of developing cervical dysplasia. The number of women in Arizona diagnosed with this disease has increased significantly in the last decade. Research has definitively shown that infection with the human papillomavirus (HPV) is a cause of most cases of cervical cancer. Although a woman's risk for cervical cancer is 10-20 fold higher if she has HPV infection, HPV infection alone is insufficient to cause cervical cancer. Approximately 28 percent of women who are infected with the HPV virus develop cervical dysplasia or cancer. The women at highest risk for cervical dysplasia and cancer are those who consistently test positive for HPV infections over time (persistent infection). We have prospectively followed 317 women ages 18-35 years for HPV persistence. Among non-smokers, 63.9 percent had persistent HPV infections and 36.1 percent cleared their infections. In comparison, 78 percent of smokers had persistent infections and only 22 percent cleared their infections. We are currently in the process of determining the association between duration of smoking and amount smoked on the HPV persistence rates. In addition, we are in the process of merging data files to determine other factors associated with HPV persistence in this cohort.

Arthur F. Gmitro, Ph.D.

University of Arizona
Award Amount FY 1999: $49,853

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

A new type of imaging system has been built for imaging cells inside the human body. This catheter-based microscopic imaging system is intended to aid doctors in the detection and diagnosis of cancer by allowing them to directly visualize cells inside the body. This may obviate the need to extract tissue for biopsy, or at least aid in the selection of which tissue to biopsy. The basic microscope and fiber-optic catheter system, including catheter optics and mechanical focusing mechanism, have been built and fully characterized. The image quality obtained with the system is excellent and consistent with that predicted by theory. Preliminary imaging results have been obtained from cell cultures, excised tissue samples, and live animals. These results demonstrate the basic capability, unique properties, and clinical potential of this imaging system.

Publications:

Sabharwal YS, Rouse AR, Donaldson L, Hopkins MF, Gmitro AF, A slit scanning confocal micro-endoscope for high resolution *in-vivo* imaging. Applied Optics In press, 1999.

Sabharwal YS, remote access slit-scanning confocal microscope for *in-vivo* tumor diagnosis. Ph.D. Dissertation, University of Arizona 1998.

T. Philip Malan, Jr., Ph.D.

University of Arizona
Award Amount FY 1999: $49,767

Role of c-fos in the Regulation of Neuropathic Pain

We studied the role of the transcription factor, Fos and the neuropeptide, dynorphin in the production and maintenance of neuropathic pain. Neuropathic pain is pain caused by injury or disease of nerves. Inhibiting production of Fos increased pain intensity, showing that normal levels of Fos help to reduce pain severity. The function of Fos is to regulate the amount of certain proteins, such as dynorphin, in the cells of the body. We showed that dynorphin is increased in the spinal cord in a neuropathic pain state and may help cause the symptoms of neuropathic pain. The importance of this work is that, if a way may be found to block the actions of dynorphin, neuropathic pain may be prevented or its severity reduced.

Bain D, Ossipov MH, Ibrahim M, Raffa RB, Tallarida RJ, Malan TP, Lai J, Porreca F. Loss of antiallodynic and antinociceptive spinal/supraspinal morphine synergy in nerve-injured rats: Restoration by MK-801 or dynorphin antiserum. **Brain Research** 831:55-63, 1999.

Malan TP, Ossipov MH, Ibrahim M, Bain D, Lai J, Porreca F. Extraterritorial neuropathic pain correlates with multisegmental elevation of spinal dynorphin in nerve-injured rats. **Pain** In press, 1999.

Yeh-Shan Peng, Ph.D

University of Arizona
Award Amount FY 1999: $50,000

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

The second cycle of the intervention study has been completed. The results were similar to those observed in the first cycle. Essentially, the smokers doubled their fruit and vegetable intake in two weeks and the high intake was maintained throughout the 6-month intervention period. The increased intake was associated with an increased concentrations of lutein, beta-cryptoxanthin, alpha-carotene and beta-carotene in the plasma. The concentration of 8-hydroxydeoxyguanosine in the peripheral nucleated blood cells was not decreased despite the increase in the concentrations of many carotenoids in the plasma of the smokers during the intervention period. The results suggest that the concentration of 80HDG was not easily changed by changing the anti-oxidant capacity of the smokers; thus, 80HDG may not be an appropriate biomarker in the intervention study. The results should be useful to the smokers in Arizona.

William A. Remers, Ph.D.

University of Arizona
Award Amount FY 1999: $90,226

Design of Non-cross Resistant Agents for Lung Cancer

The goal of this project is to discover new compounds that are effective against resistant human lung cancers. To accomplish this goal, we have synthesized an initial set of 14 compounds and tested them against the Lewis lung tumor in mice. From these results, a data base was created for use in the design of future compounds with improved activity. Among the fourteen compounds, four were active against the Lewis lung tumor, using a criterion for activity of 25 percent reduction in tumor growth compared with controls. Six compounds were inactive and three were toxic to mice at the doses used. One compound was active initially, but toxic on a retest. Among the active compounds, 6-ethoxyazonafide was tested against human lung cancer A-549 in immunodeficient mice and found to reduce growth of these tumors by an average of 45 percent.

The compound active against the human lung carcinoma is considered a candidate for a human medicine and plans are being made to obtain preclinical pharmacology on it and introduce it into a Phase I clinical trial. An effective chemical synthesis has been established for its large scale production. A manuscript is being written to describe these results.

Donato Romagnolo, Ph.D.

University of Arizona
Award Amount FY 1999: $53,072

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

The purpose of this project is to investigate the mechanisms that lead to loss of expression of the tumor suppressor gene BRCA-1 in response to exposure to a class of tobacco derivatives termed polycyclic aromatic hydrocarbons (PAHs). The loss of BRCA-1, which is involved in repair of DNA damage, may alter the balance between production and repair of DNA favoring tumor development. In 1990 the state of Arizona ranked 16 in the United States in deaths (5,700) attributable to smoking, with estimated years of potential life lost approaching 67,000 years. This project will study the molecular mechanisms by which PAHs repress normal production of BRCA-1. These findings may provide new clues for the development of preventive drugs and to improve the awareness of the public, in particular of adolescent smokers, concerning the potential long-lasting effects of exposure to tobacco derivatives as a risk factor in breast tumorigenesis.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY 1999: $49,817

Chemotherapy by Contravention of Oncogenesis in Smoking-induced Lung Cancer

Approximately one-third of lung cancers result from the action of a faulty protein that triggers unwanted cell division. To exert its detrimental effect, the faulty protein must be acted upon by a cellular enzyme. We are trying to obstruct that enzyme so that the effects of the faulty protein can be blocked. Two novel compounds tailored to bind to the enzyme were found to potently obstruct the enzyme, reducing its activity to nil within minutes. One will be evaluated by the National Cancer Institute against cancer cells grown in culture. An additional compound in this family of agents has been designed and synthesized for further testing, and the preparation of a fourth was begun. A compound representing a different class of agents was tested by the National Cancer Institute and was active against melanoma and central nervous system cancer cells. These studies may lead to the development of effective anticancer agents for the benefit of Arizona residents.

Publication:

Lefler SR, Rose SD. Synthesis of enol esters from copper (1) carboxylates generated from copper (1) trifluormethanesulfonate benzene complex. **Synth Commun** In press, 29:21, 1999.

Ann L. Baldwin, Ph.D.

University of Arizona

Award Amount FY 1999: $49,707

What Cellular Mechanisms are Responsible for Histamine-Induced Aerations in Microvascular Permeability

Our overall goal is to determine how inflammatory agents, such as those produced by smoking, damage the lining of blood vessels (endothelium) and cause leakage of molecules from the circulation. We apply an inflammatory agent, histamine, to a network of small blood vessels in the rat mesentery, a thin sheet of tissue that holds the intestine together, and measure the leakage of albumin from the blood vessels. This year, we showed that histamine produces leaks in small veins, but if the tissue is pretreated with nitric oxide, the leaks are reduced. We also demonstrated that histamine stimulates the endothelial cells to produce protein kinase C, and if the PKC is blocked with an antagonist, then the leakage is reduced. Therefore if the endothelium could be stimulated to produce more nitric oxide, or if production of PKC were blocked, the tissue would be protected from some effects of inflammation. Inflammation contributes to cardiovascular disease. In Arizona 25 percent of the population smoker, and in 1991, cardiovascular disease was responsible for 38 percent of total deaths.

Publications:

Wong R, Heimark RL, Baldwin AL. Redistribution of cadherin-5 sites of TNFα and γIFN induced macromolecular permeability in mesenteric venules. **Amer J Physiol** 276:H736-H748, 1999.

Valeski JE, Baldwin AL. Effect of early transient adherent leukocytes on venular permeability and endothelial actin cytoskeleton. **Amer J Physiol** 277:H569-H575, 1999.

Al-Maemi H, and Baldwin AL. Nitric oxide: role in venular permeability recovery after histamine challenge. **Amer J Physiol** In press, 1999.

Irwin L. Flink, Ph.D.

University of Arizona
Award Amount FY 1999: $32,513

Cardiac Cell Cycle Progression and Terminal Differentiation

The major goal of this research is to determine the molecular basis underlying the inability of cardiac muscle cells to undergo cell division after birth. Members of the retinoblastoma protein family are regulators of cellular proliferation and differentiation and are comprised of pRb (retinoblastoma protein), p107, and p130. Each protein is regulated by a phosphorylation cascade carried out by cyclins associated with their cognate cyclin-dependent kinases (cdks). In the underphophorylated form, pRb family members are inactive and interact with the transcriptional factor, E2F/DP1, which is involved in the regulation of DNA synthesis. We have shown that during early neonatal development, p130 replaces p107 bound to E2F/DP. pRb becomes underphosphorylated due to the down regulation of D cyclins and cdk 4. The cdk inhibitors, p21 and p27, were shown to increase which also contributes to the decreased phosphorylation of pRb family members. Associated with the changes in pRb family members, there is a change in β1 integrin isoforms and several of its associated α-subunits, suggesting that integrin-mediated signaling may play a role in terminal differentiation. These studies will aid in the development of therapeutic agents that will replace or modify altered cellular growth genes resulting from the use of tobacco.

Publication:

Flink IL, Oana S, Maitra N, Bahl JJ, Morkin E. Changes in E2F Complexes containing retinoblastoma protein family members and increased cyclin-dependent kinase inhibitor activities during terminal differentiation of cardiomyocytes. J Mol Cell Card 30:563-578, 1998.

Ronald J. Lukas, Ph.D.

St. Joseph's Hospital
Award Amount FY 1999: $149,383

Molecular Basis for Nicotine Dependence

Nicotine dependence is thought to drive the habitual use of 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 supports our hypothesis that extended exposure to nicotine induces long-lasting changes in numbers and function of nAChR. Doses and time-dependences of these effects differ across nAChR subtypes. We have found that different nicotine-like drugs mimic or block effects of nicotine at specific forms of nAChR. We also have made the surprising finding that the loss in nAChR function is progressively deeper as nicotine treatment times are lengthened. These studies identify nAChR forms that are most powerfully affected by chronic nicotine exposure and drugs that mimic or block those effects. This information is potential use in the design of strategies and therapies to prevent or promote cessation of the habitual use of tobacco products. 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.

Publications:

Fryer JD, Lukas RJ. Non-competitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. J Pharmacol Exper Ther In press, 288:88-92, 1999.

Fryer JD, Lukas RJ. Antidepressants non-competitively inhibit nicotinic acetylcholine receptor function. J Neurochem 72:1117-1124, 1999.

Reitstetter R, Lukas RJ, Gruener R. Dependence of nicotinic acetylcholine receptor recovery from desensitization on the duration of agonist exposure. J Phamacol Exper Thera In press, 1999.

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

I. B. Scientific

Award Amount FY 1999: $49,995

Acupuncture in Smoking Cessation: Randomized, Placebo-controlled Trial

In the third and final year of the project the major goals were accomplished. At the 18 month follow-up visit, 71 patients were retained. Final data was entered into the computer for analysis. Appropriate power was available to determine the effectiveness of acupuncture for smoking cessation.

The group that received both true acupuncture and the educational program has maintained a 40 percent cessation rate at 18 months post baseline, higher then both of the other groups. ($p<0.05$). Tthe study design did not include an actual placebo control group due to ethical reasons. Therefore, the combination of acupuncture and education was more effective than education or acupuncture alone, both of which have shown effectiveness above that of placebo in other trials.

For the most recent year of available data (1989), Arizona lost 76,021 productive years of life due to deaths associated with smoking. The economic cost of smoking-related morbidity and mortality in Arizona in 1992 alone hit $706 million. In 1994, Arizonans spend $896,492,902 in smoking related costs including health care, morbidity and mortality. Given these statistics, if acupuncture can potentially reduce the number of Arizonans that smoke, it can benefit the state's productivity, economy, and the health of its citizens.

Dean E. Carter, Ph.D.

