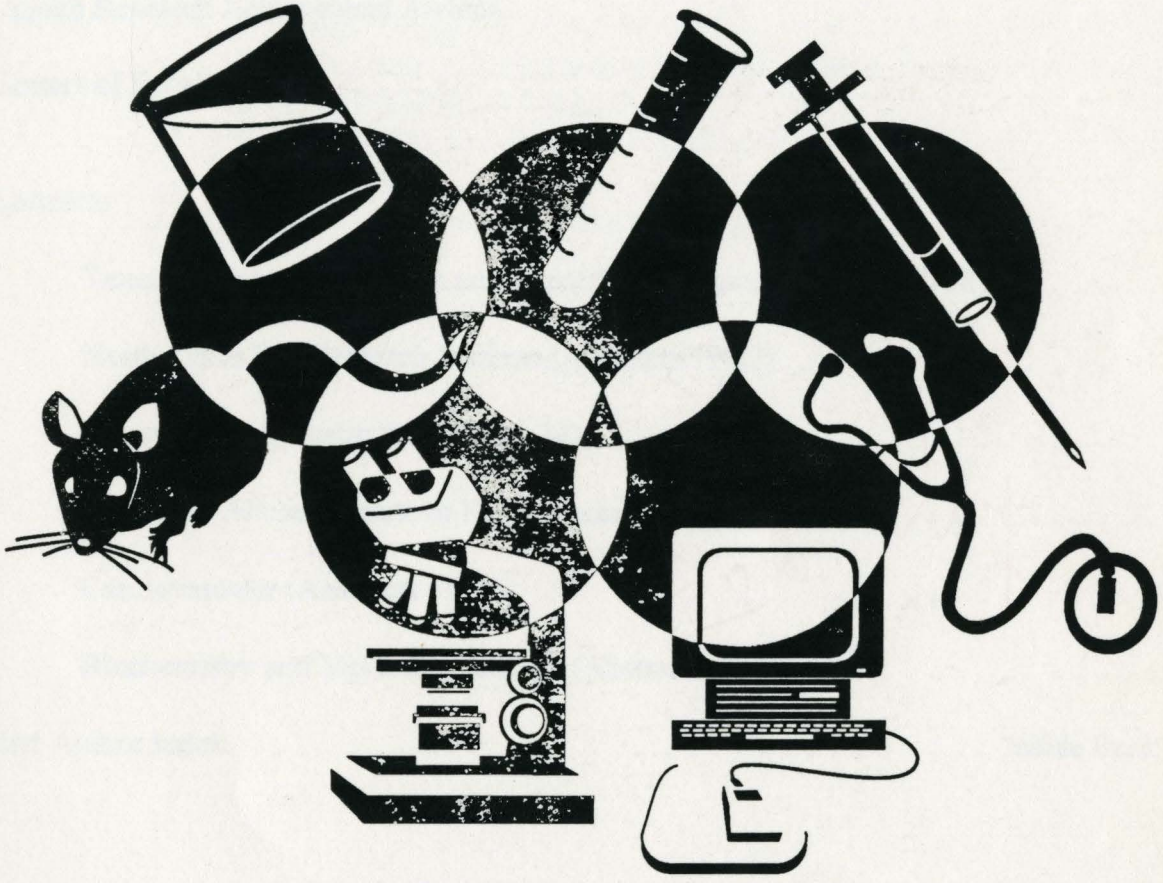
University of North Texas
Health Science Center at Fort Worth

Second Annual

Research Appreciation Day

March 23, 1994



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Graduate School of Biomedical Sciences
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University of North Texas Health Science Center at Fort Worth

Research Appreciation Day

March 23, 1994

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AGENDA

8:00 - 9:00	Assemble posters . . . . . Interdisciplinary Lab
10:30 - 12:30	Judging (Student first authors to remain next to poster)
12:00	Lunch provided by SmithKline Beecham. . . . . Kiva Lounge
	Introduction of Keynote Speaker . . . . . Robert Gracy, Ph.D. Associate Dean for Basic Science and Research
	Keynote Address . . . . . .Kiva "Prospects of Gene Therapy on Cardiovascular Disease" Robert Sanders Williams, M.D. UT Southwestern Medical Center at Dallas
1:30 - 3:00	All non-student first authors to remain next to posters
4:00	Award Ceremony . . . . . .Kiva

Robert Sanders Williams, M.D.

University of Texas Southwestern Medical Center at Dallas

Robert Sanders Williams performed his undergraduate work at Princeton University, graduating *cum laude* and received an M.D. from Duke University. An internship and residency was completed at Massachusettes General Hospital. He received a three-year cardiology fellowship at Duke University during which he completed post-doctoral research training in Biochemistry.

Currently, Dr. Williams is a Professor of Internal Medicine and Chief of Cardiology at the University of Texas Southwestern Medical Center in Dallas where he also holds an appointment as a Professor in Biochemistry and the J.T. Willerson Distinguished Chair in Cardiovascular Medicine. Dr. Williams holds memberships in numerous clinical and basic science organizations and serves on several editorial boards including the *American Journal of Physiology*, *Circulation* and the *Journal of Clinical Investigation.*

Internationally renowned for his pioneering work in molecular cardiology, Dr. Williams is currently funded as a principle investigator of several NIH grants and post-doctoral training grants as well as the State of Texas Advanced Technology Program and a Leland Fikes Foundation award for a program in molecular cardiology. A long time proponent of the use of molecular techniques in the understanding of cardiac disease, Dr. Williams has focused his work on the regulation of mitochondrial biogenesis using gene transfer and developmental biology techniques. He has two patents pending concerning particle-mediated transformation of animal tissue and RNA-mediated transfer of genetic material to mitochondria. He is the author of more than one hundred peer-reviewed publications.

Upjohn Research Achievement Awards

Upjohn is an international pharmaceutical company that has taken an active role in supporting medical and health education. The company provides funding for programs in education, health social welfare, arts and culture, civic development, environmental preservation, public policy research and the sciences.

The Upjohn Research Achievement Awards are given to the top three student poster presentations as determined by a panel of judges. The 1994 Research Appreciation Day judges are Abbot Clark, Ph.D., Alcon Laboratories, Inc.; Loy Frazier, Ph.D., Baylor College of Dentistry; and Gerard O'Donovan, Ph.D, University of North Texas.

Abe Clark is the Associate Director of Glaucoma Research at Alcon Laboratories, Inc., in Fort Worth. His research includes the development of drugs to treat and prevent the manifestations of glaucoma.

Loy Frazier is Professor of Physiology at the Baylor College of Dentistry in Dallas. His research characterizes the mechanisms involved in the regulation of H^+ and NH_4^+ secretion in renal epithelia, with an emphasis on second messenger interactions.

Gerry O'Donovan is the Chair of the Department of Biological Sciences at the University of North Texas in Denton. His research investigates the biochemistry and molecular biology of pyrimadine metabolism in microorganisms.

Special Centers of Excellence

Texas Institute for Research and Education on Aging

Contributing to the better health of the nation's aging society through innovative and interdisciplinary clinical care, teaching and research has long been a priority of the UNT Health Science Center and its sister institution in Denton, the University of North Texas. The Texas Institute for Research and Education on Aging is one of the most recent collaborations dedicated to this mission.

Established in 1992 and unique to this region of Texas, the institute now involves more than 40 faculty members from a variety of disciplines at UNT Health Science Center and UNT. Students, postdoctoral fellows, residents, visiting scientists and health and social service practioners from both institutions also participate.

The institute focuses on four primary areas: the biology of aging, geriatric care and practice, the development of a long-term care system and health promotion for older adults. Activities have already gained national recognition, attracting the attention of leading researchers from the National Institutes of Health and respected scholars from other major universities.

The institute sponsors pilot research grants as well as a number of international conferences and seminars. An external review program brings scientistis, physicians and administrators to both campuses to update faculty members on new opportunities and to review ongoing and proposed programs. The institute also publishes a newsletter and maintains a registry of volunteers available for aging research.

Institute for Forensic Medicine

The Institute for Forensic Medicine, established in the early 1980s, is an academic and research partnership between UNT Health Science Center's Department of Pathology and DNA/Identity Laboratory, the University of North Texas and the Tarrant County Medical Examiner's Office.

The institute's goals are to increase the quantity and scope of research projects in forensic medicine as well as the number of graduate students studying toxicology, molecular biology and criminalistics. The collaborative strength and variety of the institute's teaching and research activities provide students a comprehensive training arena and building the health science center's forensic medicine research funding.

Substance Abuse Institute of North Texas

The Substance Abust Institute of North Texas, established in 1993 by the UNT Health Science Center's Department of Pharmacology and the Department of Psychiatry and Human Behavior, is a consortium of professionals with expertise in substance abuse. Scientists and physicians in physiology, pathology, public health/preventive medicine, general and family practice and medicine also participate.

The institute's missions are to foster clinical and basic science research, train professionals whose efforts focus on the prevention and treatment of substance abuse and serve as an information resource for area substance abuse treatment programs. The institute hosts research conferences and cosponsors seminars with area substance abuse prevention groups and the pharmaceutical industry. The industry also regularly sponsors visits by international scholars to the health science center and the Fort Worth/Dallas Metroplex and provides faculty consultants to state, federal and international agencies. Outreach programs involving many institutions from the North Texas area are being developed.

North Texas Eye Research Institute

The North Texas Eye Research Institute, formed in 1992, includes faculty experts in anatomy and cell biology, biochemistry and molecular biology, pharmacology and medicine.

Examples of institute initiatives include a Distinguished Scientist Seminar Program cosponsored with Alcon Laboratories, a course in ocular pharmacology and research collaborations with hospitals and industries concerned with vision problems. Areas of research include aging, cataracts and diabetic complications; glaucoma and glaucoma medications; courses and therapies of retinal dystrophies; and the normal and abnormal relationships between photoreceptor cells, retinal pigment epithelium and retinal gila and retinal neovascularization problems.

Wound Care Institute

The Wound Care Institute seeks to improve and enhance the quality of health, well-being and productivity of all people through research, education and service activities.

Its five-fold mission includes: expanding knowledge of the process of injury and wound healing using novel *in vitro* models and molecular biology techniques; testing innovative applications of hyperbaric medicine, growth factor therapy and cell replacement therapy on problem wounds as alternatives to amputation and permanent disbility; training graduate and medical students, interns and residents in new and interdisciplinary approaches to problem wounds; disseminating knowledge and experience through courses, seminars, conferences and

symposia as a part of continuing medical education; and evaluating new pharmaceuticals and devices through all phases of the FDA approval process.

Support for the institute's various projects comes from federal, state and private agencies and organizations. Basic science departments of general and family practice, medicine, pathology, surgery and hyperbaric medicine are some of the participants in the institute.

Texas Institute for Research and Education on Aging

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11.	S. Dan Dimitrijevich, Ph.D.	Localization of Fibroblast Growth Factor in Infant, Adult and Aging Skin
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13.	Zhong Chen, Ph.D.	Protein Kinase C Activity Changes in Cultured Blood-Brain Barrier Cells in Aging Rats
14.	Ming-chi Wu, Ph.D.	Alteration of Colony-Stimulating Factor Production in Aging
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18.	Edward L. Orr, Ph.D.	CSF Volume and Histamine Concentration in Rats with EAE

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Jan Weaver
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Signature: Jan Weaver

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ADULT DAY CARE PROGRAMS IN DALLAS, DENTON, AND TARRANT COUNTIES. Jan Weaver, MA, University of North Texas Health Science Center.

Medical and long-term care costs are financially devastating for today's aging population. The burden on state and federal funding is also significant. Community-based care not only reduces these costs, but also improves the quality of life for program participants by allowing them to remain in their homes while receiving needed health and social services. Adult day care is being recognized as an integral component of community care and, despite the lack of adequate funding, the number of centers is increasing.

This project studied the availability and utilization of adult day care centers in Dallas, Denton, and Tarrant Counties of Texas. The study explored various structural and operational dimensions of adult day care including types of services offered, staffing, and utilization of the program. The affects of these dimensions on the facility characteristics of adult day care centers in the three counties along with the relationship of licensing and certification requirements was investigated. 216 facilities were contacted.

Of the 23 adult day care programs identified by the study, 7 were free-standing, 11 were located in nursing homes, 3 were affiliated with retirement or foster care facilities, and 2 were affiliated with hospitals. All but two of the programs are located in urban areas. Seventeen of the 23 programs are for-profit (four of these are privately owned) and six are non-profit corporations. Since licensure for adult day care is not mandatory in Texas, only six of the facilities are licensed. Four of the six licensed facilities are non-profit and free-standing.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

IMPROVING FUNCTIONAL ABILITY IN THE ELDERLY BY OSTEOPATHIC MANIPULATIVE TREATMENT

Knebl, J.A., Gamber, R.G., Gibson, K., Moss, G., and Shores, J.H. University of North Texas Health Science Center at Fort Worth, Department of Medicine, Fort Worth, Texas 76107.

The loss of functional ability of the shoulder joint due to tendonitis/bursitis/osteoarthritis/ trauma or stroke is common occurrence in the geriatric population. Treatment modalities have consisted of a combination of rest, physical therapy, medication, and perhaps surgery. The treatment of this chronic somatic dysfunction by osteopathic manipulative treatment such as the Spencer technique has not, however, received much attention. Twenty-nine elderly volunteers (≥62 years of age) were recruited who had chronic shoulder pain, decreased range of motion or decreased functional abilities of one shoulder. Subjects were randomized to receive 5 treatments of the Spencer technique or a "sham" Spencer technique (placebo) to the affected shoulder with assessment of pain, range of motion and functional abilities each week after treatment by a blinded assessor. There was an effect overall on both treatment and placebo group on pain perception and range of motion. Passive shoulder abduction and sitting flexion range of motion improved significantly in the treatment group ($P<.03$, $<.04$, respectively). Chronic shoulder dysfunction in the elderly can be improved with osteopathic manipulative treatment. (American Osteopathic Association, 91-11-343)

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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DEVELOPMENT OF CURRICULUM TO TRAIN COMMUNITY HEALTH WORKERS TO SERVE MINORITY ELDERS

J. Weaver, MA, University of North Texas Health Science Center; Anna Madison, PhD and Stan Ingman PhD, University of North Texas.

The elderly population of the Washington Senior Center neighborhood in Corpus Christi, Texas has been identified as at risk for poor health and institutionalization due to low income, minority status, the prevalence of multiple severe chronic diseases, lack of knowledge about available health services, lack of transportation to services outside the community, and social isolation due to loss of spouse or friends. In response to these problems, a program was developed and implemented by the Texas Institute for Research and Education on Aging in Spring and Summer 1993 to train well elders living in the community to provide outreach for their peers.

Goals of the program were to (1) increase knowledge on health and nutrition; (2) provide information regarding local health services; (3) increase participation in the Washington Senior Center's health clinic and recreational programs; and (4) provide information to the clinic's nurse practitioners on the needs of the home-bound residents in the community. Specific features of the program conform to recommended guidelines of similar programs including reliance on home visiting and other in-vivo intervention methods, a focus on the highest priority service recipients, and an interdisciplinary team approach.

The 20-hour curriculum was developed specifically for the project and includes content on the role of community health workers and the process of program implementation. A unique feature of the training involves team projects in which local resources are identified and accessibility is determined. Volunteers are taught to involve program participants and their family members on every level of the planning process.

Although the curriculum for the program was developed by the Texas Institute for Research and Education on Aging at the University of North Texas Health Science Center, local participation in the project was recognized as an important component of its ongoing success. Training was conducted at St. Matthew Baptist Church in Corpus Christi by a qualified instructor who is actively involved in community development.

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

IN-HOME HEALTH SCREENING AND NEEDS ASSESSMENT IN HOMEBOUND LOW-INCOME BLACK ELDERLY-SECOND YEAR EXPERIENCE T. Ware, J. DeLoach, J. Knebl, DO*, J. Weaver, MA, UNT Health Science Center, 3500 Camp Bowie, Fort Worth, TX 76107.

A previous study in Tarrant County revealed unmet medical needs in 60% of the forty subjects evaluated who were African-American elderly, low-income and homebound. This followup study included 80 new evaluations by medical students who received the referrals from three Area Agency on Aging contract providers: Meals on Wheels, Errand and Assurance, and ACCESS programs. An additional 20 subjects from the prior year study were also re-evaluated and included. Over 40% were over the age of 80; almost 75% were women, and 50% lived alone. Almost all had a primary care physician they felt they could get to. However, a third felt that their medical needs were not being met. Over half had difficulties with IADL's, but maintained their basic ADL abilities. Preventive health services were lacking with over 2/3rds not receiving flu shots, mammograms, proctosigmoidoscopy, Pap smears, pneumonia vaccine, tetanus shots or dental care. Medical followup was required in over 60% of subjects and a home health care referral was recommended in half of the subjects. Unmet social needs were identified in most subjects to include housekeeping and personal care services. A medical case management approach may improve health and preventive care for low-income, homebound minority elders.

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

IN-HOME HEALTH SCREENING AND NEEDS ASSESSMENT IN HOMEBOUND LOW-INCOME BLACK ELDERLY

Ferree, S., Knebl, J.A., and Weaver, J.
University of North Texas Health Science Center at Fort Worth, Department of Medicine, Fort Worth, Texas 76107.

There are currently over 2.5 million black Americans aged 65 years and over living in the United States, but little is known about those that are homebound with low socioeconomic status. A case-finding approach was utilized to identify individuals believed by three Area Agency on Aging providers (i.e., Meals on Wheels, Errand and Assurance, Access programs) to have unmet medical needs. The project involved an in-home health screen performed by a medical student with follow-up medical care coordination and management. Forty homebount low-income black elders were identified and assessed. More than 50% were over the age of 80 years and over half lived alone. This population had chronic health conditions resembling data from national surveys, but most felt that their medical needs were not being met. Assessment of functional status revealed primary difficulties and IADL'S and ADL's. Most were at nutritional risk. None of the individuals had received any dental care or services. Based on the health screen, it was determined that over 60% of subjects needed medical follow-up. A medical case management approach was utilized with 50% of the subjects requiring home care referrals, 55% needing home health referrals, 10% requiring adult protective services, and 7.5% requiring hospitalization. The in-home health screen revealed unmet medical and functional needs. (Administration on Aging)

University of North Texas Health Science Center
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ABSTRACT FORM

First Author: Dr NY Zachariah PhD
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

PROSTATE SPECIFIC ANTIGEN (PSA) AS A MARKER FOR CARCINOMA OF THE PROSTATE - CASE STUDIES, N.Y. Zachariah, J. Thomas O'Shea (Osteopathic Medical Center of Texas, Dept. of Pathology, Fort Worth, Texas 76107) Robert Hagan Jr., John Pung (University of North Texas Health Science Center at Fort Worth)

Serum PSA levels were found to be extremely useful to diagnose and manage patients with adenocarcinoma of the prostate. PSA was determined by RIA in 505 patients, ages ranging from 40-90 yrs. Of these, 60.0% were Caucasian, 8.6% African American, 10.0% Hispanic, and the rest of unknown origin. In normal patient population, PSA was detectable up to 4 ng/ml. In attempting to establish the risk in different ethnic origins, it was found that 19.00% of Caucasians, 66.7% African American, and 28.6% Hispanics had PSA levels of >4 ng/ml. Following is the table with distribution of PSA with age, indicating an increased risk (PSA>4ng/ml) with age.