University of Arizona

Award Amount FY 1999: $102,728

Synergism Between Smoking and Arsenic Exposure in Lung Injury

Our research is attempting to define the relative risk of lung injury following exposure to arsenic and cigarette smoke. We found a synergism between cigarette smoke and arsenic in producing lung injury that may lead to increased lung cancer. However, changes in DNA were not accompanied by increases in inflammatory cells or mediators in the lung indicating that lung injury is not due to inflammation as we had originally thought. This indicates that treatments based on reducing inflammation would not be effective in lowering the risk of lung injury caused by exposure to both arsenic and cigarette smoke. This year, we also report results on identification of potential sensitive molecular markers of arsenic exposure. Establishing unique markers will allow us to identify people who may be at risk for adverse health affects. This would be particularly important for Arizona residents that work and live in copper mining and smelting towns.

Richard L. Friedman, Ph.D.

University of Arizona
Award Amount FY 1999: $50,000

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

Tuberculosis is the most important infectious disease which afflicts mankind. Over three million people die yearly from tuberculosis, the largest single infectious cause of mortality worldwide. For citizens of the state of Arizona, tuberculosis is a serious and rapidly increasing health problem.

The goal of this research is to identify, clone, and characterize genes of *M. tuberculosis* which are required for intracellular survival within macrophages. Using a human macrophage-like cell line, U-937, we have begun to screen a plasmid library containing DNA from virulent *Mycobacterium tuberculosis* in a avirulent strain of mycobacterium (*Mycobacterium smegmatis*). In our screening we have identified recombinant clones from the plasmid library which demonstrate enhanced intracellular survival. One plasmid clone in particular, clone 69, has a four to six-fold enhanced intracellular survival as compared to vector controls. Further genetic analysis has shown that the enhanced intracellular phenotype of clone 69 is associated with *M. tuberculosis* DNA containing the *eis* gene. Additional plasmid clones with enhanced intracellular survival have been also identified and are presently being further analyzed.

Using genetic and molecular biology approaches to study the role of intracellular survival in tuberculosis pathogenesis will expand our understanding and knowledge of the disease process. Study results will lead to the development of improved vaccines and to more effective treatment and prevention of this deadly disease. This will in turn reduce the extra risks that smoking citizens of the state of Arizona face when they encounter the agent of tuberculosis in their daily lives.

Publication:

Friedman RL, Wei J, Dahl JL, Moulder JW, Roberts EA, O'Gaora P, Young DB. Expression of a *Mycobacterium tuberculosis* gene (*eis*) in *Mycobacterium smegmatis:* Enhanced survival in human macrophage-like U-937 cells and the location of the putative gene product in a surface-exposed position. Proceedings of the 34th Annual U.S.-Japan Cooperative Medical Science Program, Leprosy and Tuberculosis Conference pp. 235-239, 1999.

Michael P. Habib, M.D.

University of Arizona
Award Amount FY 1999: $36,820

The Effect of Micronutrient Antioxidants on Exhaled Ethane in Cigarette Smokers

Only 15-20 percent of cigarette smokers develop significant lung disease which is costly to Arizona. Antioxidant vitamin ingestion may determine disease susceptibility permitting early intervention. Ethane gas is released in breath during oxidant injury. Our previous work indicates that antioxidants (vitamins E, C, and β-carotene) reduce ethane and may predict disease susceptibility.

Questions: Can β-carotene alone reduce ethane in and predict smokers at risk for lung disease? We gave β-carotene to 20 smokers for three weeks.

Results: β-carotene slightly increased the amount of exhaled breath ethane showing a slight pro-oxidative effect.

Conclusion: β-carotene alone fails to explain changes found in our initial study. This was also the case when vitamins E and C were administered previously. It may be that a combination of all three vitamins is required to demonstrate a large effect on exhaled ethane.

Anne L. Wright, Ph.D.

University of Arizona
Award Amount FY 1999: $85,608

Passive Smoke Exposure, Immunologic Function and Lower Respiratory Tract Illness in Infancy

Lower respiratory tract illnesses (LRIs) such as bronchiolitis, bronchitis and pneumonia, are major causes of infant illness and hospitalization. This project is designed to investigate whether passive smoke exposure alters the immune system response of infants so as to increase their risk of developing LRIs. To date, we have enrolled 178 mothers (51 percent of those eligible). Baseline questionnaire data have been obtained on all mothers and the majority of fathers, and blood specimens obtained for the majority of subjects. On average, participating mothers have 13.6 years of school, most (63 percent) are married, 8 percent smoked during their pregnancy and 59 percent are Anglo. In part because of our success in enrolling and following study subjects, we have obtained five years of funding from the National Institute of Allergy and Infectious Disease. This grant will be used to expand the project, by increasing enrollment to 400 subjects and expanding the length of follow-up to five years. Continued long term success in this project will permit identification of preventable risk factors for this common type of illness, which could have a major impact on the health of infants and on health care costs in Arizona as well as elsewhere.

Charlene A. McQueen, Ph.D.

University of Arizona
Award Amount FY 1999: $46,038

Genetic Variation in N-Acetyltransferase and the Development Toxicity of Aromatic Amines

The body has specialized proteins, called enzymes, that help eliminate chemicals. These enzymes change the chemical structure, making it more water soluble. As part of this process both toxic and harmless products may be formed. Many enzymes vary from one person to another because of an individual's genetic make-up. The capacity to detoxify chemicals can affect the risk of toxicity. This project is investigating enzymes called N-acetyltransferases (NAT). Genetic variation in NAT results in different abilities to detoxify 4-aminobiphenyl (4AB), a component to tobacco smoke. It was found in a mouse model that NATs are expressed during embryonic development. Consequently, the embryo as well as the mother has active enzymes which may effect risk for 4AB toxicity. Furthermore, exposure to 4AB during pregnancy resulted in DNA damage in the mother and embryo. Such damage during development may be related to an increased cancer risk in later life.

Publication:

Mitchell K, Futscher BM, McQueen CA. Developmental expression of N-acetyltransferases in C57B1/6 mice. Drug Metabolism and Disposition 27:261-264, 1999.

SECTION D

PROPOSALS RECEIVED

TOBACCO-RELATED RESEARCH

FY 1999

Ahmad	University of Arizona	Effect of Tobacco Smoking on HIV-1 Pathogenesis and Disease Progression in Mothers and Infants Following Perinatal Transmission	$50,000 50,000 50,000
Baldwin	University of Arizona	Mechanisms by which Noise Stress Elicits Microvascular and Epithelial Changes in Intestine	$49,420 49,663 49,672
Barton	University of Arizona	Imaging Tumor Development and Invasion Using Optical Coherence Tomography	$49,995
Beach	Sun Health Research Institute	Smoking as a Disease Modifier in Alzheimer's Disease: An Autopsy Study	$36,025 36,025
Bier	I.B. Scientific	Auricular Acupuncture Education and Smoking Cessation: A Randomize Sham-controlled Trial	$46,666 49,846 39,097
Bloom	University of Arizona	The Airway Epithelium as a Target for the Anti-inflammatory Action of Inhaled Steroid Therapy	$49,975 49,979 49,993
Boswell	University of Arizona	Genetic and Molecular Regulation of Angiogenesis	$115,150 129,302 96,073
Brower	University of Arizona	Genetic Probes for the Study of Integrin Structure- Function Relationships	$48,781 45,593 45,286
Camhi	University of Arizona	K-ras and P53 Mutations as Markers of Tobacco-induced Genetic Damage in Airways of Smokers	$49,988 49,863 49,688
Chen	University of Arizona	Signal of Oxidant Induced Cardiomyocyte Hypertrophy	$50,000 50,000 50,000

Chen	University of Arizona	Smoking, Body Composition and Bone Mineral Density in Urban Premenopausal Native American Women	$45,397 41,215 43,831
Consroe	University of Arizona	Cannabinoid Receptor G-Protein Coupling	$49,522 49,522 49,522
Dallas	University of Arizona	"Visual Human" Based Validation of Right-Ventricular Measurements	$46,701 43,859
Davis	Arizona State University	The Effects of Smoking and Menopause on Physiological Stress Responses in Middle-Aged Women	$47,156 49,991 18,924
DeLuca	University of Arizona	Nicotine Effects on Human Stem Cell Differentiation *In Vitro*	$50,000 50,000 50,000
Disney	University of Arizona	Identifying Key Indicators of Resumption of Tobacco Use in Post-Partum Women	$48,069 48,590 42,595
Drumm	Sun Health Research Institute	Effects of Smoking and Smoking Cessation on Cognition after Carotid Endarterectomy	$115,029 118,134 122,272
Epner	University of Arizona	Cyclin D1 Deregulation in Lung and Other Smoking Related Cancers	$48,961 49,181 49,181
Flink	University of Arizona	The Effects of Nicotine and Catecholamines on Integrin Expression and Retinoblastoma Protein Regulators of the Cardiomyocyte Cell Cycle	$50,000 50,000 50,000
Fregosi	University of Arizona	Physiology of Pharyngeal Airway Obstruction	$49,999 48,850 49,999

French	University of Arizona	Nicotine Dependence Brain Reward Systems and Dopamine Neurons: Electrophysiological and Molecular Studies	$137,935 135,320 138,436
Friedman	University of Arizona	Further Investigations on *Mycobacterium tuberculosis* Genes Contributing to Survival in Macrophages	$50,000 50,000 50,000
Giuliano	University of Arizona	Effects of Antioxidant Nutrients and Smoking on Type-Specific HPV Persistence	$148,892 147,501 149,964
Habib	University of Arizona	The Effect of the Combination of Vitamin C, Vitamin E and B Carotene on Exhaled Ethane in Cigarette Smokers and the Relation to Lung Function	$49,152 49,744 49,942
Hakim	University of Arizona	The Role of High Tea Consumption in the Modulation of DNA Oxidative Damage in Smokers	$125,061 136,091 102,692
Hall	University of Arizona	The Role of Emotions in Countering the Initiation of Adolescent Smoking Behaviors	$82,437 73,541 68,679
Harris	University of Arizona	Development of High Level Gene Expression Systems for Gene Therapy	$49,772 49,772
Hill	University of Arizona	The Nutritional Impact of Smoking Cessation in Hispanics	$44,924
Jacobs	Arizona State University	Specific Induction of dsRNA-Mediated Suicide in Lung Cancer Cells	$150,000 150,000 150,000
Johnson	University of Arizona	Effect of Nicotine on Neuronal and Glial Development: Interaction with Ethanol	$46,779 45,493 46,427

Kelly	Northern Arizona University	Is Chelation Therapy for Cadmium Toxicity Effective?	$46,568 43,498
King	Samaritan Regional Medical Ctr.	A Study of Journal Writing as an Intervention to Assist Individuals with Cancer Who Smoke or Have Smoked to Remain Nonsmoking and Increase Their Quality of Life	$12,036
Lindstedt	Northern Arizona University	Respiratory Muscle Training with Nonrespiratory Tasks: Intervention for COPD Patients	$49,995 49,977
Lorton	Sun Health Research Institute	Nicotine-induced Effects on Immune Functions: Neural-Immune Mechanisms	$50,000 50,000 50,000
Lukas	St. Joseph's Hospital	Molecular Bases for Nicotine Dependence	$149,954 149,713 149,802
Lukas	Barrow Neurological Institute	Structure and Function of a Model Receptor for Nicotine	$149,218 148,972 149,606
Lynch	University of Arizona	Effect of Nicotine on Hypothalamic Glucose-responsive Neurons	$49,936 48,640 49,998
Martinez	University of Arizona	p53-Dependent Apoptosis in Lung Cancer	$49,990 49,990 49,990
Martinez	University of Arizona	Tobacco Exposure, Genetic Susceptibility and Risk of Colorectal Cancer	$149,668 149,811 137,119
Matsunaga	ImaRx Pharmaceut-ical Corp.	Targeted Micro-bubbles for Diagnosis and Treatment of Vascular Thrombosis	$50,000 50,000 50,000

McQueen	University of Arizona	N-Acetyltransferases: Genotype and Genotoxicity	$119,804 119,324 121,695
Miller	University of Arizona	The Effectiveness of Counseling and Bupropion Hydrochloride in Prevention of Postpartum Smoking Recidivism	$148,362 149,202 139,720
Miller	University of Arizona	New Strategies for Achieving Smoking Cessation in Pregnancy: A Comparison of Counseling and Sustained-release Bupropion	$148,647 149,891 145,411
Morkin	University of Arizona	Grafting of Stem Cell-Derived Cardiomyocytes to Repair Myocardial Infarction	$150,000 150,000 150,000
Patel	Maricopa Medical Center	Implementation of a Cardiac Wellness Program and Evaluation of Effects on Health; Quality of Life; and Cost Containment	$147,840 144,870 144,870
Payne	University of Arizona	Aberrant Expression of Redox-Associated Proteins NF-kB(p65)Thioredoxin and Inducible Nitric Oxide Synthase as Biomakers of Colon Cancer Risk	$150,000 150,000 150,000
Reems	Blood Systems	A New Therapeutic Option for Patients Who Develop Cancers Caused by Cigarette Smoking	$49,885 49,838 49,679
Romagnolo	University of Arizona	Transcriptional Repression of the Breast Cancer Gene BRCA-1 by Tobacco Polycyclic Aromatic Hydro-carbons	$48,675 49,415 49,500
Rose	Arizona State University	Enzyme Active Site Tailored Anticancer Drugs	$50,000 50,000 50,000
Salt	Northern Arizona University	Tobacco Enrichment with Anticarcinogenic Selenium	$49,651 49,644 49,644

Simon	University of Arizona	Role of Gap Junction-Mediated Communication in Preventing Endothelial Dysfunction	$50,000 50,000 50,000
Slepian	University of Arizona	Microtubule-Dependent Integrin Function: Role in Atherosclerosis and Restenosis	$49,393 50,000 50,000
Smith	AZ Heart Institute & Hospital	Ginkgo Biloba in Intermittent Claudication: A Randomized, Double-Blind, Placebo-Controlled Trial	$49,962 49,962
Sparks	Sun Health Research Institute	The Effect of Nicotine in an Animal Model of Both Cardiovascular Disease and Alzheimer's Disease	$40,6286 37,7140 38,8971
Taren	University of Arizona	Why Do Women Gain Weight With Smoking Cessation: Understanding the Role of Diet and Metabolism	$142,793 146,053 149,412
Watson	University of Arizona	Investigation and Treatment of Pulmonary Disease from Tobacco Smoke in Murine Model of AIDS	$150,000 150,000 150,000
Weinert	University of Arizona	Genomic Instability and Cell Cycle Controls in Yeast	$47,223 48,155 49,110
Wessells	University of Arizona	Smoking-induced Erectile Dysfunction: Characterization and Repair with Gene Therapy	$49,960 49,879 49,901
Xia	University of Arizona	The Mechanisms of Cigarette Smoke-Induced Early Menopause in Humans	$49,917 49,952 49,712

SECTION E

NEW CONTRACT AWARDS

TOBACCO-RELATED RESEARCH

BEGINNING IN FY 2000

Beach, M.D., Thomas G.

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

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

The relationship of cigarette smoking to Alzheimer's disease (AD) is still controversial after 17 year of research. Much of the work to date has suggested that cigarette smokers, as compared to non-smokers, are less likely to develop Alzheimer's disease. An important new study has, however, come to an opposite conclusion. All of these studies were done with living patients, and therefore are likely to be inaccurate with respect to the diagnosis of AD. Alzheimer's disease can only be definitively diagnosed at autopsy, by microscopic examination of the brain. There are many conditions which can be identical to Alzheimer's disease in terms of their effect on the living patient. A diagnosis of Alzheimer's disease which is based on examinations of the living patient is only correct in from 50-90 percent of patients, depending on the expertise of the evaluating clinicians. The study proposed here can clarify this issue by using only cases of AD which have been microscopically diagnosed after autopsy. This will allow a detailed examination of the effects of smoking on the age of onset, duration and severity of Alzheimer's disease. It is of utmost importance that this issue be resolved. Alzheimer's disease becomes increasingly prevalent with age, affecting approximately 5 percent of individuals at age 60, 15 percent at age 80 and 40 percent at age 90. The toll, in terms of both human suffering and economic expense, is immense. Arizona, with its large population of retired people, is especially vulnerable to this affliction. If cigarette smoking increases the risk of Alzheimer's disease, as suggested by the most recent and reliable study, it should be possible to reduce the attack rate of the disease by public health campaigns against smoking. We have already completed a pilot study, the results of which show both a greater severity and earlier onset of the disease in smokers. A larger study with more cases is needed to determine the strength of this finding.

The *goal* of this research is to determine whether smoking affects the severity or course of Alzheimer's disease.

The *hypothesis* is to be tested, based on our preliminary results, is that smokers will have an earlier age of onset of dementia and an increased severity of AD-specific histopathologic lesions.

Our objectives are:

1) To determine, in brains from individuals neuropathologically diagnosed as Alzheimer's disease, the relative severity of the specific histopathologic lesions of the disease in smokers and non-smokers. This will be done by quantifying the numbers and extent of the characteristic brain lesions.
2) To determine, using the clinical histories of cases, if the course of neuropathologically-diagnosed Alzheimer's disease differs in smokers versus non-smokers. The makers of disease course will be age of onset, durations of disease and age at death.

Bier, ND, MS, Ian D.

I. B. Scientific
Award Amount FY 2000: $146,666

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

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

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

In the United States, research on acupuncture as a treatment for addictions is still in an early stage of development. The treatment protocols tested have been based on clinical experience rather than systematic research and only a handful of the studies have adequate follow-up data. This proposal follows a pilot trial funded by the ADCRC, based on the successful model for chronic alcoholism set forth by Bullock *et al.* and is designed to evaluate the efficacy of a multi-component treatment program involving acupuncture alone, and in combination with a smoking cessation educational program, over a significant follow-up period. Pilot data indicated that acupuncture, in combination with a smoking cessation educational program a can be effective in helping 40 percent of the population quit smoking, even at 12 months after treatment. Smokers with the longest pack-year history, and therefore the greatest risks for tobacco related disease, benefitted the most from the protocol.

The primary goal of this research is to acquire a fundamental understanding of the role of acupuncture in a treatment protocol for smoking cessation. The protocol will be used with cigarette smokers that have (a) smoked one or more packs for a minimum of five years, (b) have attempted and failed to quit smoking at least once, and (c) have over a 20 pack-year history of smoking. Objectives of this study are as follows:

1) Determine the effects of acupuncture given at true acupuncture sites as compared to

acupuncture given at sham acupuncture sites on the following dependent variables before treatment immediately following treatment, and 3, 6, 9, 12, 15, and 18 months after treatment:

(a) number of cigarettes;
(b) level of cigarette craving;
(c) carbon monoxide cigarette use makers;
(d) participant's health and psychological status;
(e) participant drop-out rates from treatment.

2) Determine the efficacy of acupuncture alone, versus acupuncture in combination with a smoking cessation educational program.
3) Determine if smokers with over a 20 pack-year history have a greater cessation rate than other groups.
4) Identify predictor variables to determine who might or might not respond to acupuncture and educational intervention based on psychological and health status, and demographic information.
5) Identify possible adverse effects of acupuncture treatment.

Hypotheses to be tested are as follows:

1) No difference will be observed between groups during, immediately after, and at 3, 6, 9, 12, 15, and 18 months following completion of treatment in:
   (a) cigarette use
   (b) cigarette craving
   (c) carbon monoxide cigarette use makers
   (d) health and psychological status
   (e) drop out rates.
2) No difference in demographic information will be observed between participants who respond to treatment and those who do not.

Brower, Ph.D., Danny L.

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

Genetic Probes for the Study of Integrin Structure-Function Relationships

Coronary heart disease is the number one killer in the U.S. It is now well known that smoking greatly enhances the formation of the precursors of coronary blood clots, atherosclerotic plaques, with concomitant increase in mortality from heart attacks. 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. Thus, agents that can reduce the ability of integrins to make these connections can be very important in reducing the incidence and severity of heart attacks (for example, aspirin exerts its effects as a blood thinner by inhibiting, albeit slightly, the activity of the platelet integrins), and there is considerable research underway with this goal in mind. We propose studies 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, either chronically in persons at risk for heart attack or for the treatment of acute coronary thrombosis.

The overall goal is to generate a set of mutations that disrupt the gene encoding the βPS integrin subunit, and to identify mutants that are especially likely to be useful in understanding the structure-function relationships of the intact proteins. We will select for mutations that have no integrin function as well as mutations that compromise function without eliminating it. The latter may be especially useful for dissecting the various functional domains of the complex proteins. All of the mutants will be characterized using a number of genetic tests. The molecular lesions will be identified in the integrin structural gene on each of the mutant chromosomes. Finally, selected mutations will be further analyzed in more sophisticated genetic and cell biological experiments. Its is anticipated that this work will provide the basis for many future studies in our lab and in others interested in this basic problem.

Davis, Ph.D., Mary C.

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

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

Smoking is the single most damaging risk factor for coronary heart disease (CHD) among women, such that women who smoke heavily have 4 times the risk of heart attack as nonsmokers. Often women who smoke have other CHD risk factors as well, a combination that may be particularly harmful to cardiovascular health. Among the most common CHD risk factors for women smokers are menopause and stress, both of which have a profound physiological impact. Yet little is known about the potentially damaging physiological consequences of smoking when it coincides with menopause and stress. This is important because large stress-related changes in factors such as blood pressure, clotting components, and stress hormones are thought to play a role in CHD progression.

Roughly 25 percent of women in Arizona smoke. The situation may worsen, however, because the rates at which young girls initiate smoking appear to be on the rise, and women smokers have a particularly difficult time quitting. Thus, substantial numbers of women are likely to continue smoking into middle-age and beyond and will, therefore, experience the joint negative effects of menopause and long term exposure to cigarette smoke. Further, women report that they smoke more when under stress. Although tobacco use, menopause, and stress each have important biological effects, their combined impact on physiological systems may be even more detrimental.

Smoking and menopause together may exacerbate women's CHD risk at least party by increasing their physiological responses to stress. The current study is designed to examine how smoking, menopause, and stress combine to affect women's physiology. To explore this question, middle-aged women smokers and nonsmokers, who are either premenopausal or postmenopausal, will perform a series of stress-inducing tasks. During task performance, cardiovascular measures, blood lipids (*e.g.* cholesterol), blood clotting factors, and stress hormones will be assessed.

The primary hypothesis of the project is that physiological responses to stress will be most extreme in women who have both risk factors for CHD, menopause and smoking, relative to those who have only one risk factor, either menopause or smoking, or neither risk factor. This hypothesis builds on earlier research and is consistent with the notion that the combination of smoking with menopause have negative health effects through their influence on physiological stress responses.

Giuliano, Ph.D. Anna R.

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

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

In the U.S. in 1995, approximately 15,800 new cases of invasive cervical cancer were diagnosed and 4,900 women died. Although cervical cancer can be prevented through routine participation in Pap smear screening programs, there are tremendous costs associated with the need for diagnostic follow-up and treatment when abnormalities are found. In Arizona, the number of women diagnosed with abnormal Pap smear in the last decade has significantly increased. To decrease health care costs and patient burden, strategies which could prevent cervical dysplasia are needed. These approaches include identifying relevant risk factors such as nutritional factors and smoking and modifying these factors to decrease overall cervical dysplasia risk.

Research has definitively shown that infection with the human papillomavirus (HPV) is a cause of most cases of cervical cancer. Although a woman's risk for cervical cancer is 10-20 fold higher if she has HPV infection, HPV infection alone is insufficient to cause cervical cancer. The women at highest risk are those who consistently test positive for HPV infections over time (persistent infection) and have a higher concentration of the virus (viral load).

However, there is little information currently available on what factors allow HPV infections to persist and progress to cervical cancer. Nutritional status, in particular antioxidant nutrients, and smoking may be such factors. Both factors have consistently been associated with cervical dysplasia and cervical cancer. In addition, smoking has been shown in numerous studies to adversely affect antioxidant nutrient status. Therefore, to adequately investigate HPV persistence, nutritional status and smoking status must be simultaneously assessed and considered.

The central hypothesis of this proposal is that low serum and dietary concentrations of certain carotenoids and Vitamin E will be significantly associated with higher cervical HPV viral load and increased risk of HPV infection persistence. Furthermore, we hypothesize that smoking status modifies this association.

The proposed study, using previously archived serum and cervical cell samples from a cohort of 350 reproductive age women, will efficiently further our understanding of the role of antioxidant nutrients, smoking, and cervical carcinogenesis. The aims of this study are to: 1) Determine the association between circulating concentrations of carotenoids and vitamin E and persistent HPV infection; 2) Determine the association between dietary concentrations of these antioxidant nutrients and persistent HPV infection; 3) Determine whether smoking status modifies these associations; 4) Assess the relationship between circulating and dietary concentrations of antioxidant nutrients and HPV 16 and 18 viral load; and, 5) Determine whether smoking status modifies the association between antioxidant nutrient status and HPV viral load.

We believe that the results of our research will provide much needed information about modifiable non-infectious risk factors, diet and smoking, that may be associated with the progression of HPV infection to cervical cancer. This information could form the basis of education outreach programs to decrease smoking and increase fruit and vegetable consumption among reproductive age women, thus decreasing the number of women at risk for cervical dysplasia/cancer.

Hakim, M.D., Iman

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

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

The single most effective means of cancer prevention among smokers would be to eliminate tobacco use from society. However, this is currently an unrealistic goal. Therefore, alternate cancer prevention strategies targeting this at-risk population are necessary. Changes in dietary habits with the intake of more cancer-chemopreventive agents appears to be a practical approach for cancer prevention in smokers, with tea as one promising agent. Several studies have shown decreased risk among regular tea consumers for lung, breast, colon and rectum, gallbladder, nasopharynx, liver, pancreas, uterus, skin and stomach cancers. Because tea is one of the most popular beverages consumed in Arizona and worldwide, the relationship between tea consumption and human cancer incidence is an important finding. Tea can be easily consumed with one's ordinary meals making compliance and adherence to dietary intervention more likely to succeed. Thus, the role of tea drinking as a potential inhibitor of carcinogenesis merits careful evaluation. We believe that a program of nutritional intervention by realistic dietary modifications that are effective, safe, and acceptable should be the cornerstone of any cancer prevention strategy.