NO. & AGE	% OF TOTAL	<4 NG/ML(%)	>4 NG/ML(%)	CONCEN.NG/ML
(61) 40	12.2	95.1	4.9	0.2-4.7
(90) 50	18.0	84.4	15.6	0.2-280.4
(145) 60	28.0	80.0	20.0	0.1-458
(144) 70	28.8	65.9	34.1	ND-116.5
(56) 80	11.2	50.0	50.0	0.2-215.2
(9) 90	1.8	22.2	77.8	5.3-294

Two year follow-up of 11 patients is as follows. Seven underwent radical prostatectomy with their PSA levels reaching <4 ng/ml within 30 days. Four patients had combined prostatectomy/chemotherapy/steroid therapy.

These patients had no specific pattern in the decline of PSA levels to reach <4 ng/ml, even though the half life of PSA is established as 2.2 days. They all have normal PSA at this time.

A 56 year old patient with adenocarcinoma of the prostate had a PSA of 76 ng/ml. Treatment included a combined radiation LHRH,
and antiandrogen (Flutamide) therapy. PSA came down to 13.5 in 2 months, and further down to 2.4 ng/ml within the next 30 days. Radiation therapy was reported to take several months to a year for PSA to reach normal levels. This case report suggests a faster decline in PSA levels when treated with a combined radiation and antiandrogen therapy. A two year follow-up of the patient indicates a normal PSA of 0.2 ng/ml.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

PLASMA LIPIDS AND CHOLESTEROL ESTERIFICATION IN ALZHEIMER'S DISEASE.

Janice Knebl, Michael Clearfield, Walter J. McConathy, Ruth McPherson and Andras G. Lacko, University of North Texas Health Science Center, Fort Worth TX, USA.

Eight patients and eight age matched controls were recruited to study lipoprotein metabolism in Alzheimer's disease. Plasma lipids, lecithin: cholesterol acyltransferase (LCAT) assays and cholesteryl ester transfer protein (CETP) levels were determined. HDL cholesterol was higher (~20%) while plasma triglycerides were lower in Alzheimer's patients. The fractional rate of plasma cholesterol esterification was lower in Alzheimer's patients (~16%) compared to the controls. However, none of these differences were statistically significant.

Correlational analyses of endogenous LCAT activity and plasma lipids revealed marked and significant differences between the Alzheimer's patients and control subjects. These differences were particularly striking between the two groups when the correlations of the endogenous LCAT activity vs total cholesterol were compared (r= -0.28 for Alzheimer's subjects; r= 0.71) for controls. These findings are nearly identical to those obtained earlier with Down's syndrome patients (Lacko, A. G. et al. *Clin Chim. Acta* **132,** 133 [1983]). The data obtained during this study suggest that reverse cholesterol transport in general and LCAT or CETP activity in particular may be altered in Alzheimer's disease.

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ABSTRACT FORM

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IDENTIFICATION OF COGNITIVE AND MOTOR DEFICITS AMONG OLDER DRIVERS

Noreen L. Goggin and M. Jean Keller, University of North Texas, Denton, TX, 76203.

Older drivers constitute the most rapidly growing segment of the driving population and are involved in a high percentage of non-fatal automobile accidents (Waller, 1991). However, much of the research literature on driving performance of older adults is limited to survey and/or questionnaire information (Cutler & Coward, 1992). Older adults were examined during realistic driving situations, in this study. The study was designed to identify nervous system (cognitive vs. motor) problems during simulated driving performance and to determine if any gender differences exist in overall performance. Twenty-four older adults (11 males and 13 females) ranging in ages from 65-83 years (M=72.9, SD=5.4 years) volunteered to participate in this study. In general, subjects self-reported their health (20/24) and corrected vision (13/24) to be very good. Overall, study participants were well educated (10 had college degrees and 4 attended graduate school). The study was conducted in two phases. In phase one, older drivers responded to a demographic questionnaire, familiarized themselves with the driving simulator, and completed a paper/pencil test of 15 driving situations presented on videotape. The purpose of phase one was to assess the subject's cognitive or decision making capabilities. Phase two was conducted within a week of the first phase and examined the older drivers' motor responses during simulated driving. The same 15 driving situations were presented, but during the second phases, respondents executed their responses in driver simulation units. The actions performed by older drivers were recorded by a computer connected to each driver simulation unit. The results indicate that males and females are quite different in overall driving ability. Although no differences were found between males and females in age or years driving, men tend to drive more miles per year, and predominantly drive on highways. In phase one, no differences were found between males and females in cognitive abilities. However, in phase two, males performed significantly better in the driving simulation unit than females. Results of this study seem to indicate difficulties experienced by older drivers may be attributed to cognitive or decision making abilities, while individual motor system seems to be flexible and able to adapt (i.e., subjects performed significantly better in the driving simulation). Regression analysis indicated that highway driving and miles driven were the best predictors of driving simulation ability. Our results corroborate much of the psychomotor literature on aging that suggest that older adults have difficulty in response selection or decision-making processes during situations where quickness of response is required (Stelmach & Nahom, 1992).

References

Cutler, S.J. & Coward, R.T. (1992). Availability of personal transportation in households of elders: Age, gender, and residence differences. The Gerontologist, 32, 77-81.

Stelmach, G.E., & Nahom, A. (1992). Cognitive-motor abilities of the elderly driver. Human Factors, 34, 53-65.

Waller, P. (1991). The older driver. Human Factors, 33, 499-505.

Supported by the Texas Institute for Research and Education on Aging.

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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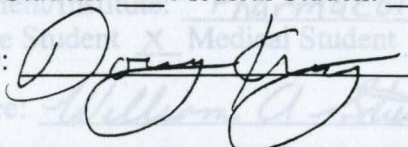
Signature: 

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

COMPARISON OF ADLIBITUM VS CHRONIC DIETARY RESTRICTION FEEDING SCHEDULES ON COGNITIVE AND SENSORIMOTOR PERFORMANCE IN MICE. Douglas E. Krug, Apostolos Lekkos, Shaunielle Cotton, Susan Summers, Carla Elsken, Amar Dhillon, Harbans Lal, and Michael J. Forster. Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

For several years, our research group has focused upon the design of procedures for measurement of age-differences in cognitive and motor performance in rodents, to be used specifically in evaluations of long-term chemical interventions. Dietary restriction has been used to test the sensitivity of the cognitive and motor tests in the context of an intervention capable of extending longevity. These experiments have revealed a number of effects which may have implications for the use of dietary restriction in long-term toxicity studies. One of the tests for cognitive functions consisted of a multiple-phase, 2-choice, footshock avoidance task (a "delayed reversal" paradigm) (Forster and Lal, *Behav Pharmacol.* 3:337, 1992) in which a number of neurobehavioral components were analyzed, including capacity for simple escape, avoidance, discrimination, reversal, conceptual learning capacity, and working memory. A water maze task has been used for assessment of spatial discrimination learning and spatial memory. Experiments with the delayed reversal paradigm have indicated that dietary restriction (to 60% of ad-libitum) produces a marked increase in the motivational effects of foot shock. Both long-term (4 to 24 months) and short-term (4 to 6 weeks) exposure to the restricted diet was sufficient to yield this effect. After titration of shock intensity among restricted and ad libitum groups to correct for diet-related differences in motivation, diet-restricted C57BL/6 and B6D2F$_1$ mice showed a decelerated decline of working memory capacity with age when compared with ad libitum fed mice. However, among young mice, there was evidence that diet restriction reduced accuracy of working memory performance. The ability to acquire a spatial discrimination in the water maze task was impaired with increasing age in ad libitum fed C57BL/6 mice, and recent data suggest little difference in spatial discrimination performance of mature mice as a function of short-term dietary restriction. Dietary restriction was found to have both short-term and long-term effects upon several measures of motor performance, including spontaneous locomotion and rotorod running capacity (Forster and Lal, *Biomed. Environ. Sci.* 4:144, 1991). Short-term diet restriction resulted in increased spontaneous locomotion and markedly improved performance on the rotorod running task after training. Long-term dietary restriction resulted in a substantial deceleration in the rate of performance decline as a function of age in C57BL/6 and B6D2F$_1$ mice, although the performance advantage conferred by short-term dietary restriction decreased as a function of age. The behavioral effects of short- and long-term dietary restriction suggest that neurological functions differ markedly between ad libitum fed and diet-restricted mice. These differing neurological conditions are reflected in cognitive and motor performance capacity at any point across the lifespan, as well as in the rate of change in performance capacity as a function of age.
[This work was part of the NIA-sponsored Biomarkers of Aging program, conducted in collaboration with the NCTR Project on Caloric Restriction; it was supported by NIA grants AG07695 and AG06182].

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

AGE-RELATED CHANGES IN SPATIAL LEARNING AND SENSORIMOTOR SKILLS IN C57BL/6 MICE.

W. A. Stutts, H. Lal, and M.J. Forster, Dept. of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX. 76107

Aging changes many functional abilities including memory and sensorimotor skills. The deficits in memory for spatial information have been reported in studies of aged humans using tasks that have some formal similarity to those used for testing rodents. In previous investigations, old rats were found to acquire accurate spatial information more slowly than younger animals, and these age-related impairments in spatial memory were independent of those in sensorimotor skills. The purpose of this study was to determine the cross-species generality of that phenomenon, by testing for similar effects in mice. Thirty-six C57BL\6J mice were tested as two groups, ages 4 and 23 months. Spatial learning was measured using a water maze task which required the animal to locate a platform in a circular tank hidden by opacified water. The procedure consisted of 3 consecutive phases of assessment: acquisition, retention and reversal. The acquisition phase allows the rate of learning to be measured while the retention phase provides a delayed recall measure. The reversal phase was designed to assess cognitive flexibility in learning new information in conflict with the previously learned material. Analysis of the acquisition phase results indicated that both groups started at equal levels but the young mice learned much more rapidly and reached a higher asymptotic performance. That pattern of results closely paralleled the data obtained previously for rats with the water maze. Upon completion of the water maze all mice were given a battery of sensorimotor tests. A Digiscan apparatus was used to provide a profile of spontaneous locomotor activity and an accelerating rotorod test was used to measure sensorimotor coordination. A motor/reflex battery was administered to assess swimming reflex, walking initiation, alley turning, wire suspension and elevated path ambulation. The results suggested that old mice were impaired in their ability to perform rotorod, wire suspension and elevated path tests. Mutivariate analysis performed on the learning and motor data indicated that mice impaired on measures of cognitive performance were not necessarily impaired in their motor performance and vice versa. Furthermore, the results suggest that age related declines in different functional anatomical systems (e.g., the limbic system and the basal ganglia) may progress independently. (Supported by NIH grants AGO7695(HL) and AGO6182(MJF) and the Texas Institute for Research and Education in Aging.)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

LOCALIZATION OF FIBROBLAST GROWTH FACTOR IN INFANT, ADULT AND AGING SKIN

S. Dan Dimitrijevich*, R. Agarwal*, R.W.Gracy* and R. Wordinger, Depts. of *Biochem. & Mol. Biol., **Anatom. & Cell Biol., UNTHSC at Ft. Worth**

Fibroblast growth factors (FGFs) are a family of mitogenic proteins the amino acid sequences of which are well established. The basic FGF (bFGF) in particular affects a wide range of cellular activities, some of which are important during wound healing.
Interest has recently focused on the acidic growth factor (aFGF) because, when bound to heparin sulphate, its activity increases about 100-fold to equal that of bFGF. Without systematic evaluation of of the distribution of aFGF and bFGF in human skin, it is difficult to ascertain the sources of these factors. Our studies examine the immunoperoxidase localization of both aFGF and b FGF in fixed paraffin embedded sections of infant, adult and aging skin. Distribution of bFGF is more prominent in young dermis than older dermis, and will be compared with that in the human dermal and skin equivalents and in the primary cultures of firbroblasts and keratinocytes. Age related changes in FGF expression have immplications related to both the normal skin functions and the wound healing of skin in the elderly.

Supported by grants from Texas Adv. Tech Program (09768-008) and NIA (AGO1274).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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DEVELOPMENT OF THE METHODOLOGY FOR COMPARATIVE TOTAL PROTEIN MAPPING OF HUMAN TISSUES. J.M. Talent, S.D. Dimitrijevich, K.Ü. Yüksel, and R.W. Gracy. Dept. Biochem. & Mol. Biol., U. North Texas Health Science Center.

Evaluation of age related changes at different organizational levels in living organisms is difficult because: a) the changes are relatively small in magnitude and require very sensitive probes for detection; b) the changes take place gradually with genetic and physiological modifiers superimposed on to the chronological effects, requiring a large sample size for statistical significance of an observed variable. A unique difficulty in ageing studies of humans is that some of the methods are not ethically acceptable.

Over the past several years we have been exploring methods for studying skin aging *in vitro*. A considerable effort has been invested in an approach based on two-dimensional gel electrophoresis and computer assisted image analysis of the resulting total protein profiles. We have established that this system can reproducibly discern minor differences. In the first instance we show that discernible differences may be observed between the total protein maps of the epidermis obtained from the donors of different ages. We are in the process of demonstrating that these changes are significant. Using this approach we are probing the *in vitro* models of human skin for retention of *in vivo* characteristics.

This work was supported in part by a grant from the Texas Advanced Research and Technology Program (09768-008).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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PROTEIN KINASE C ACTIVITY CHANGES IN CULTURED BLOOD-BRAIN BARRIER CELLS IN AGING RATS.

Zhong Chen, Pam Brett, and Walter McConathy.
Department of Medicine, University of North Texas Health Sciences Center at Fort Worth, Fort Worth, Texas

The functional role of the blood-brain barrier (BBB) is critical in understanding the genesis and progression of aging and Alzheimer's disease (AD). Protein kinase C (PKC), plays a prominent role as a regulatory enzyme in the nervous system. Preliminary experiments to characterize microvascular PKC activity have been performed using endothelial cells derived from cerebral microvasculature. There is a significant difference ($p < 0.05$) in PKC activity observed in crude extracts of cultured cells between adult (3 months) and aged rats (24 months). In an attempt to optimize the measurement of PKC activity, we used Q-Sepharose to fractionate the extract of cerebral endothelial cells. The recovered PKC activity was 10-15 times higher than the level in crude microvessel preparations. These results demonstrated the utility of Q-Sepharose in enrichment of PKC activity and suggest that the Q-Sepharose may remove modulators of microvessel PKC activity. These studies point to alterations in PKC activity which may contribute to altered cell responsiveness in aging, and play a critical role in the regulation of BBB functions. (Suppport:TIREA).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ALTERATION OF COLONY-STIMULATING FACTOR PRODUCTION IN AGING Wu, M.-C.[1], Wu, F.M.[1], and Mills, J.G.[2], Dept. of Biochem. and Mol. Biol., and Dept. of Public Health and Preventive Med.[2] UNT-HSC, Fort Worth, TX

Elderly populations display a greater susceptibility to infection, anemia, and other hematological diseases, probably due to a decrease in hematopoietic function. Studies on human populations of different ages have indicated an altered hematopoietic mechanism occurs in the elderly; however, little detailed information on the causes has been conclusively obtained. Animal model studies have provided information to augment evidence that the hematopoietic ability is lower in aged groups than in the young, but systematic approaches to determine the source of the defect have been lacking. In this proposal, we will use cultured fibroblast cells to investigate the altered function of different ages and different passages by measuring their responsiveness to interleukin-1 in producing colony-stimulating factors. Since the decrease in their ability to respond to stimulation is age-related in interleukin-2 production, similar deficiency may also occur for CSFs. Once we have the preliminary results from the study of fibroblast cells, we will then investigate the responsiveness of peripheral monocytes and lymphocytes. Since monocytes and lymphocytes are very important cells in immune response/defense system, their function in producing monokines and lymphokines such as CSFs is a good criteria for their functions. CSFs not only mediate the immune responses, but also regulate myelopoiesis and control myeloid cell functions. The monocytes and lymphocytes will be obtained from the peripheral blood of young and old persons. The level of CSFs production in response to 1L-1 stimulation will be determined. It is anticipated that the CSFs production from old fibroblast cells and monocytes and lymphocytes from old people will be decreased. The results obtained here should provide some insight into the age-related alterations in myelopoiesis and immune response and allow further biochemical characterization of the system. Preliminary results were obtained from mice and rats and shown in this poster. These data indicated that indeed old animals produced less activity. The stimulation of CSF activity produced by human fibrobrast cell line CCL 202 were also presented to provide a system for this study. (Supported by a TIREA Pilot Project).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

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MACROPHAGE COLONY-STIMULATING FACTOR FROM SPINAL NEURON CULTURES Wu, M.-C.[1], Wu, F.M.[1], Azzazy, H.M.[1] and Gross, G.W.[2] Dept. of Biochem. & Mol. Biol. UNT-HSC, Fort Worth, TX and Dept. of Biol. Sci. UNT Denton, TX[2].

Macrophage colony-stimulating factor (M-CSF) is a potent mitogen which is required for the proliferation, maturation and survival of macrophages. Because of the alternative splicing of mRNA and various forms of post-translational modification, it appears as a heterogeneous group of protein growth factor. Cultured mouse embryonic spinal cord neuron cells have been used as a system to study the neuronal development and activities. We found the conditioned medium from the cultured neuron cells contains M-CSF activity as assayed by the method of marrow cell colony formation on soft-agar. Fractionation of the M-CSF activity by Ultrogel AcA44 gel filtration column revealed exclusively a high molecular weight of greater than 100,000. Morphological analysis of the colonies showed exclusively of monocyte/macrophage colonies. Incubation of the high Mr M-M-CSF with chondroitinase ABC reduced the Mr of M-CSF to 80-90 Kd. This result is similar to the previously reported proteoglycan-M-CSF from mouse L cells and human recombinant M-CSF produced in CHO cells. Western blot analysis further confirmed the nature of proteoglycan conjugate of M-CSF from neuron cell culture. Histochemical stain of the cultured cells with anti M-CSF antibody has shown the presence of M-CSF in neuron cells. However, whether the M-CSF is produced by neuron cells, or the surrounding glial cells and bond to neuron cells, remain to be clarified. The function of this proteoglycan M-CSF in neuronal development will be investigated by its effect on network activity as well as protein F-1 expression. (Supported by a grant from TEC, Inc.)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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PROTEIN F1 EXPRESSION AND FUNCTIONS IN SPINAL NEURONAL CULTURES Azzazy, H.M.E.,[1] Gross, G.W.,[2] Wu, M.-C.[1] Dept. of Biochem. & Mol. Biol. UNTHSC, Fort Worth, TX [1] and Dept. of Biol. Sci. UNT, Denton, TX [2].