The overall goal of this study is to reduce the incidence of tobacco-related diseases by establishing an efficient and feasible intervention approach that targets smokers who are at particularly high risk for lung caner. We propose a 4-month randomized controlled chemopreventive trial in a group of current smokers who do not intend to quit. These smokers will be randomized to a green or black tea or a control intervention (n=45 per group). Levels of urinary 8-OhdG will be used to measure oxidative DNA damage. We hypothesize that regular tea consumption (black or green) in smokers is associated with decreased oxidative DNA damage and that adherence to a regular pattern of tea consumption is feasible. The study population will be composed of current regular smokers, males and females, who are residents of southeast Arizona (metropolitan Tucson). The population of regular smokers is selected for this trial for two reasons. One is to test interventions potentially feasible among high risk smokers who do not want stop smoking. The second reason is the use of high risk population, like smokers, increases study efficiency for a Phase II prevention trial.

Hill, Ph.D., Anabel L.

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

The Nutritional Impact of Smoking Cessation in Hispanics

Health related data indicate that the health status of Hispanics is poor compared to non-Hispanic whites. Hispanics are reported to have higher rates of nutritionally related diseases including chronic diseases such as cancer and cardiovascular disease. The health status of Hispanics is influenced by their higher prevalence of risk factors such as smoking, alcohol consumption, and obesity. Thus, the economy of the state is affected as poor health status translates to higher health costs for the state. The sub-group of Hispanics in Arizona consists mostly of Mexican-Americans who are rapidly becoming acculturated to the U.S. The prevalence of smoking among Mexican-American males is higher than non-Hispanic whites and comparable to Black American males (44 versus 41 percent) and is increasing for Mexican-American females. In studies primarily of non-Hispanic whites, smoking has been shown to be related to decreased circulating levels of essential micronutrients which are themselves independently associated with chronic disease risk. Smokers also consume fewer serving of fruits and vegetables known to contain these nutrients. Currently, there is minimal nutritional data collected specifically for the population of Hispanic smokers; thus, it is unknown if the dietary and plasma levels of micronutrients are the same as measured in non-Hispanic whites. This project will measure dietary changes and plasma changes in energy intake, macronutrients, and selected micronutrients in Hispanic smokers and successful quitters. Thus, this project has the unique potential to impact future directions in chronic disease prevention research for Hispanics, as well as address nicotine dependence in this understudied population of smokers.

This study will compare dietary intake and plasma levels of Vitamins A and E, carotenoids nd folate in Hispanic smokers, before and after quitting smoking. In addition, comparisons will be made between two methods of dietary assessment: the Southwestern Food Frequency Questionnaire and Three-day diet food records. The data to be analyzed in this project was collected in a randomized, placebo controlled, clinical trial evaluating the efficacy of nicotine patch use in Hispanics. I am requesting funding for laboratory and statistical analyses of the plasma samples and salary support for data entry and statistical analyses of the dietary data. Results from this project will provide pilot data so that I can request extramural support for a larger study evaluating diet and nutritional status in Hispanic smokers and quitters. Specific aims include:

1) to assess changes in plasma levels of carotenoids, Vitamins A and E, and folate in Hispanic smokers who quit compared to unsuccessful quitters;
2) to assess changes in total caloric intake, dietary intake of macronutrients, and dietary intake of specific micronutrients in Hispanic smokers who quit compared to unsuccessful quitter; 3) to correlate the intake of selected micronutrients measured in the plasma to

dietary intake to the same micronutrients in Hispanic smokers who quit compared to unsuccessful quitters; and

4) to compare two methods of dietary assessment for energy intake, macronutrient and selected micronutrient intakes in Hispanic smokers.

The study hypotheses to be evaluated include:

(1) quitting smoking will result in increases in plasma levels of carotenoids, folate, and Vitamin E;

(2) quitting smoking will result in increases in dietary intake of energy, fat and carbohydrate:

(3) correlations between dietary and plasma micronutrient levels will be increased as a result of quitting smoking due to increased dietary consumption and potentially metabolism of selected micronutrients; and

(4) dietary food records will show higher correlations with plasma levels of selected micronutrients compared with food frequency questionnaires.

Jacobs, Ph.D., Bertram L.

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

Specific Induction of dsRNA-Mediated Suicide in Lung Cancer Cells

Most anti-cancer agents, including agents that kill tobacco-caused cancers, work by making cancer cells commit suicide. Over time, many cancers become resistant to the suicidal effects of the anti-cancer agents, which leads to failure of the anti-cancer treatment. We have been investigating induction of suicide in cancer cells by a novel potential anti-cancer agent, dsRNA. dsRNA induces suicide in a very different manner form most other known anti-cancer agents. dsRNA appears to induce suicide even in cancer cells that have become resistant to treatment with many traditional anti-cancer agents. The problem with using dsRNA as an anti-cancer agent is that it causes both cancer cells and normal cells to commit suicide, leading to unacceptable side-effects. We a proposing to develop a method to specifically make dsRNA only in cancer cells (including lung cancer cells), thus allowing specific killing of cancer cells while minimizing injury to normal cells in the body. We plan to do this by engineering an otherwise harmless virus that will make dsRNA only in cancer cells. Tumors could then be infected with this virus which would specifically kill the cancer cells without harming surrounding normal cells.

The overall goal of the research is to develop an otherwise harmless virus that can specifically kill lung cancer cells. This virus could then be potentially used as a novel, specific, anti-cancer agent. This research will test the hypothesis that dsRNA can be made specifically in the cytoplasm of cancer cells, and can specifically kill cancer cells without damaging normal cells. To develop this system, we will first optimize cell killing with two well characterized systems, human cervical carcinoma cells and human glioma cells. We will then use the knowledge we have gained from study of these systems to develop tools to specifically kill human lung cancer cells. The *Specific Aims* are to:

1. develop a virus that can specifically kill human cervical carcinoma cells in culture as a test of the hypothesis;
2. optimize killing of human cervical cancer cells in culture;
3. develop a virus that can specifically kill rapidly migrating human brain cancer cells as a test of the hypothesis;
4. develop a virus that can specifically kill human lung carcinoma cells as a test of the hypothesis.

Johnson, M.D., Mary I.

University of Arizona
Award Amount FY 2000: $46,779

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

Multiple drug use is the most common pattern of abuse. As a result, newborn infants may be exposed *in utero* to two drugs commonly used by pregnant mothers, nicotine *via* cigarette smoking and alcohol. Over 1 million Arizona citizens smoke; of these some 8,500 pregnant women will give birth yearly to infants three times more likely than those of non-smoking mothers to be premature with the attendant complications of increased infant mortality and morbidity. Prenatal exposure to cigarette smoking has also been associated with significant cognitive and receptive language disorders in older children. Additionally, in Arizona with approximately 70,00 births a year, conservatively 200-300 infants may have sufficient exposure to develop either the fetal alcohol syndrome or, while not fulfilling the complete diagnostic criteria, significant effects of maternal alcohol use. Prenatal alcohol exposure results in growth retardation (particularly the brain), craniofacial abnormalities and other congenital malformations, as well as significant cognitive and attentional disorders that will adversely impact these children's long-term ability to function in society. Because a clear association between drinking and smoking exists, many infants will have co-exposure to both nicotine and alcohol with possible magnification of the adverse effects of either exposure alone. Little is currently known concerning the effect of nicotine in concert with alcohol, a combination that may be particularly damaging to the developing nervous system.

The first goal of this project is to define the effects of nicotine on developing nerve cells. We propose to use a tissue culture model of nervous system development where nerve cells can be exposed to nicotine and observed directly as they first develop an axon, a process that interacts with target cells, and then dendrites, processes that are a major site of incoming information. The model will also allow the study of nicotine's effect on the interaction between the nerve cells and their glial companions, the Schwann cells. We believe on the basis of our preliminary experiments that nicotine may be particularly noxious to this interaction and thus to the normal development of the nerve cell. The second goal will be to study the combined effect of nicotine and alcohol on both nerve and glial cells. With these studies we will test the following hypotheses: 1) nicotine adversely affects the development of both axons and dendrites; and 2) nicotine causes death of the rapidly dividing supporting glial cells, the Schwann cells, and Co-exposure to nicotine and alcohol together results in augmented adverse effects on glial and neuronal development. Information obtained in this study would heighten the awareness of the public and health care community to the potential hazards of the combined prenatal exposure of infants to nicotine and alcohol and provide the basis for further studies concerning the basic mechanisms of injury by these two most commonly used elicit drugs.

Lorton, Ph.D., Dianne

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

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

Elevated levels of circulating nicotine have been shown to suppress immune function. Nicotine from tobacco use results in the release of several hypothalamic-pituitary-adrenal axis (HPA) hormones and alters release of norepinephrine by sympathetic nerves. The immunosuppressive effects of nicotine are likely to result by interaction of nicotine with nicotinic receptors expressed by hypothalamic neurons, sympathetic ganglion neurons, and/or immune cells. This proposal explores effects of nicotine on immune function following challenge with an autoimmune disease causing antigen. We propose that nicotine will alter the cytokine production. Cytokines are signaling molecules of the immune systems that direct immune responses. Nicotine will effect cytokine production either by direct interaction with immunocytes or by changing corticosteroid levels via interaction with the hypothalamus or by changing norepinephrine/adrenaline levels via interaction with sympathetic ganglia. The resulting pattern of cytokines produced by immunocytes will promote disease induction and progression.

At present relatively little is known about the effects of nicotine of the hypothalamic-pituitary-adrenal axis, sympathetic nervous system, and immune function. This research will provide us with a better view of the role played by nicotine in the effects of tobacco on immune function and potential side effects that nicotine patches and gum aimed at reducing tobacco addiction may have on immune function. Treatment with nicotine has been shown to influence all aspects of the immune system, including alterations in humoral and cellular immunity.

Immune function is compromised with increasing age, and the incidence of autoimmune disease, such as rheumatoid arthritis, increases with advancing age. Since two of the most concentrated geriatric communities in the world are in the state of Arizona, this is likely to represent a significant health problem for many of its residence. The ultimate goals of this research are to gain a better understanding of how nicotine effects immune function; to determine if the nicotine, the psychoactive component in tobacco, promotes autoimmune disease in susceptible strains of rats by its immuno suppressive properties, and to determine potential effects nicotine treatments to reduce tobacco addiction may have on immune function.

The proposal has two specific aims. The first specific aim tests the hypotheses that treatment with chronic nicotine will alter cytokine production patterns of immunocytes from animals challenged with a relevant antigen. Cytokine production patterns in nicotine treated Lewis rats will more strongly favor induction and progress of autoimmune disease than cytokine patterns in nicotine treated Fischer rats. The second specific aim of this proposal is to determine whether the observed nicotine-induced changes in immune function in these two strains of rats is the result of nicotine-nicotinic receptor interaction at the level of the hypothalamus, sympathetic ganglia, or lymphocytes.

Lukas, Ph.D., Ronald

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

Molecular Bases for Nicotine Dependence

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

The central hypothesis of the project is that extended exposure to nicotine induces long-lasting changes in numbers and function of nAChR. These changes are postulated to contribute to the unique effects on nervous system function of chronic nicotine use and to underlie nicotine dependence.

The broad goal of this project is to establish whether and how chronic nicotine exposure induces long-term changes in numbers and function of several different and important forms of nAChR, focusing particularly on the numerically predominant forms of human nAChR found in the brain.

The project's specific objectives (aims) are based on strong preliminary findings. The findings suggest the working hypothesis that chronic nicotine exposure causes both 1) an unusually long-lasting loss of nAChR function, and 2) an increase in numbers of nAChR. These effects are postulated to occur for all forms of nAChR. However, they also are postulated to occur at nicotine doses that differ across nAChR forms. Furthermore, different nicotine-like drugs are predicted to mimic or block effects of nicotine at specific forms of nAChR. Studies will be done focusing on the two numerically predominant forms of nAChR found in the brain. SPECIFIC AIM 1 is to identify the effects of exposure to nicotine or related substances on function of these nAChR. SPECIFIC AIM 2 is to determine whether and how exposure to nicotine or related substances affects numbers of these nAChR.

Lynch, Ph.D., Ronald M.

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

Effect of Nicotine on Hypothalamic Glucose-Responsive Neurons

Cessation of chronic nicotine exposure is linked with changes in feeding behavior and caloric intake in humans and other animals. Neurons in the hypothalamic region of the brain receive sensory information to set caloric intake and thereby maintain body weight. It is likely that changes in the ability of hypothalamic neurons to sense this information underlie feeding changes which lead to weight gain and obesity. One of the suspected sensory inputs is the level of blood glucose. It is likely that changes in sensitivity of the hypothalamic neurons to glucose underlie the obesity that is often observed with diabetes. We propose that chronic nicotine exposure also alters the normal sensitivity of the hypothalamic neurons to glucose, which thereby elicits at least a portion of the changes in feeding behavior which are observed following cessation of smoking. One of the major obstacles to quitting smoking is the weight gain experience by many individuals. Moreover, smoking is a significant concern which influences weight gain observed in diabetics. Our studies will provide basic information regarding the role specific hypothalamic neurons may play in linking these events. Based on this understanding, specific and rational drug therapies may be developed to reverse the effects of nicotine on feeding behavior and thereby weight gain following cessation of smoking.