Protein F1 (Gap-43) has been mainly studied in neurons and implicated in axonal growth, neurotransmitter release, and plasticity. Protein F1, which is enriched in the growing tips of extending neurites, is a prominent protein kinase C (PKC) substrate. In an attempt to investigate protein F1, we synthesized a 21-amino acid polypeptide (position 204-224) which contains the PKC phosphorylation sequence SXR. The synthetic peptide was phosphorylated by rat brain PKC in a concentration-dependent manner suggesting that this site can be phosphorylated by PKC in vivo. Polyclonal antibodies against the polypeptide were produced in rabbit and used to recognize protein F1 purified from rat brain, and to immunoprecipitate protein F1 phosphorylated by PKC in vitro. In addition, the antibodies were used to immunostain cultured mouse spinal neurons derived from 13-14 day old embryonic tissue. The anti-protein F1 antibodies stained cell bodies and neurites of mouse spinal neurons without staining glial cells. The antibodies were also used to quantitate the levels of protein F1 during the development of monolayer networks of cultured spinal neurons. The highest levels of protein F1 were detected, by enzyme-linked immunosorbent assay, at 48 hr after seeding. Protein F1 levels then dropped to basal levels by 96 hr. These results are in accordance with previous reports that correlate high expression of protein F1 to neurite outgrowth. Attempts to deliver anti-protein F1 antibodies into cultured neurons to study the possible effect of protein F1 on the electrical activity of monolayer neural networks as well as its effects on neuronal development have also being conducted by liposome-delivery methods. The preferential uptake of liposome by neuron cells over glial cells provides a useful tool for neuronal delivery. The function of Protein F-1 is now being investigated with this system.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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OLIGODENDROCYTE-OLIGODENDROCYTE INTERACTIONS IN VITRO. Stephen J. Moorman, Department of Anatomy and Cell Biology, UNT Health Science Center at Fort Worth, Ft. Worth, TX 76107.

Within the vertebrate central nervous system oligodendrocytes serve a critical role, they insulate axons with a sheath of myelin. The node of Ranvier, demarcates the boundary between segments of myelin contributed by different oligodendrocytes. Little is known about how these nodes are established during development. During development, the node of Ranvier is a site of potential contact between adjacent oligodendrocytes. It is unclear how the segments of the sheath contributed by adjacent oligodendrocytes are kept apart, rather then growing together to make a continuous myelin sheath. My central hypothesis is: Cell-cell interactions between oligodendrocytes play a key role in establishing the gap between oligodendrocytes that characterizes the node of Ranvier.

In previous experiments I have examined the response of neonatal-rat oligodendrocytes to contact with myelin extracts prepared from the central and peripheral nervous system. Contact with either CNS-myelin or PNS-myelin resulted in collapse of the fine structure of the leading edge of oligodendrocytes in-vitro. The collapse of the fine structure of oligodendrocyte processes was preceded by a substantial (approximately fivefold) increase in intracellular free calcium concentration. The calcium concentration increase was due, at least in part, to a release of calcium from internal stores, since it persisted when extracellular calcium was removed by chelation by EGTA. The increase in calcium concentration and the coincident morphological change suggest that oligodendrocytes might be able to recognize and react to specific molecules on the surface of other oligodendrocytes. To test this idea I have manipulated oligodendrocytes so that they came in contact with other oligodendrocytes in vitro. Oligodendrocyte-oligodendrocyte contact resulted in a substantial increase in intracellular free calcium concentration in the leading edge. This contact also resulted in a coincident collapse of the fine structure of the oligodendrocyte leading edge. Neither the calcium increase nor the collapse of the oligodendrocyte leading edge was induced by contact with either neurons or astrocytes. These data suggest that the effect is oligodendrocyte specific.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
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ABSTRACT FORM

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CSF VOLUME AND HISTAMINE CONCENTRATION IN RATS WITH EAE. Orr, E. L., Mallick, S., Taylor, M. T. and deSchweinitz, L.H., U.N.T. Health Science Center at Fort Worth, Fort Worth, TX 76107

Lewis rats with experimental autoimmune encephalomyelitis (EAE) exhibit significant changes in brainstem and spinal cord histamine and water content (edema) in concert with the clinical course of EAE. To determine if CSF levels of histamine (HA) are also altered in EAE, we measured the concentration of HA present in CSF obtained from the cisterna magna of control and EAE rats. **METHODS**: EAE was induced by inoculation of female Lewis rats with an emulsion of guinea pig spinal cord homogenate (gpsch) in complete Freund's adjuvant; control rats received an identical inoculum lacking gpsch. Beginning on day 7 post inoculation (pi), rats were monitored daily for clinical signs of EAE. Whole spinal cords, brainstems, and samples of cisternal CSF were obtained from anesthetized control and EAE rats on days 7, 9, 11, 14, and 16 pi. The tissues and CSF samples were stored frozen until assayed for HA using radioenzymatic methods. **RESULTS**: Significant changes in spinal cord and brainstem HA levels and wet weight occurred in parallel with the clinical course of EAE. Similarly, CSF concentrations of HA also fluctuated in concert with the clinical course of EAE. This increase in CSF HA was accompanied by a significant decrease in the volume of CSF one could obtain from the cisterna magna. **CONCLUSIONS**: We conclude that changes in cisternal CSF HA concentration parallels the changes in CNS HA levels which occur during EAE in Lewis rats. In addition, the decrease in CSF volume obtained from the cisterna magna of rats with clinically significant EAE is consistent with the significant CNS edema observed in rats with EAE. (Supported by a grant from the National Multiple Sclerosis Society.)

North Texas Eye Research Institute

19.	Tamara Reese	Acidic and Basic FGF in the Human Cornea
20.	Michael B. Berman, Ph.D.	Quantitation of Rabbit and Human Corneal Epithelial Urokinase-Like (uPA) mRNA
21.	Debra Shade	Effect of Muscarinic Agents on Human Trabecular Meshwork Intracellular Calcium Levels
22.	Ying Jin, Ph.D.	Pharmacological Characterization of $Alpha_2$ Adrenergic Receptors in the Rabbit Ciliary Body
23.	Ying Jin, Ph.D.	Sodium Ion and Guanine Nucleotide Modulate Binding of ^{125}I-Iodoclonidine to $Alpha_2$ Adrenergic Receptors in Rabbit Ciliary Body Membrane
24.	Patrick Cammarata, Ph.D.	Hypertonicity-Induced Upregulation of Na^+/Myoinositol Cotransporter mRNA Denotes an Early-Onset, Interactive, Protective Mechanism Against Water Stress in Cultured Bovine Lens Epithelial Cells (BLECs)
25.	Sarita L. Sharma, Ph.D.	Müller Cell-Derived Growth Factor(s) Promote Endothelial Cell Proliferation
26.	Alberta Davis, Ph.D.	Human Müller Cells Grown in Tissue Culture Proliferate in Response to Medium Conditioned by a Human RPE Cell Line
27.	David Jaynes	Effects of Media Conditioned by a Human Retinal Pigment Epithelial Cell Line on Rat Müller Cells
28.	Ning Lin, M.D., O.D.	Early Photoreceptor Cell Repair is Initiated by RPE Transplants in RCS Dystrophic Rats: Outer Segment Regeneration
29.	Harold J. Sheedlo, Ph.D.	Effects of Retinal Dystrophy on Müller Cell Markers in Rodents
30.	Wei Fan	Microscopic Examination of Retinas of Aged Fischer Rats
31.	Michael H. Chaitin, Ph.D.	Immunolocalization of CD44 in the Vertebrate Retina
32.	Neeraj Agarwal, Ph.D.	Diurnal Expression of a "Zinc-Finger" DNA-Binding Protein, NGF1-A mRNA in Retinal Degeneration Slow (*rds*) Mutant Mouse Retina
33.	Scott Krueger	The Effects of Meso-Tetra-(4-Sulfonatophenyl)-Porphine Dihydrochloride (TPPS) on the suceptability of the Sprague Dawley Rat to Light-Induced Retinal Damage

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ACIDIC AND BASIC FGF IN THE HUMAN CORNEA

T. Reese,* S. Dan Dimitrijevich*, R.Wordinger and R.W. Gracy*, Depts of *Biochem. & Mol. Biol., and **Anatomy & Cell Biol., UNTHSC at Ft. Worth. North Texas Eye Research Institute.**

Therapeutic potential of growth factors, EGF and FGF in particular is based on their ability to promote ocular wound healing. Regulation of the events involved in the tissue repair process depends on the understanding of the autocrine as well as paracrine responses which follow an injury. Our interest in FGF is directed towards utilization of the Human Corneal Epithelial Equivalent (HCEE) as a physiological *in vitro* model of human corneal tissue. Immunoperoxidase localization of FGF in the fixed paraffin embedded sections of the human cornea shows significant bFGF in the suprabasal layers of the epithelium, in the basement membrane and the endothelium . In contrast aFGF is diffuse throughout the epithelium but very strongly localized in the basal limbal epithelium. The presence of both aFGF and bFGF in the stroma is most pronounced in the limbal region. It is likely that the bFGF stimulates basal cell proliferation as a ligand immobilized on the basement membrane. The differentiation signal is presumably maintained by the presence of the bFGF at the cell surface of the suprabasal epithelial cells. The above findings will be compared with those found in the HCEE where bFGF is expressed by the keratocytes during the early stages of adaptation to the collagen matrix. FGF expression by all three corneal cell types cultured *in vitro* was also demonstrated.