In proposed studies, neurons will be isolated from the hypothalamus of rats and classified for their ability to sense glucose, nicotine and the hormones which modulate blood glucose (*e.g.*, insulin). Once the neurons are characterized we will test the hypothesis that: The activity of Glucose-Responsive Neurons of the hypothalamus is modulated by insulin and nicotine.

The Specific Aims of the proposed studies are to:

- Develop methods to isolate and maintain neurons isolated from the rat hypothalamus in culture.
- Characterize the mechanism by which hypothalamic neurons sense glucose.
- Determine the influence of nicotine on the sensitivity of isolated hypothalamic neurons to glucose.
- Determine the influence of the metabolic hormones insulin and leptin on the sensitivity of these isolated hypothalamic neurons to glucose.
- Develop a rapid technique for isolating only Glucose-Responsive Neurons from the hypothalamus, to set the basis for future studies on the effects of chronic exposure in adult animals.

This work will set a foundation for studies of changes in feeding centers in the brain that occur during development of diabetes and following chronic nicotine exposure. Long term studies will focus on animal models of these altered states. However, the proposed studies will directly test

this issue in the isolated situation. Findings made here will provide an understanding of how nicotine influences the metabolic set point in animals and, therefore, potential strategies to block or reverse these influences.

Martinez, Ph.D., Jesse

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

p53-Dependent Apoptosis in Lung Cancer

Lung cancer is the number one 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. Linear relationships exist between cigarette consumption and lung cancer risk. As with other cancers, lung cancer is thought to develop as a consequence of multiple genetic mutations that collectively dysregulate cell growth and promote the development of tumors. A frequent target of those mutational events is the p53 tumor suppressor gene which acts as the guardian of the genome and stops cell growth whenever the cell's DNA has been damaged. p53 helps to initiate the repair process, but if DNA damage is extensive and unrepairable, it initiates programmed cell death or apoptosis. This process eliminates those cells with the kind of mutations that could lead to tumors. Hence, p53's capacity to induce apoptosis is of the utmost importance for its function as a tumor suppressor. Importantly, tumor cells that lack p53 are less sensitive to chemotherapeutic drugs because they are more resistant to the apoptosis induced by these treatments. Therefore, mutations in the p53 gene increase the probability of developing a tumor and decreases the chances of successful treatment with conventional therapies. Unfortunately, how p53 functions is not completely understood. Hence, a prime objective of the proposed work is to identify the molecular mechanism by which p53 induces apoptosis.

Our long range goal is to develop a therapeutic strategy that sensitizes lung cancer cells that lack functional p53 tumor suppressor genes to the cytotoxic effects of conventional therapies. We propose that p53 induces apoptosis by regulating the activity of the critical cell cycle regulatory proteins, Cdc2, Cdc25, and 14-3-3, which also play important roles in apoptosis. Specifically, we propose p53 induces apoptosis by causing inappropriate activation of Cdc2 in growth arrested cells by modulating the activity of Cdc25 via 14-3-3 proteins. We will test this hypothesis by: 1) examining the relationship between Cdc2 kinase and p53-dependent apoptosis induced in lung cancer cells, and 2) characterizing the functional consequences of the interactions between p53, Cdc25, and 14-3-3 in lung cancer tissue. Our expectation is that at the end of these studies we will have developed a mechanistic explanation for the induction of p53-dependent apoptosis that occurs in response to ionizing radiation and/or chemotherapeutic drugs and have determined whether 14-3-3 proteins can serve as useful bio-markers for lung cancer.

McQueen, Ph.D., Charlene

University of Arizona
Award Amount FY 2000: $119,804

N-Acetyltransferases: Genotype and Genotoxicity

An individual's susceptibility to the hazardous effects of a chemical is due to the interaction between his/her genetic make-up and environmental factors. The proposed research will investigate the role of genetic variation in tobacco-induced cancer. One component of cigarette smoke is a chemical called 4-aminobiphenyl (4AB). This chemical causes urinary bladder tumors and may be linked to breast cancer in humans. There is a genetic difference in how 4AB is changed in the body. Individuals are classified as having high or low activity for this reaction. In Arizona, about 50 percent of the population has low activity. Studies are planned to develop genetically defined mice that differ is this trait. These animals will be used to study how high or low activity alters whether 4AB reacts with DNA, one step in changing normal cells to tumor cells. The information will help to determine if this trait could be used as a genetic marker of susceptibility to a tobacco carcinogen.

The overall goal is to investigate if there is a relationship between genetic make-up and risk of developing cancer caused by one component to tobacco smoke, 4-aminobiphenyl (4AB). The genetic trait is designated N-acetyltransferase (NAT) and individuals have high or low NAT activity. The hypothesis to be tested is that susceptibility to DNA damage (one step in developing cancer) induced by 4AB is associated with the expression of NAT genes. Strains of mice that have defined NAT genes expressed in particular tissue will be developed. These animals will be exposed to 4AB and DNA damage will be measured . Target tissues known to develop 4AB tumors and nontarget tissues will be evaluated. These studies will show if high or low activity is associated with more or less damage. This information would ultimately help to predict individual risk.

Morkin, M.D., Eugene

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

Grafting of Stem Cell-Derived Cardiomyocytes to Repair Myocardial Infarction

The major goal of this proposal is to develop a strategy for repair of myocardial infarction using cardiomyocyte grafting. The damaged heart is unable to maintain an adequate output of blood and heart failure ensues. In the United States about half a million individuals present with newly diagnosed heart failure each year and over 4 million persons are currently affected. The proportion of the Arizona population affected is similar. The relationship of tobacco smoking to this deadly chain of events is well established. The damaged heart is unable to repair itself because in adult animals heart cells can not divide. At present, there is no means of reversing the damage short of transplantation of a new heart. Unfortunately, the limited availability of donor hearts leaves the vast majority of heart attack victims in need of an alternative therapy. Ideally, heart cells around an infarction might be stimulated to divide and replace the damaged area. Until this goal can be attained, an alternative strategy is to graft fetal heart cells or heart cells derived from embryonic stem (ES) cells into the heart to fill in the damaged region. Our hypothesis is that cardiomyocyte transplantation into the infarcted heart wall will result in repair of the scar and enhanced cardiac function.

The two major aims of this proposal are: 1) to investigate the control of the cell cycle in developing heart cells with the long-term goal of discovering ways to stimulate adult heart cells to divide and the short-term goal of stimulating grafted heart cells to proliferate sufficiently to fill in the damaged area; and, 2) to assess the survival of cardiomyocyte grafts in animals with experimental infarctions and to determine their contribution to improved function. The need for expertise in several areas, including cardiac cell culture, molecular biology, experimental animal surgery, and cardiovascular physiology, requires a Program Project approach. Accordingly, in Section 1 Dr. Morkin and his colleagues will study control of the cardiac cell cycle and prepare fetal rat heart cells and mouse ES cells that have been diverted to form cardiomyocyte. In Section 2 Dr. Goldman and coworkers will inject these cells into the infarcted hearts of rats. The survival of fetal rat cell grafts will be compared with grafts of cardiomyocyte derived from ES cells. The effects of treatment with growth factors on survival will be analyzed. Both the regional function in the area of grafting and the overall function of the heart will be measured in grafted animals and controls.

Payne, Ph.D., Claire M.

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

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

On the basis of current mortality rates, approximately 64,000 of Arizona's current residents (about 2 percent of the population) are projected to die of colorectal cancer. At least 90 percent of colorectal cancer deaths (or 57,600 in Arizona) can be attributed to lifestyle factors including diet and smoking and are potentially preventable. A recent authoritative estimate is that approximately 20 percent of colon cancers in men are due to smoking. Individuals who have had colon cancers removed are at increased risk for recurrence of colon cancer. Individuals having certain kinds of premalignant colonic polyps, or with one or more first degree relatives who have had colon cancer, are also at increased risk for colon cancer. Our research goal is to develop a practical biomarker to assess the degree of colon cancer risk on an individual basis. The biomarker could be used to identify individuals within moderate to high risk groups (for example, smokers or past smokers) allowing them to take appropriate preventive action. If this biomarker were available, deaths from colon cancer in Arizona would drop, as individuals with previously undetected precancerous changes in their colon are identified and can modify their lifestyle as well as be followed by colonoscopy to remove early stage pre-cancerous polyps.

Cells excessively damaged in the colon undergo a controlled process of cell death called apoptosis. This is beneficial because it removes cells with unrepaired DNA damage. Such cells, if allowed to survive, tend to mutate and give rise to cancer. We and others have found that colonic cancer cells are defective in apoptosis. We also made the novel finding that the normal-appearing colonic mucosa of individuals in high risk groups are deficient in the ability to undergo apoptosis. Our published and preliminary data indicate that aberrant levels of three specific proteins (associated with oxidation levels of the cell) can contribute to apoptosis resistance. Our preliminary data imply that such aberrant protein levels in the colon may indicate pre-cancerous changes in high risk individuals. Since levels of these specific proteins can be measured in routinely prepared biopsy specimens, we hypothesize that they should prove useful as biomarker for colon cancer risk. One research objective is to test altered patterns of expression of these proteins within the colonic mucosa of individuals in different risk groups to determine whether they would be reliable as biomarkers for assessing colon cancer risk. Our other research objectives are to determine whether expression of these proteins becomes more aberrant during colon cancer progression, whether the oxidant promoting smoke components (nicotine, catechol and hydroquinone) affect their expression, and whether aberrant expression of these oxidant proteins is associated with heavy smoking. Our preliminary data indicate that nicotine activates one of these oxidant-related proteins and could contribute to cancer risk, in part, through this type of mechanism.

Romagnolo, Ph.D., Donato

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

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

The state of Arizona ranks 16 in the United States in death (5,700 in 1990) attributable to smoking with estimated years of potential life lost approaching 67,000 years and a state medical-care expenditure related to smoking of about $52,000/death. Recent findings substantiate that tobacco usage is a risk factor in the etiology of breast cancer. Research conducted in our laboratory during the last two years has generated preliminary data suggesting that specific tobacco derivatives such as polycyclic aromatic hydrocarbons alter the production of protective proteins defined as tumor suppressors whose loss is known to be a predisposing event in the etiology of breast cancer. Understanding how these tobacco derivatives alter at the cellular and molecular levels the function to tumor suppressor genes is a key step in defining strategies targeted to preventing the onset of breast malignancies. In turn, new knowledge acquired through completion of this project will improve the awareness of the public, in particular of adolescent smokers, and assist in understanding the consequences of tobacco exposure on breast cancer development and therapeutic interventions.

The overall goal of this proposal is to understand how polycyclic aromatic hydrocarbons present in tobacco smoke regulate the production of the tumor suppressor protein BRCA-1 (BR= Breast, CA=Cancer) and the significance of this modulation in the etiology of sporadic breast cancer. In pursuit of this goal, the primary objective of this application is to detail the molecular mechanisms through which polycyclic aromatic hydrocarbons influence the activity of the tumor suppressor gene BRCA-1. The central hypothesis of this proposal is that polycyclic aromatic hydrocarbons cause at the same time DNA damage and disrupt the normal production of BRCA-1 which is involved in DNA repair. Under the current model of tumorigenesis presented in this proposal, loss of BRCA-1 would result in increased frequency of DNA damage and sporadic tumor development.

Rose, Ph.D., Seth D.

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

Enzyme Active Site Tailored Anticancer Drugs

Lung Cancer is the leading cause of cancer death among both men and women. The American Cancer Society estimates that in 1998 there will be about 171,500 new cases of lung cancer nationwide, accounting for about 14 percent of all new cancers and about 29 percent of deaths from cancer. In Arizona the 1996 cancer death rate was 119.3 per 100,000, the lowest rate in the 11-year period from 1986 to 1996. In Arizona lung cancer accounted for 31.5 percent of all cancer deaths among males and 25.1 percent among females. The one-year survival rate for lung cancer is about 40 percent, up from 32 percent in 1973, largely as a result of better methods of surgery and some progress in chemotherapy and radiation therapy. It is estimated that 87 percent of lung cancer cases are smoking related. A significant number of lung cancers result from unrestrained growth that can be traced to the action of a protein that triggers the cell division machinery. This protein normally acts as a "switch" that cycles between "on" and "off" states, but in cancer cells, it is continually "on," so the cells continue to multiply.