Supported by the Texas Adv. Tech. Program (009768-008). Procurement of human tissue by Dr. W. E. Howe of ALCON Laboratories Inc. is gratefully acknowledged.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

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North Texas Eye Research Institute
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QUANTITATION OF RABBIT AND HUMAN CORNEAL EPITHELIAL UROKINASE-LIKE (uPA) mRNA M. Berman Departments of Anatomy & Cell Biology and Biochemistry & Molecular Biology UNT Health Science Center at Ft. Worth. Fort Worth, TX 76107.

Urokinase-like plasminogen activator (uPA) has a critical role in epithelial migration; and faulty regulation of this enzyme is thought to result in a cascade of events that result in persisent epithelial defects and stromal ulceration. **Purpose**: To develop a uPA cDNA probe for use in quantitating rabbit and human corneal epithelial uPA mRNA in wound healing studies. **Materials and Methods**: The human uPA cDNA probe, pH UK-8, obtained in phagemid pEMBL8(ATCC#57328) was grown up in E. coli; the phagemid with uPA insert was purified and then restricted with PstI to recover the 1.5 kB insert. The insert was then restricted by DrdI and Bam HI to yield a 548 bp, intron-free cDNA probe. Using this probe ^{32}P-labeled by random priming (6.0×10^6dpm/ng), and under high stringency, Northern Blots were prepared from total cell RNA from cultured rabbit corneal sheets that were wounded and treated with phorbol myristate acetate(PMA) to stimulate uPA gene transcription in comparison to human osteosarcoma cell RNA, known to contain uPA mRNA (positive control); and densitometry was used to quantitate Slot Blots of the rabbit corneal epithelial and osteosarcoma RNA. The probe was used also to corroborate identification of RT/PCR cDNA products derived from rabbit and human corneal epithelial total cell RNA. **Results**. Northern Blots demonstrated that rabbit corneal epithelial RNA contains uPA mRNA of the same approximate size (2.5 kB) as human osteosarcoma uPA mRNA and human corneal epithelial uPA mRNA. Slot Blot analysis demonstrated a linear relationship between optical density and 0.2 ug-30 ug for the rabbit RNA and 0.2 ug-7.5 ug for human osteosarcoma RNA. Southern Blots demonstrated that the 548 bp human probe labels both rabbit and human corneal epithelial RT/PCR uPA cDNA products. **Conclusions**. Although the rabbit uPA gene has not been sequenced, and its cDNA clones are not available, the 548 bp human cDNA probe described above can be used to quantitate steady-state levels of uPA mRNA in wounded cultures of both rabbit and human corneal epithelia, in relationship to wound closure and as modulated by growth factors and cytokines.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

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ABSTRACT FORM

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EFFECT OF MUSCARINIC AGENTS ON HUMAN TRABECULAR MESHWORK INTRACELLULAR CALCIUM LEVELS.

Debra L. Shade, Abbot F. Clark, and Iok-Hou Pang. Alcon Laboratories, Inc. and University of North Texas Health Science Center at Fort Worth, Fort Worth, TX.

Muscarinic cholinergic agonists such as carbachol are widely used for the treatment of elevated intraocular pressure (IOP) associated with glaucoma. Their mechanism of action is believed chiefly due to contraction of ciliary muscle; the resultant widening of the trabecular meshwork's (TM) intercellular spaces presumably allows increased aqueous humor outflow and thus a decrease in IOP. Recently, however, Lepple-Wienhues, et al, 1991, have shown that the TM tissue itself contracts following muscarinic stimulation, hence direct muscarinic effects on the TM may also play a role in the regulation of outflow and IOP. In order to delineate the potential role(s) of muscarinic agents in regulating TM cell function, we have studied the effects of muscarinic agents on intracellular calcium concentration ($[Ca^{++}]_i$) in cultured human TM cells.

Measurements were performed using cells seeded on glass coverslips. Cells were exposed to 5 μM fura 2-AM for one hour, followed by washing to remove exogenous dye. Fluctuations in the cells' $[Ca^{++}]_i$ were evaluated by means of a microscope-based ratio fluorescence system. Carbachol increased $[Ca^{++}]_i$ in a dose-dependent manner (EC_{50} ~7.5 μM); $[Ca^{++}]_i$ was also increased by oxotremorine-M and pilocarpine. The effect of 100 μM carbachol was blocked by the non-selective muscarinic antagonist atropine (IC_{50} ~12 nM), as well as by muscarinic receptor subtype-selective antagonists such as pirenzipine (IC_{50} ~8 μM; M1-selective), p-fHHSiD (IC_{50} ~118 nM; M3-selective), and 4-DAMP (IC_{50} ~5.6 nM; M1 and M3 subtypes). Comparison of the antagonists' experimental values versus published data for other tissues strongly indicates that changes in TM cell $[Ca^{++}]_i$ are linked to activation of an M3-like receptor subtype. The existence of m3 receptor subtype mRNA has previously been demonstrated in human TM tissue (Gupta, et al, 1991), and an M3-like receptor has been postulated to mediate the activation of phospholipase C in cultured human TM cells (WoldeMussie, et al, 1990). Our findings confirm these results and further suggest that the muscarinic M3 receptor may be important in regulating TM cell function.

(Supported by Alcon Laboratories, Inc.)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

PHARMACOLOGICAL CHARACTERIZATION OF ALPHA$_2$-ADRENERGIC RECEPTORS IN THE RABBIT CILIARY BODY. Y. Jin, A. Verstappen and T. Yorio. TCOM\University of North Texas, North Texas Eye Research Institute, Ft. Worth, TX 76107

The agonist- and antagonist-binding properties of α_2-adrenergic receptors in the rabbit ciliary body membrane preparation were studied. Binding of agonist ligand, p-[^{125}I]iodoclonidine ([^{125}I]PIC), was characterized by a single high affinity binding site (Kd = 1.92 $\pm$ 0.24 nM and B_{max} = 627 $\pm$ 92 fmol/mg). Inclusion of Gpp(NH)p in the assay decreased the specific binding by 25% but did not alter the K_d. Inhibition of [^{125}I]PIC binding by yohimbine, idazoxan and amiloride was determined to differentiate between α_2-adrenergic receptors and imidazoline preferring receptors (IPR). Yohimbine and idazoxan inhibited all of the [^{125}I]PIC binding and their inhibition curves were consistent with a single class of binding sites suggesting that the [^{125}I]PIC binding sites in the rabbit ciliary body were α_2-adrenergic receptors but not IPR. Subtypes of α_2-adrenergic receptors were further studied by competition for [^{125}I]PIC binding with subtype-selective compounds. [^{125}I]PIC binding sites showed the pharmacologic characteristics of the **α-2A** adrenergic subtype (oxymetazoline >chlorpromazine >>prazosin) and competition by oxymetazoline and chlorpromazine was best fit by a single class of binding sites, indicating that the binding sites detected by this agonist ligand were **α-2A** subtype. Binding of the antagonist ligand, [^{3}H]rauwolscin, in the rabbit ciliary body membrane preparation also showed linear Scatchard plots (Kd = 6.79 $\pm$ 1.5 nM and Bmax = 653 $\pm$ 52 fmol/mg). However, inclusion of Gpp(NH)p in the assay did not affect either K_d or B_{max} of [^{3}H]rauwolscin binding. Competition for this antagonist ligand by α_2-adrenergic receptor subtype-selective compounds was also studied and compared with the ligand, [^{125}I]PIC.

Supported in part by a grant from Alcon Laboratories, Inc.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Signature: _____

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

SODIUM ION AND GUANINE NUCLEOTIDE MODULATE BINDING OF ^{125}I-IODOCLONIDINE TO ALPHA$_2$ ADRENERGIC RECEPTORS IN RABBIT CILIARY BODY MEMBRANE Y. Jin[1], J.R. Gooding[2] and T. Yorio[1]. Department of Pharmacology, North Texas Eye Research Institute, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, 76107[1]; University of North Texas[2].

Purpose. To examine the effects of monovalent cations and guanine nucleotide on binding of α_2-adrenergic agonist ligand to rabbit ciliary body membrane.

Methods. Radioligand binding assays were performed using the α_2-adrenergic agonist ligand, ^{125}I-iodoclondine ([^{125}I]PIC), with the membrane from rabbit ciliary body.

Results. NaCl dose-dependently-decreased the specific [^{125}I]PIC binding in the range of 0 - 200 mM, with a maximum inhibition of 60% at 200 mM of NaCl concentration. The IC_{50} was 35mM. In contrast, maximum inhibition by KCl was only 24% with an IC_{50} of 170 mM. In addition, Gpp(NH)p was found to decrease the specific [^{125}I]PIC binding in a dose-dependent manner, with a maximum decrease 45% of at 100 μM of Gpp(NH)p concentration. To determine if these effects of Na^+ and Gpp(NH)p were at the same site, saturation experiments were performed in the presence and absence of 150 mM of NaCl 100 μM of Gpp(NH)p and the combination of the two. Neither Na^+ nor Gpp(NH)p had effect on the K_d but B_{max} was decreased by about 50% by either 150 mM NaCl or 100 μM Gpp(NH)p. Moreover, the simultaneous presence of both Na^+ and Gpp(NH)p produced a decrease in the B_{max} of [^{125}I]PIC binding which was greater than the effect of either modulator alone.