Our long-term goal is to produce new medicines that will turn off the switch that causes lung cancer cells to multiply, providing a cure or at least a long-term remission of the disease. The switch is a protein that can exert its effect on cell multiplication only after it has been acted upon by a cellular enzyme and converted into an active form. In effect, blockage of the enzyme that carries out this conversion has the effect of preventing the protein from acting as an "on switch." This prevents the cancer from growing, which allows normal processes in the tissue to reduce the size of the tumor. We plan to develop new substances that block the enzyme that converts the switch protein into a functional form. The new substances will be tailored to the molecular structure of a part of the enzyme known as the active site, which is the site in the enzyme where it carries out its job of altering the switch protein. Preparation of the new substances, their purification and identification will be by known laboratory methods of organic chemistry. The hypotheses to be tested are: 1) whether the new substances can be tailored in such a way as to target them to the enzyme's active site for blockage of the enzyme's action; and 2) whether compounds designed to block the enzyme active site can inhibit cancer cell growth. Testing of the compounds in the laboratory will be carried out against the target enzyme to see whether the enzyme's action is blocked. Further testing will also be carried out against cancer cells grown in cell culture at the National Cancer Institute.

Simon, Ph.D., Alexander M.

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

Role of Gap Junction-Mediated Communication in Preventing Endothelial Dysfunction

Heart disease, which affects more than 56 million Americans, is the leading cause of death in the United States, accounting for 40 percent of all deaths. In Arizona alone about 10,000 people died from heart disease in 1996. Cigarette smoking has been found to be a major risk factor for the development of heart disease, especially atherosclerosis, or hardening of the arteries. Heavy smokers are 2 to 4 times more likely to have a heart attack than nonsmokers. How smoking leads to atherosclerosis is not well understood, but studies suggest that chemicals in cigarette smoke cause dysfunction of endothelial cells which line the interior of blood vessels. Their abnormal state triggers a cascade of events leading to inflammation and eventually plaque formation that can block blood flow. How do we know what smoking causes endothelial cell dysfunction? Research in animals and humans has shown that smoking causes blood vessels to constrict and be less responsive to chemicals that normally make them open up in response to changing metabolic demands. One possible explanation for this lack of responsiveness is that structures called gap junctions in the vessel wall may be adversely affected by smoking. Gap junctions are aggregations of pore-forming proteins that connect adjacent cells so that they can exchange small molecules. These intercellular channels, which are present between cells in the blood vessel wall, are thought to be important in regulating blood vessel contraction and expansion. This study proposes to examine the effects of disrupting gap junction communication on the health of blood vessels. This knowledge should help to better understand the relationship between smoking, atherosclerosis and heart disease.

Since smoking causes endothelial dysfunction and is a major risk factor for atherosclerosis, it is important to learn more about what influences the health of blood vessels. We hypothesize that one of the effects of smoking-induced endothelial dysfunction may be to interfere with intercellular communication. The long term goal of this project is to determine the effects of interfering with the ability of blood vessel cells to communicate through gap junction channels. To investigate this question, we will take advantage of two genetically engineered mouse lines previously generated and characterized by the applicant. In each mouse line, a specific connexin gene, encoding a gap junction channel protein, has been genetically ablated. First, we will determine if communication is inhibited in the connexin-deficient mice. This will be tested by injecting dyes into cells of blood vessels and watching the movement of dye from cell to cell. Second, we will test if the ability of blood vessels to adaptively relax or constrict in response to signaling chemicals is affected in these animals. A diminished ability to respond would support the idea that interfering with communication causes endothelial dysfunction similar to that induced by cigarette smoking. Third, we will test the idea that blocking communication in the vessel wall may also trigger an inflammatory response which could lead to atherosclerosis. We will determine if endothelial cells from the mice display classic signs of inflammation such as changes

in the cell surface proteins which are important in attracting immune cells to inflamed areas. Lastly, we will examine the effects of eliminating both genes by interbreeding the mouse lines. Preliminary studies indicate that the resulting newborn mice die during the first day of life and have difficulties maintaining the integrity of their blood vessels. We will investigate the nature and location of the leakage sites in order to better understand the relationship between communication and vascular permeability. It is hoped that these studies will lead to new approaches and treatments for smoking-induced atherosclerosis and heart disease.

Slepian, M.D., Marvin J.

University of Arizona
Award Amount FY 2000: $49,393

Microtubule-Dependent Integrin Function: Role in Atherosclerosis and Restenosis

Cigarette smoking and other forms of tobacco use have been identified as a major causative risk factor for atherosclerosis (hardening and degeneration of the arteries). Smoking-induced atherosclerosis may involve multiple arterial beds leading to peripheral vascular, cerebrovascular and coronary artery disease (CAD). CAD in particular has significant morbidity and mortality being the leading cause of death in the United States and a major health problem in Arizona. Smoking has been determined to result in up to 30 percent of all deaths due to cardiovascular disease. Cigarette smoke has been demonstrated to initiate and propagate atherosclerosis via multiple mechanisms including direct endothelial damage, enhanced platelet adhesiveness, lipoprotein oxidation and smooth muscle cell activation, migration and proliferation. In addition to the connection between smoking and atherosclerosis, a direct link exists between smoking and post angioplasty restenosis, arterial renarrowing following balloon angioplasty, the primary mode of therapy for advanced atherosclerosis. In smokers restenosis occurs in >40 percent of patients, *i.e.* at even greater rates than in the nonsmoking population. The impact of restenosis is considerable with increased patient morbidity, repeat procedure risk, and increased health care cost. In order to develop effective therapies for atherosclerosis and restenosis retardation, regression and prevention understanding of basic mechanisms is essential.

At the cellular level a common mechanism underlying the initiation and progression of both atherosclerosis and restenosis is smooth muscle cell (SMC) migration. SMCs are the predominant cell type composing the wall of the artery. Following the initial inciting injury, either from noxious chemical initiators, *i.e.* smoking associated substances, or from mechanical injury (balloon angioplasty), these cells move to the lumenal surface of the artery, proliferate and deposit extracellular matrix (ECM), filler proteins between cells, providing scaffolding for tissue integrity. This process of lesion formation occupies space, narrowing the artery reducing blood flow, leading to heart attack and death. Recently new insights into mechanisms responsible for SMC migration have begun to emerge. SMCs have on their surfaces molecules called integrins. Integrins function as linker or liaison molecules allowing cells to interact with their surrounding extracellular matrix protein environment. Migrating SMCs have been shown to alter the integrin types on their surfaces with the onset of migration, allowing cells to detach from their ECM environment and migrate more readily through the artery wall. Despite demonstration of the significance of integrins in SMC migration, our understanding of mechanisms that control and modulate integrin function is limited.

Recently it has been demonstrated by my lab and that of others that a connection may exist between cellular cytoskeleton elements and integrins. Upon binding of SMCs to extracellular matrix *via* surface integrins, specific alterations in cytoskeleton organization have been

demonstrated. As its name implies, the cytoskeleton consists of s structured network of protein filaments including microtubules and actin which provide a physical scaffold or skeleton for maintenance of cell shape, control of cell division and control of migration. Integrin activation is known to result in dramatic alterations in cytoskeletal organization, but very little is known about the role microtubules play in integrin function and in the responses of arterial wall SMCs in atherosclerosis and post angioplasty restenosis. This proposal seeks to understand the completely-undefined mechanism by which interaction of specific integrins with microtubules in vascular SMCs following injury contributes to their pathologic migration and proliferation.

The goal of this project is to examine the role of microtubules in cell surface integrin function. Central in our studies will be the novel natural product drug, Combretastatin A4 (CA-4), which inhibits the function of cellular microtubules. In addition to providing an important tool for biologic studies, Combretastatin has potential as well for becoming a practical local therapeutic for local atherosclerosis control (local drug delivery therapy) and in treating post angioplasty restenosis. We and others have demonstrated that CA-4 disrupts the proliferative blood vessels which supply solid tumors with little effect on normal, established vessels. The biology underlying this activity is unknown, but we hypothesize that a predominant mechanism for its selectivity involves the disruption of specific microtubule-dependent integrin functions which are essential for the survival and proliferation of migrating cells. In order to evaluate this hypothesis, we will examine the effect of CA-4 on the adhesion, migration and survival of SMCs plated on various matrix substrates in tissue culture. The ability of integrins to activate their physiologic signaling pathways and turn on expression of genes related to growth and survival in CA-4 treated cells will also be defined. Lastly, with a view toward future clinical utilization, the effect of local application of CA-4 onto the arterial wall following balloon injury in an animal model, using an *in situ* hydrogel delivery system developed in our lab, will be studied.

SECTION F

PROPOSALS RECEIVED

ANTI-CANCER DRUG DISCOVERY RESEARCH

FY 1999

Canfield	University of Arizona	Anticancer Activity of B-Carotene Oxidation Products	$151,381 115,732
Chang	Arizona State University	Efficacy, Mode and Toxicity of Newly Developed Anti-cancer Drugs	$248,156 240,211
Epner	University of Arizona	Pharmacologic Approaches to Turning Off De-regulated Cyclin D Expression in Lymphoid	$49,951 49,951
Gunatilaka	University of Arizona	Discovery of Novel Anticancer Drugs from Rhizosphere Microflora of Desert Plants An Unexploited Source of Bioactive Natural Products	$249,949 205,440
Jacobs	Arizona State University	Viral Vectors for Treatment of Brain Cancer	$250,000 250,000
Katsanis	University of Arizona	Improvement of Anticancer Immune Responses Generated by Chaperone Protein Associated Tumor Peptides	$230,603 201,467
King	Samaritan Regional Med. Ctr.	Clinical Trials Network	$88,503 87,194
Mash, Jr.	University of Arizona	Rational Design of Thioredoxin	$249,874 249,874
McGovern	University of Arizona	Stress-Inducible Gene Constructs and Stress-sensitive Liposome Delivery Systems for Targeted Cancer Therapy	$244,800 247,242
Pettit	Arizona State University	Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs	$749,982 749,980
Polt	University of Arizona	Synthesis and Methodology for New Glycosyl-transferase Inhibitors with Potential Anti-Metastatic Activity	$52,325 59,374

Powis	University of Arizona	Arizona Cancer Center Novel Anticancer Drug Discovery & Development	$750,000 750,000
Rose	Arizona State University	Enzyme Active Site Tailored Anticancer Drugs	$50,000 50,000
Scheck	Barrow Neurological Institute	Rational Design of New Treatment Regimens for Human Malignant Brain Tumors	$50,000 50,000
Skibo	Arizona State University	Preclinical Development of the PBI'S	$155,990 141,145
Watson	University of Arizona	T Cell Receptor Peptide A Novel Immunotherapy to Prevent Retroviral Promotion of Lymphoma Growth	$50,000 50,000
Whitesell	University of Arizona	Development of Antisense Oligonucleotides as Chemotherapeutic Agents for Intratumoral Administration	$226,456 194,880

SECTION G

NEW CONTRACT AWARDS

ANTI-CANCER DRUG DISCOVERY RESEARCH

FY 1999

Gunatilaka, Ph.D., A.A. Leslie

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

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

Cancer accounts for a vast number of human deaths and suffering. Each year 6.4 million people are diagnosed with cancer worldwide. In the U.S. more than 10 million people are living with a history of cancer, and in excess of one million new cancer cases develop annually. According to the Arizona Cancer Registry published by the Arizona Department of Health Services, in 1992 cancer was responsible for 7,400 deaths and 19,378 newly diagnosed cases in Arizona. Cancer incidence and deaths in our state is on the rise as many elderly continue to move here due to its desirable climate.

Cancer treatment currently relies on surgery, radiation and chemotherapy (treatment with anticancer drugs). Chemotherapy is the only effective way to treat a disseminated cancer. Unfortunately there are no good anticancer drugs available to treat major solid tumors such as cancers of the lung, breast and colon effectively without any side effects. There is thus an urgent need for the discovery and development of new, effective and non-toxic anticancer drugs to treat these cancers.

The majority of anticancer drugs in use today are from natural sources. The goal of this project is to investigate a novel and an unexploited natural source of anticancer drugs. This source consists of microorganisms, bacteria and fungi, that grow in the roots of desert plants. This region is called the rhizosphere and each plant supports unique types of microorganisms in its rhizosphere. Micro-organisms are known to be a valuable source of pharmaceuticals including antibiotics and anticancer agents. Desert plants are unique compared to other plants growing elsewhere in the United States and thus will support different microorganisms. This novel source has not previously been exploited for anticancer drugs.

Microorganisms associated with the roots of selected desert plants will be assayed for the production of anticancer active compounds using our in-house anticancer bioassays. Those microorganisms showing potential activity will be grown, cultured, on a large scale in order to isolate compounds responsible for their anticancer activity. The chemical structures of these promising leads will be elucidated by modern instrumental techniques and chemical methods as needed. Those active compounds with novel structures will be further developed for future animal testing and if promising for clinical trials and further development as anticancer drugs. We are hopeful that having access to this unique source of bioactive in our laboratories will lead to discovery of effective new drugs to treat currently untreatable forms of cancer, thus increasing the survival and quality of life of victims of cancer not only in Arizona but worldwide.

Jacobs, Ph.D., Bertram L.