Conclusion. Sodium ion and guanine nucleotide modulation of the α_2-adrenergic system in rabbit ciliary body is mediated by different molecular components.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

HYPERTONICITY-INDUCED UPREGULATION OF Na^+/MYO-INOSITOL COTRANSPORTER mRNA DENOTES AN EARLY-ONSET, INTERACTIVE, PROTECTIVE MECHANISM AGAINST WATER STRESS IN CULTURED BOVINE LENS EPITHELIAL CELLS (BLECs) ((P.R. Cammarata, N. Agarwal, R. Reeves and C. Zhou)) Anatomy and Cell Biology, University of North Texas Health Science Center, Fort Worth, TX.

Purpose. Does hypertonicity induce *de novo* synthesis of MI carrier protein and/or Na^+/MI cotransporter mRNA? **Methods**. BLECs were maintained from 2-72 h in physiological medium made hypertonic by supplementation of 116 mmol/l NaCl (473 $\pm$ 6 mosm). The effect of the protein synthesis inhibitor, cycloheximide (Cyc), was studied. A Na^+/MI cotransporter 626 bp PCR product amplified from lens cell RNA and aldose reductase (AR) cDNA probes were used to measure their respective mRNA content by Northern blot analysis. **Results**. At least 12 h of hypertonic exposure was necessary to enhance MI uptake; switching to isotonic media (257 $\pm$ 2 mosm) for 24 h reversed this enhancement. Cells exposed to hypertonic conditions and Cyc showed marked impairment of enhancement of MI uptake. A time course of hypertonic exposure further revealed a maximal increase in Na^+/MI cotransporter mRNA by 8 h *which thereafter steadily declined*; while the level of AR mRNA was maximally increased *after* 24 h *but remained elevated throughout the remainder of the experiment*, 72 h. Hypertonic exposure resulted in a steady-state accumulation of MI and sorbitol over six days. Inhibition of sorbitol formation prompted the intracellular MI content to a higher level. **Conclusions**. Enhanced MI accumulation is an early protective mechanism against water stress, succeeded by a second protective mechanism, activation of maximal AR activity. Lens water stress management is interactive as MI and sorbitol levels are regulated in concert. Sorbitol accumulation affects intracellular MI content via multiple mechanisms; (1) polyol-mediated inhibition of MI uptake via suppression of MI carrier protein transport sites, (2) polyol-induced downregulation of Na^+/MI cotransporter mRNA transcript and (3) polyol driven MI efflux from cell to medium. Supported by National Health Public Service Award EY05570 (PRC).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

MÜLLER CELL-DERIVED GROWTH FACTOR(S) PROMOTE ENDOTHELIAL CELL PROLIFERATION. **Sarita L. Sharma and Rouel S. Roque.** Dept. of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, TX 76107.

Although morphological studies suggest that Müller cells affect the growth and distribution of retinal vessels during development and disease, the exact mechanisms remain vague. Müller cells may affect endothelial cell growth by altering the matrix composition around retinal vessels or by releasing factors which promote the proliferation of endothelial cells. We have begun to investigate these mechanisms by studying the effects of Müller cell products on cultured endothelial cells. Müller cells prepared from normal rats were positive for glial cell markers cellular retinaldehyde binding protein, S-100, and carbonic anhydrase-C. Conditioned medium (MCCM) was collected from confluent Müller cell cultures maintained in basal medium (DMEM + bovine serum albumin, 0.1%) for 48 hr. MCCM was concentrated by ultrafiltration; heated at various temperatures (56°C, 30 min; or 100°C, 5 min); or treated with trypsin (250mg/L) for 4 hr. Microvascular endothelial cells derived from rat brain were incubated for 23 hr in either basal medium or various treatments of MCCM. Cells counts were taken using trypan blue and a hemocytometer, while proliferation was measured using a non-radioactive colorimetric assay (Promega, Madison, WI) based on the conversion of a tetrazolium salt into a blue formazan product by dehydrogenase enzymes present in metabolically active cells. All experiments were done in triplicate. Counts of endothelial cells cultured in the presence of MCCM were 2.6-fold higher than those grown in basal medium. The mitogenic activity, however, decreased concomitantly with serial dilutions of the MCCM. Treatment of MCCM with heat did not inhibit its activity. However, trypsin-treatment resulted in a loss of about 30% of its activity. The consistent increase in endothelial cell population in response to Müller cell conditioned media strongly suggests that Müller cells secrete a soluble product(s) which promotes the survival and proliferation of endothelial cells. Moreover, initial characterization of this substance(s) suggests that cultured Müller cells may secrete a heat-resistant peptide growth factor(s). Although the nature of this mitogenic activity is still under investigation it may prove important in understanding the role of Müller cells in vascular growth during development or disease.

Supported by a UNTHSCFW BRSG and a TCOM Faculty Grant.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

HUMAN MULLER CELLS GROWN IN TISSUE CULTURE PROLIFERATE IN RESPONSE TO MEDIUM CONDITIONED BY A HUMAN RPE CELL LINE A. A. Davis, S. Brady, and J.E. Turner Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, North Texas Eye Research Institute, Fort Worth, TX. 76107

Purpose. (1) Establish a pure population of human Muller cells in tissue culture; (2) devise an assay to test their proliferative response to RPE conditioned medium (CM) and (3) begin to characterize the factor (s) responsible for these proliferative effects. **Methods.** Human eye tissue was obtained from the Fort Worth Lions Organ and Eye Bank within 12-20 hrs. postmortem. The neural retinae were removed, cut into approximately 1mm pieces, triturated and plated in dishes coated with fibronectin/collagen type I/laminin. Cells which grew out of this tissue were analyzed using immunohistochemistry and immunoblotting. Conditioned medium obtained from D407, a human RPE cell line, was fractionated using Centricon microconcentrators and used in a non-radioactive cell proliferation assay with human Muller cells. **Results.** Human Muller cells in tissue culture express typical markers for Muller cells, including carbonic anhydrase and cellular retinaldehyde binding protein (CRALBP). These cells also survive and proliferate in CM from a human RPE cell line in a dose-dependent manner. Using concentrated medium from a Centricon microconcentrator with a molecular weight cutoff of 30 kD, the material greater than 30 kD gave a more pronounced proliferative response than that below 30 kD. **Conclusions.** Human Muller cells can be grown in tissue culture and these cells express known markers for Muller cells. Most significantly, human Muller cells survive and proliferate in medium conditioned by a human RPE cell line.
NIH Grant EY04337 .

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

EFFECTS OF MEDIA CONDITIONED BY A HUMAN RETINAL PIGMENT EPITHELIAL CELL LINE ON RAT MULLER CELLS. C.D. Jaynes and J.E. Turner Department of Anatomy and Cell Biology, North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, Texas 76107.

Pathologic conditions of the retina often result in proliferative vitreoretinopathy (PVR), partially in response to Muller cell (MC) activation. Studies from this lab have shown that factors secreted by rat retinal pigment epithelium (RPE) elicit MC proliferation, and therefore may be responsible for the MC role in the PVR process. This study was undertaken to determine whether media conditioned by a transformed human RPE cell line (hRPE-CM) elicits a similar MC growth response. A pure population of MCs was isolated (Hicks and Courtois, 1990) from two day Long-Evans rats and cultured in 0, 10, 30, 50, 80, 100, and 150% hRPE-CM. In addition, using the Centricon-30 microconcentrator, fractions of the hRPE-CM above and below 30kD were tested in the bioassay. A stepwise increase in cell number was observed from wells with the lowest concentration of hRPE-CM to those with the highest. Treatment of the cultures with the fraction of hRPE-CM greater than 30kD induced a two-fold increase in cell number by day four of the assay when compared to that of control wells. In contrast, the fraction of hRPE-CM less than 30kD was no different than control wells. This study demonstrates that, similar to the effects of rat RPE-CM, medium conditioned by a transformed human RPE cell line promotes neonatal rat MC survival and proliferation in a dose-dependent manner. Our findings also suggest the fraction of hRPE-CM responsible for MC proliferation is greater than 30kD. (Supported by NIH grant EY04377).

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Ning LIN, M.D.,O.D.
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

EARLY PHOTORECEPTOR CELL REPAIR IS INITIATED BY RPE TRANSPLANTS IN RCS DYSTROPHIC RATS: OUTER SEGMENT REGENERATION. N. Lin, H.J. Sheedlo, W. Fan and J.E. Turner. Department of Anatomy and Cell Biology/North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107.