Arizona State University
Amount Awarded FY 2000: $250,000

Viral Vectors for Treatment of Brain Cancer

We propose in this grant application to begin a concerted effort to develop viruses that can specifically kill brain cancer cells. There are three compelling reasons to launch and sustain a concerted interdisciplinary brain cancer research effort in Arizona: 1) the incidence of primary brain cancer is on the rise, with disproportionate increases in the aging population (of which Arizona claims, indeed recruits, a burgeoning proportion of our nation's senior citizenry); 2) survival rates for patients diagnosed with the most common forms of primary brain cancer have not improved in the past 25 years; and 3) strong basic and clinical science programs have matured in the state to the degree that interdisciplinary projects merging these efforts stand to make a unique contribution to the field. We believe that progress along the lines of the studies outlined in this proposal will serve to advance treatment opportunities for brain cancer patients and to raise the awareness nationally of the outstanding cancer research in Arizona. Such awareness will attract further research, and (hopefully) patients, who will be successfully managed for their disease.

Ten percent of primary brain tumors reported in national clinical studies in 1999 will occur in Arizona residents (close to 2,000 patients). Clearly this state harbors a disproportionate number of victims of this aggressive disease. By being situated in the brain, these tumors engender special and challenging constraints on treatment planning. These tumors also demand rapid and effective interventions due to the limited ability of the brain to tolerate a growing cancer. Strategies that seek to exploit specific unique properties of the brain cancer cells, properties that in effect make the cells identifiable targets, are championed in this project. The described experiments will determine the degree to which brain cancer cells can be specifically hit by novel virus delivery systems, and will assess the outcome of such hits in rendering the cancer vulnerable to control. Development of viruses for treatment of cancer was recently championed in a *Science* "News Focus" (Training Viruses To Attack Cancer, *Science* 282;1244-1246, 1998).

We hypothesize that specific properties of brain tumor cells can serve as development beacons for targeting new forms of treatment. Several such properties have been described, including particular susceptibility to infection by brain-homing viruses, heightened or unusual expression of genes, and overly active cell growth. We believe these observations demand focused attention as ultimate, exploitable targets for anticancer drug discovery. Using the research tools of molecular and cellular biology, extended to small animal model systems of human brain cancer, we will develop and test viral treatments targeted at key molecular processes in brain cancer cells. The goal is to educate ourselves, and our colleagues, as to optimal methods for delivering molecular therapy against brain cancer. We aim to develop the working parameters by which engineered viruses can rendered safe for the brain but deadly for the brain tumors. It is our objective to apply our various specific scientific expertise in an interdisciplinary manner to advance ways to control the lethal behavior of human brain cancers.

Katsanis, M.D., Emmanuel

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

Improvement of Anticancer Immune Responses Generated by Chaperone Protein Associated Peptides

Despite improvements in surgery, chemotherapy agents and radiation therapy, far too many patients die from refractory cancers. Although the concept of stimulating the body's immune system to treat cancer has existed for many years, recent discoveries in how the body recognizes foreign proteins offer promising new approaches to cancer that can be combined with conventional treatment. The T cells of the immune system are normally able to recognize and react against foreign tissue. A good example of how powerful T cells are in attacking foreign tissues is their rejection of organ transplants. Drugs that markedly suppress T cell function are currently required for organ transplants to survive. Although cancers also differ significantly from the normal host tissues, they have a way of being invisible to the T cells. Studies have shown that tumors express unique foreign proteins or antigens. Despite that, the body is unable to present these antigens to T cells and stimulate them to attack. Moreover, some cancers secrete substances called cytokines, some of which block the immune system's ability to fight back. One of these factors is called transforming growth factor beta or (TGF-β). Fortunately, the body has cells that are professionally antigen presenting cells called dendritic cells. Their role is to pick up foreign proteins such as bacterial or viral antigens and present them to T cells. Dendritic cells are also capable of presenting tumor antigens to T cells, but this does not appear to happen in an effect-ive way. To overcome the immune system's apathy toward cancer and apply immunotherapy more widely, one would need the following: 1) to have accessible and effective antigens from every tumor; 2) to improve the presentation of these antigens to T cells in a recognizable form so that T cells can react against them: and 3) to overcome the immunosuppressive effects of TGF-β produced by some tumors.

Our objectives are to further study promising immunotherapeutic approaches by developing an effective way to isolate antigen from tumors, improving tumor antigen presentation to T cells and overcoming potential immunosuppression generated by the tumor. 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 antigens. The conventional way of isolating HSP-antigens is very labor intensive and not practical for future clinical use. Therefore, we are proposing to test a new rapid method for isolating HSP-antigens from tumors. To improve the presentation of HSP-antigen to T cells, we will use dendritic cells obtained from bone marrow of mice. These dendritic cells are grown outside the body, activated and mixed with HSP-antigens. The HSPs are taken up by the activated dendritic cells which are then given as a vaccine to tumor bearing mice to stimulate the immune system to recognize, attack and destroy their growing tumors. In summary, the studies proposed will determine the immuno-stimulatory potential of different tumor derived HSP-antigens isolated by two methods and how the

presentation of HSP-antigens can be improved by dendritic cells. Moreover, we will also study the role of tumor derived TGF-β in suppressing the antitumor response by its action on dendritic cells, and develop treatment strategies to overcome this immunosuppression. The specific aims of the proposed research project therefore are:

1. To develop a practical method for isolating and using immunologically active chaperone protein-associated peptides (HSPs) from tumors.
2. To use dendritic cell vaccines to augment the antitumor effect of chaperone protein-peptide complexes.
3. To improve the efficacy of dendritic cell vaccines by eliminating tumor-derived TGF-β-mediated immunosuppression.

If the proposed experiments in animal tumors are successful, it may lead to future use of these treatments in human tumors.

King, M.D., David K.

Good Samaritan Medical Center
Amount Awarded: $88,503

Clinical Trials Network

The ADCRC has requested proposals for Anticancer Drug Discovery Research Projects directed at all phases of drug discovery. All drugs discovered during this research process will need to undergo human subjects research trials, or clinical trials.

Once the human study design or protocol has been completed, the clinical trial can be implemented. However, successful implementation of a clinical trial requires the following:

An Institutional Review Board that will approve all protocols and consent forms.
A referral source of subjects.
An onsite pharmacy that can distribute study drugs.
A data manager who can determine eligibility criteria and who can obtain informed consent from subjects.
Physicians who will work with the data manager to make sure that the protocol is followed and adverse events documented.
An administrative network to collect and compile the patient data from Healthcare facilities, doctor's offices and other sites.

The Greater Phoenix Community Clinical Oncology Program (GPCCOP) currently has an extensive oncology clinical trial distribution network in place to meet the above requirements. GPCCOP's expertise is in the distribution and data management of oncology research trials. GPCCOP has been an oncology research network for 15 years and has developed a strong rapport and excellent reputation in the Phoenix and Tucson communities.

With a grant from the ADCRC the Greater Phoenix Community Clinical Oncology Program will be able to expand its services to include anticancer drugs developed in the state of Arizona. GPCCOP physicians and patients expect cutting edge research trials from us, and we are dedicated to providing them.

It is our goal to recruit subjects, accrue accurate date, and report findings to the investigator in charge of the study design, assuring protocol compliance, data management and research network communication.

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

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

Rational Design of Thioredoxin Active Anticancer Drugs

The link between smoking and lung cancer has been clearly established. We are studying human thioredoxin, a protein that is synthesized at a rate higher than normal in lung and colon tumors. Addition of thioredoxin to cultured breast cancer cells results in a high rate of growth for these cells and provides greater cell-growth stimulation than some other growth factors. Over-expression of thioredoxin in these cells causes them to be more tumorigenic in mice. Inhibition of thioredoxin in these cells, either through mutation or by the addition of a thioredoxin inhibitor, reduces and in some cases eliminates tumorigenicity. Thus, human thioredoxin is a promising target for anticancer drug therapy. We have begun a three-laboratory project to design specific inhibitors of thioredoxin. Several promising lead compounds have been identified. We propose to rationally apply biological, chemical, and structural techniques to improve upon these lead compounds to the point where they are potent and specific inhibitors of thioredoxin that merit pre-clinical study as anticancer drugs.

Our experimental studies have shown that the protein thioredoxin transforms normal cells into tumor cells, accelerates the growth of tumors, and inhibits apoptosis, or normal cell senescence and death. Thioredoxin is overexpressed by a number of human tumors where it is associated with aggressive tumor growth. The hypothesis upon which this project is based is that thioredoxin is a rational target for anticancer drug discovery.

The goals for this research program are to :

1) rationally design specific inhibitors of thioredoxin;
2) chemically synthesize a large number of potential inhibitors of thioredoxin along the lines of the design model;
3) test these inhibitors for activity and to use the data obtained to better understand the catalytic mechanism of thioredoxin and the role of thioredoxin in normal and tumorigenic cells;
4) to design, prepare, and test through an iterative process, ever more potent and specific inhibitors of thioredoxin; and
5) conduct pre-clinical development of the most active compounds as anticancer drugs.

The specific aims of the project are to :

1) To use the X-ray crystal structure of thioredoxin to model and design specific inhibitors of thioredoxin based on preliminary leads we have developed;
2) use chemical synthesis to produce a large number of analogs of lead compounds which possess a range of structural and chemical properties;
3) test the ability of these analogs to inhibit thioredoxin and to characterize the mechanisms

of action of the most potent compounds;

4) employ a recursive process to develop chemical agents with potent pharmacological activity; and

5) conduct pre-clinical development studies of the most active agents. To achieve the state goals, the specific aims will be addressed by an interdisciplinary research team with expertise in protein crystallography, computer-assisted protein modeling, chemical synthesis, enzyme biochemistry, and cancer biology.

This team has previously worked together and is responsible for much of the current knowledge about the role of thioredoxin in cancer.

McGovern, Ph.D., Kathy A.

University of Arizona
Amount Awarded FY 2000: $244,800

Stress-Inducible Gene Constructs and Stress-Sensitive Liposome Delivery Systems for Targeted Cancer Therapy

There are many therapies for the treatment of cancer. All are plagued by the problem of targeting the therapy to the tumor tissue without damage to normal tissue. In this proposal, we describe methods for treating cancer with gene therapy which deal with the issue of tissue specificity in several ways. In gene therapy, a gene must first be delivered to and taken up by the target cells. It must then express itself by using the machinery of the cell to produce the specific protein which the gene encodes.

In Part I of this proposal, we will design gene constructs in which gene expression can be regulated by physical means. This means that while the gene may be delivered throughout the body, it would only be expressed in the target tissue after treatment of that tissue with energy, e.g., heat. This would limit the gene's expression to the treated area and thus avoid untoward effects as a result of gene expression in other tissues.

In Part II, we will develop novel liposome delivery systems for these constructs. Liposomes are tiny droplets of lipid which surround or encapsulate the material to be delivered. The material carried by these novel lipids will be delivered throughout the body; however, these liposomes are designed such that their contents will only be released at the target tissue after treatment with energy. *e.g.*, light. This could provide a second level of spatial control, and may prove useful in delivering conventional cytotoxic agents with decreased toxicity to normal tissues as well as the novel gene constructs developed in Part I.

In Part III, these novel gene constructs and lipid delivery systems will be characterized both in cells and in animal models, and compared to conventional therapies. The gene delivered in these studies will express Interleukin-2 (IL-2), a small protein which stimulates the immune system to reject the tumor cells. Sufficiently stimulated, this immune response could result in therapeutic benefits at distant metastatic sites as well as at the target site.

The overall goal of this project is to improve the specificity of cancer therapy by targeting therapy to tumor cells using several different approaches. The project is divided into three parts distributed amongst five investigators.

Part 1: The goal is to design and optimize a novel gene construct which permits conditional expression of the target gene by a specific stress such as heat, *i.e.*, the specific application of heat will induce expression.

Part 2: The goal is to design and optimize a novel lipid delivery system suitable for intravenous delivery of a gene construct and sensitive to a specific stress such as light, *i.e.*, exposure to light at a specific wavelength will result in release of the liposomal contents.

Part 3: The goal is to evaluate the efficacy of the systems developed in Parts 1 and 2 in a series of tumor cell lines as well as in animal models. These systems will be used to deliver the therapeutic gene for IL-2 and the results compared to conventional therapies. Given promising results, selected systems will be carefully characterized and protocols optimized in order to lay the foundation for clinical studies.

Pettit, Ph.D., George R.

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

Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs

In the coming year, some 600,000 people in the United States will die from devastating attacks by one or more types of human cancer. That most tragic death toll in the United States, and on a much larger scale internationally, will not be reduced until more generally effective and curative anticancer drugs are discovered and developed. Although a small number of anticancer drugs are now available that have greatly improved cancer treatment and provided various levels of curative treatments for about 20 types of human cancer, twelve of the major types of human cancer have continued to remain generally refractory to current anticancer drugs and urgently require discovery and development of generally curative anticancer drug treatments. Illustrative are diagnosis and death rates statistics available last year: lung cancer, 177,000 new cases diagnosed and 158,700 deaths; colorectal cancer, 133,500 diagnosed and 54,900 deaths; breast cancer, 185,700 new cases diagnosed and 44,560 deaths; prostate cancer 317,100 new cases diagnosed and 41,400 deaths; pancreatic cancer, 26,300 new cases diagnosed and 27,800 deaths; non-Hodgkin's lymphoma, 52,700 diagnosed and 23,300 deaths; leukemia, 27,600 diagnosed, 21,000 deaths; and ovarian cancer, 26,700 diagnosed and 14,800 deaths expected. With continuation of these tragic death rates for the overall 200+ types of human cancer it is abundantly clear that a great acceleration in the discovery and development of new anticancer drugs is vitally important and urgently needed.

In May 1998 Governor Hull signed legislation directing the Arizona Disease Control Research Commission to accelerate the discovery and development in Arizona of new anticancer drugs to clinical trials. That forms the overall objective and sharp focus of this research proposal. Specifically, ten new anticancer drugs discovered in the ASU Cancer Research Institute and now at various levels of development have been selected for accelerated preclinical research leading to clinical trials. As these very promising anticancer drug candidates are moved toward the clinic, resources (as available) will also be devoted to the scale-up, procurement, and processing of very promising plants, marine organisms, and microorganisms that have given evidence of potentially important anticancer constituents. The availability of these leads in larger quantities will greatly speed up actual isolation of the new anticancer drugs. In turn, that will ensure a steady stream of new anticancer drug projects moving toward eventual clinical trials. The ten anticancer drug candidates selected for vigorous research directed at making them available as quickly as possible for clinical trials comprise structurally new, powerful and very potent anticancer substances that we have isolated primarily from terrestrial plants and marine animals. They are bryostatin 5, DABIS maleate, phyllanthoside/phyllanthostatin 1, pancratistatin, dolastatin 15, auristatin 15-PE, auristatin PYE, auristatin E, phenstatin prodrug, and cephalostatin 1. The rapid introduction of these new anticancer drugs into human cancer clinical trials should lead to a series of very important advances in improving human cancer treatment.

Polt, Ph.D., Robin

University of Arizona
Amount Awarded FY 2000: $49,578

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

Tobacco-induced cancer is a major health threat to the people in Arizona, especially so due to the relatively large percentage of smokers in the state, greater than 20 percent according to the American Lung Association. While advances in early cancer detection and in treatments such as chemotherapy, radiation therapy, and surgery have improved the survival rate of many victims, the spread of cancer, metastasis or malignancy, still represents a major threat to survival. Recent discoveries linking increased metastasis rates to the presence of particular carbohydrates displayed on the surface of cancer cells led this research group to the hypothesis that if one could block the expression of these metastasis-linked carbohydrates, then one could block the spread of cancer throughout the body.

An experimental drug, PDMP, has now been shown to block metastasis by inhibiting the display of carbohydrates on tumor cell surfaces. Further work with PDMP and related drugs to prevent the spread of cancer now seems justified. Rapidly growing insect nerve cells can serve as a model for cancer in humans, and simple assays based on this approach should permit the testing of a large number of new drugs based on PDMP. A new chemical approach, which simultaneously produces an array of new PDMP-based drugs, will permit our research group to produce the required number of drugs and rapidly test them against the growing insect nerve cells. Drugs that block the outgrowth of the insect nerve cells should also block the outgrowth of cancer cells in humans or other vertebrate organisms. Using this approach, we should be able to rapidly develop potent new drugs which will block metastasis in human cancers. In other words, these drugs will convert malignant cancers into benign cancers that can be removed surgically, or treated without fear of them spreading to other parts of the body. This represents a fundamentally new approach to the treatment of cancer and could save thousands of lives and billions of treatment dollars in the state of Arizona.

1. We will optimize the parallel chemical synthesis of PDMP analog on solid-phase polymer supports. This chemical methodology will allow us to produce up to 96 different PDMP analog in a single chemical operation.
2. We will develop a biological assay based on insect nerve cells for the parallel screening of the PDMP analog. This biological methodology will allow us to screen up to 96 different PDMP analog in a single biological operation.
3. Use of methods 1 and 2 above will permit us to rapidly develop potent new drugs that are capable of inhibiting the growth and/or adhesion of insect nerve cells during metamorphosis, a process which mimics the metastatic process of cancer in higher organisms in several key aspects. The best compounds discovered will be tested further against actual cancer in future studies.

Powis, D. Phil, Garth

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

Arizona Cancer Center Novel Anticancer Drug Discovery & Development

Available contemporary therapies have failed to cure the most common human cancers. New types of agents to prevent and treat cancer are urgently needed. The Arizona Cancer Center is nationally and internationally known for its anticancer drug development efforts; the present proposal is a multidisciplinary program project that continues the Center's commitment to new anticancer drug discovery and is aimed at all phases of cancer drug development including new anticancer drug discovery, preclinical development, and clinical trials. To discover potential new drugs we will use state of the art automated mechanism-based anticancer drug screens. We will also use the power of functional genomics using arrays of up to thousands of human genes on a chip the size of a postage stamp, a technology which is revolutionizing biomedical research. As a source of new compounds, we are exploiting nordihydroguaiaretic acid (NDGA) which is a product of the creosote bush (*Larrea tridentata*) which is widely distributed throughout the desert regions of southern Arizona and has been previously used by American Indians and in folk medicine to treat cancer. We have shown that NDGA has promising antitumor and chemopreventive activity against skin cancer. We will also study up to 2,000 other plant derived widely diverse natural products as potential new anticancer drugs. We are proposing a series of core services to carry out the development of the anticancer agents we discover. The services of these core functions will provide the infrastructure for current and future drug discovery efforts and will be available to cancer researchers throughout the state. We are also proposing two Phase I clinical trials of anticancer drugs, one for therapy and one for prevention. Thus, the program project is an integrated and comprehensive drug discovery effort that exploits the natural and intellectual resources in the state of Arizona to provide lasting benefits to its citizens through the development of new treatments for the prevention and treatment of cancer. It will also provide an infrastructure that can be used by other cancer investigators in the state in their cancer drug discovery and development efforts.

The goal of this Category III multidisciplinary program project "Novel Anticancer Drug Discovery & Development" is to discover and develop novel agents for the prevention and treatment of cancer and to move the resulting agents into clinical trials. The hypothesis to be tested is that mechanism-based drug screening employing defined molecular targets relevant to cancer development and progression is a rational mechanism for the discovery of novel agents for preventing and treating human cancer. The objectives of the program project are: 1) to synthesize a series of novel analogues of nordihydroguaiaretic acid, a product of the creosote bush or chaparral found in Arizona desert regions, and to acquire other pure novel natural products; 2) to screen these compounds as inhibitors of relevant cancer-related proteins that regulate the growth and development of cancer cells, either as direct inhibitors or as inhibitors of gene transcription using the new technique of DNA microarray that allows many hundreds or even

thousands of genes to be studied at a single time; 3) to conduct preclinical development studies including tumor cell and animal antitumor and prevention testing, pharmacology and toxicology on the most active compounds; and 4) to conduct Phase I clinical trials of one new chemotherapy drug and one chemopreventive drug. It is anticipated that 1000 to 2000 compounds will be acquired or synthesized in 1) and tested in 2), and that 3) will lead to at least two anticancer agents ready for clinical testing during the two-year funding period.

Skibo, Ph.D., Edward B.

Arizona State University
Amount Awarded FY 2000: $155,900

Preclinical Development of the PBI's

The ideal cancer drug would be a magic bullet able to kill only cancer cells. For example, a drug which 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. Many cancer cells possess high concentrations of this enzyme, which is known to activate cancer drugs by reduction. However, we believe the enzyme can vary by cancer cell type and that a suitably-substituted drug could be activated only in certain cancer cells. The pyrrolo[1,2-α]benzimidazoles(PBIs) developed in this laboratory are activated by DT-diaphorase with cell kill resulting from the reaction of the activated drug with DNA. Our approach for selecting cancer cell types has been to utilize the drug's stereochemistry (i.e. right versus left handed drug) and its substituents. Furthermore, the activated drug is designed to react with DNA efficiently so as to maximize cell kill.

Since their discovery in the early 1990's, the PBI's have been undergoing analogue development and screening against the National Cancer Institute's 60-cell line cancer panel and in animal tumor models. Finally, in the Summer of 1998 the National Cancer Institute informed this laboratory that one of our compounds was the second most active among the hundreds of natural products and synthetic compounds screened in their assays. A short time later, we were able to develop a new analogue a hundred-fold more active!

This new series of compounds has a good possibility of seeing use in the clinic, and we would like to develop these compounds further. We will develop the synthetic methodology further, carry out additional animal studies, and design more active analogues using our structure-activity relationship.

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 DNA cleavage. The enzyme Dt-diaphorase is found in many types of cancer cells and is a means of achieving selective cancer cell toxicity. Furthermore, the cancer cell Dt-diaphorase may be heterogeneous permitting differential cytotoxicity by an appropriately substituted drug. By modifying the 3-substituent of the PBI and utilizing the r(+) and S(-) enantiomeric forms, highly selective cancer drugs with outstanding cytotoxicity have been designed. Some of the PBI's from this laboratory are among the most active screened at the National Cancer Institute. The research project described herein will exploit these discoveries and prepare the PBIs for

clinical trials.

The specific aims of the proposed project are as follows: 1) The efficient bulk synthesis of highly active PBI antitumor agents as a prelude to comprehensive *in vivo* studies; 2) Stability and other chemical studies of active PBI antitumor agents; 3) *In vivo* and toxicological studies of the PBIs; and 4) The design of new, and perhaps more active, PBIs, employing the structure activity relationship developed in this laboratory. Achieving these specific aims will prepare these compounds for clinical trials by the end of the two-year project period as well as test the PBI structure activity relationship.

Whitesell, M.D., Luke

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

Development of Antisense Oligonucleotides as Chemotherapeutic Agents for Intratumoral Administration

Human cancers result from the accumulation of specific alterations in genes called mutations which occur within a particular organ of the body such as the breast, nervous system or bowel. Many mutations result in abnormal activity for genes involved in regulating cell growth and survival. One particular gene product implicated in the malignant behavior of many human cancers that are refractory to current conventional chemotherapy treatments is the type 1 insulin-like growth factor receptor (IGF-1R). Extensive laboratory research has demonstrated that blocking the function of this gene product has profound inhibitory effects on cancer cells isolated from patients and grown in dishes. More recently, we and others have used genetic methods to demonstrate that decreasing the level of this receptor in tumors growing in rodents not only inhibits tumor growth but also causes the animals to mount a potent immune response against the cancer leading to its complete regression. Unfortunately, no clinically useful drugs currently exit which selectively target IGF-IR. Because the entire DNA sequence of the IGF-1R is known, however, a potentially useful approach to disrupting the function of the receptor is to synthesize an artificial piece of DNA which is exactly opposite or antisense to a small portion of the gene's sequence and, as a result, in a very selective manner, interferes with its ability to direct the production of the IGF-1R. Such small pieces of DNA or oligonucleotides (oligo) have already been shown to inhibit the growth of cancer cells growing in dishes, but for a variety of reasons, none have yet been developed which cure actual tumors growing in laboratory animals such as mice. In an effort to bridge the gap between these exciting biologic insights and clinical medicine, we are proposing a collaborative effort between investigators with expertise in cell biology, tumor immunology, pharmacology and clinical oncology to develop a synthetic antisense oligonucleotide which when administered into tumors will decrease IGF-1R levels, inhibit tumor growth and induce a host immune response leading to eradication of the tumor.

The goal of the proposed research is to identify and develop antisense oligo with sufficient activity in pre-clinical animal models of human cancer to justify evaluation in human patients. *In theory,* oligo possess the specificity desired for a new anticancer therapy which could complement the non-specific actions of the conventional cytotoxic drugs currently in use. *In practice,* however, it has been very difficult to develop an oligo as useful anticancer drugs because they are quite specific, and the appropriate genes to target for effective anticancer activity have not been clear. In addition, oligo have a number of intrinsically undesirable properties as drugs designed to treat the whole body of a patient, *e.g.* they are unstable in biologic fluids such as blood and they are not taken up well by cells. The research we propose seeks to overcome these problems by a) using an appropriate, well-defined target (IGF-1R) for antisense oligo therapy and b) using a new system for administering the oligo directly into the tumor. Previous work by us

and others has shown that localized administration of anti-IGF-1R reagents into a tumor can exert both a direct antitumor effect and induce a whole animal response. These findings suggest that by direct administration of the oligo into the tumor we can target the IGF-1R and avoid the need for systemic administration to the whole animal. To test the **hypothesis** that direct administration of an oligo into tumors growing in mice can induce a sufficiently potent host immune response to eradicate the tumors, we propose a systematic plan designed to achieve the following **objectives**: 1) To screen a library of 100 distinct oligo directed at several different areas of the IGF-1R gene in order to identify the sequences most effective in reducing receptor levels in cancer cells; 2) To synthesize novel, modified versions of the most active sequences using a method we have previously shown increases oligo stability and cellular uptake and confirm the activity of these modified versions against cancer cells; 3) To label the modified oligo with a fluorescent marker so that we can visualize its distribution and retention within a tumor following administration via a needle or a new high pressure compressed gas delivery system; and 4) To administer modified oligo into tumors, measure the induction of an immune response in the mice and quantitate effects on tumor growth. The proposed research will provide important new information on how to use oligo, a whole new class of anticancer agents, in the most effective manner. If our underlying hypothesis proves true, it will also identify specific new compounds for clinical trial in cancer patients.

Principal Investigators, Continuing Contracts