We have shown previously that transplanted normal RPE cells rescued photoreceptor cells in RCS rats from two months to one year. In this study, we investigated the photoreceptor cell rescue effects in RCS rats within the first three weeks following surgery in an attempt to determine if RPE transplants initiated repair mechanisms such as outer segment regeneration. Freshly isolated RPE cells (80 - 100K cells/1ml) from neonatal normal rats were transplanted into the subretinal space of 22-23 day-old RCS rats using a transscleral approach. For controls, 1 ml of vehicle was similarly injected. In order to limit or avoid RPE-cell rejection during the early transplantation period, some of the RCS rats in transplant and control groups were injected daily with cyclosporine (10 mg/kg, i.m.) from the day prior to transplant-ation through their termination at 2, 4, 7, 10, 14 and 21 days post-operation. Tissues were subsequently prepared for light and electron microscopy. When analyzed at 10 days post-transplantation, long inner segments were observed with buds of outer segment growth in the area of the RPE-cell transplants. The outer segments were 2-4 fold longer at 14 days than at 10 days, and reached adult length by 21 days. The thickness of the outer nuclear layer (ONL) was 8-10 cells at 10 days and remained 6-8 cells thick at 14 and 21 days. Transplanted RPE cells were occasionally seen attached to Bruch's membrane, while others were found free in the subretinal space. Most interestingly, only a few transplanted RPE cells were sufficient to stimulate significant outer segment regeneration. In control retinas from 10 days to 21 days post-injection, outer segments were not observed, the inner segments were shorter than in RPE-transplanted retinas, while the ONL was only 4-5 cells thick . This study has shown that transplants of neonatal normal RPE cells have the capacity to support not only photoreceptor cell survival but also initiate early repair mechanisms as exhibited by outer segment regeneration in RCS retinas. (Supported by NIH grant EY 04337)

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Dr Harold J. Sheedlo
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

EFFECTS OF RETINAL DYSTROPHY ON MULLER CELL MARKERS IN RODENTS. Sheedlo, H.J., C.D. Jaynes and J.E. Turner. Department of Anatomy and Cell Biology, North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, Texas 76107.

Retinas of Royal College of Surgeons (RCS) dystrophic rats and retinal degeneration (rd/rd) mice undergo Muller cell alterations concomitant with rapid photoreceptor cell loss. In this study, retinas of these rodents at various stages of retinal degeneration were examined immunocytochemically for two Muller cell markers, carbonic anhydrase-C (CAC) and cellular retinaldehyde-binding protein (CRALBP). In retinas of 1 to 12 month-old RCS dystrophic rats, putative Muller cells were immunostained for CAC. In addition, a nondescript material in the region of the retinal pigment epithelial (RPE) cell layer, possibly processes of Muller cells in the subretinal space, was immunolabelled for CAC in retinas of 2 month-old and older RCS rats. The CAC-immunoreactive Muller cells seen in retinas of 1 year-old RCS rats were disorganized, that is not observed in a laminated pattern, as significant degeneration had occurred by this time. Furthermore, in retinas of 6 week-old RCS rats, Muller cells and their processes were immunolabelled for CRALBP. These processes spanned from the nerve fiber layer through the outer nuclear layer. The density of this immunostaining increased, especially in the area of the RPE-cell layer, with advancing age in RCS rats, particularly in retinas of 9 month-old RCS rats. In retinas of rd/rd mice beginning by day 14, when rod photoreceptor cell degeneration was well underway, CAC- and CRALBP-immunoreactive material was observed in the vicinity of the RPE-cell layer. By 6 weeks, when only cone photoreceptor cells are present, representing only about 2% of total photoreceptor cells in rodent retinas, the immunostaining pattern for these two proteins appeared thin and patchy. The immunostained structures in the subretinal space of rd/rd mice were possibly Muller cell processes which extended into this region following photoreceptor cell degeneration as was also shown in retinas of RCS rats. In this study, we determined that immunostaining for CRALBP increased in degenerating retinas of RCS rats, but not rd/rd mice, while CAC immunostaining did not appear to increase in either of these retinas in the advanced stages of photoreceptor cell degeneration. This work was supported by NIH grant EY 04337.

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University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

MICROSCOPIC EXAMINATION OF RETINAS OF AGED FISCHER RATS. W. Fan, N. Lin and J. E. Turner Department of Anatomy and Cell Biology/North Texas Eye Research Institute, UNT Health Science Center at Fort Worth, Fort Worth, TX 76107

Fischer-344 rats exhibit a selective photoreceptor cell loss from the ora serrata to central retina resulting in a pronounced peripheral retinopathy. In this study, we examined retinal sections by light microscopic immunocytochemistry using antibodies to glial fibrillary acidic protein (GFAP) and carbonic anhydrase II (CA II) to show changes of Muller cells and retinal pigment epithilial (RPE) cells of the retina with advancing age in Fischer rats. Our study showed intense GFAP immunostaining of Muller cell processes in the superior peripheral retina and some GFAP positive paravessel staining could be seen along these Muller cell processes where photoreceptor cells were degenerating at 18, 23 and 27 months of age. At the later time periods these processes extended into the subretinal space just below the RPE cells. However, in the central retina, where the photoreceptor cell population was more stable, GFAP-immunolabelled Muller cells were not detected. Immunoblots of retinal homogenates from 18, 23 and 27 month-old Fischer rats confirmed an elevated GFAP content when compared to retinas of 6 month-old Fischer rats. During photoreceptor cell degeneration, Muller cell processes were also prominently immunostained for CA II and were seen to occupy the subretinal space. When the photoreceptor cells were completely lost, the number of CA II staining Muller cells gradually decreased in the inner nuclear layer. After photoreceptor cells were totally lost, vascularization of the RPE cells and RPE-associated vessels were observed in the inner retina. Neovascularization was also seen in the corresponding area of the choroid capillary layer. Our results suggest that Muller cells respond to the age-related peripheral retinopathy in Fischer rats by increased GFAP content and growth of their processes into the subretinal space to form a glial scar, particularly in the area of photoreceptor cell loss. RPE and Muller cell close opposition and new vessel growth may contribute to RPE proliferation and migration subsequently enhancing the age-related peripheral retinopathy. Supported by NIH grant EY 04337 and The Texas Institute for Research and Educational Aging.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

IMMUNOLOCALIZATION OF CD44 IN THE VERTEBRATE RETINA. Michael H. Chaitin, Helen S. Wortham, Matt T. Ankrum, and Anne-Marie Brun-Zinkernagel. Dept. of Anatomy and Cell Biology, University of North Texas Health Science Center, Fort Worth, TX 76107.

The transmembrane glycoprotein CD44 is a cell adhesion molecule which has recently been localized in a variety of cell types. It mediates cell attachment to extracellular matrix components and also binds to the actin cytoskeleton within the cell. In this study, we investigated the presence of CD44 in mouse and rat retinas to determine if this molecule might be important for retinal cell adhesion. With immunoperoxidase techniques, positive labeling for CD44 was found at the level of the outer limiting membrane and in the region just above it. However, from these light microscope results it was not clear if the label was in the photoreceptor inner segments, Muller cell microvilli, or both. In order to answer this question, cryoultramicrotomy and immunogold labeling were used to demonstrate that CD44 is specifically localized to the Muller cell microvilli which appose the interphotoreceptor matrix. Western blotting showed that the anti-CD44 is specific for a protein of approximately 90 kilodaltons which is the correct molecular weight for CD44H, the most abundant form of CD44. These results demonstrate the presence of CD44 in Muller cell microvilli and suggest that CD44 could play a role in mediating the attachment of the neural retina to components of the interphotoreceptor matrix.

(Supported by NIH grant EY-06590 and by a grant from the National Retinitis Pigmentosa Foundation, Inc.).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

Diurnal Expression of a "zinc-finger" DNA-binding protein, NGF1-A mRNA in retinal degeneration slow (*rds*) Mutant Mouse Retina.

Neeraj Agarwal, and Katherine O'Rourke
Department of Anatomy and Cell Biology
North Texas Eye Research Institute
University of North Texas Health Science Center, Fort Worth, TX 76107

The *retinal degeneration slow (rds)* mutant mouse is a model for studying the retinal dystrophy for human disease, retinitis pigmentosa (RP). To continue our effort towards a possible mechanism of photoreceptor cell death in retinal dystrophies, we have studied the impact of the *rds* mutation on diurnal expression of a "zinc-finger" DNA-binding protein, NGF1-A mRNA in the isolated retinas of *rds* mutant mice compared to those of BALB/c mice. Background levels of NGF1-A mRNA were maintained during the subjective light period. Higher levels of NGF1-A mRNA were observed immediately after the light offset and peaked two hours into the light offset for both the BALB/c and the *rds* mutant retinas and remained higher for several hours in the dark. If the animals were left continuously in light during the subjective dark period, NGF1-A mRNA levels were not induced and remained lower. On the other hand NGF1-A mRNA levels were transiently induced during the transition of the dark to light phase. These data suggest that NGF1-A mRNA is differentially regulated by light and dark stimuli in the retina and an absence of rod outer segments in the *rds* mutant retina does not alter the normal diurnal cycle of NGF1-A mRNA expression.

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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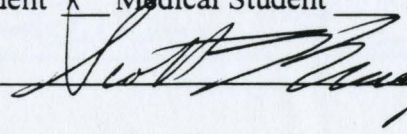
Signature: 

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

THE EFFECTS OF MESO-TETRA-(4-SULFONATOPHENYL)-PORPHINE DIHYDROCHLORIDE (TPPS) ON THE SUCEPTABILITY OF THE SPRAGUE DAWLEY RAT TO LIGHT-INDUCED RETINAL DAMAGE.

D.S. Krueger, M.S., S.A. Clark, S.A. English and R.L. Collier, Ph.D. UNT Health Sciences Center at Fort Worth, Dept. of Anatomy and Cell Biology, Fort Worth, Texas 76107 and Alcon Laboratories, Retinal Research, Fort Worth, Texas 76134.

Light-induced retinal damage is believed to be associated with the ability of energies of light to generate singlet oxygen and associated free radicals which subsequently react with intra-cellular molecules and thereby disrupt cellular structure and metabolism. Porphrins are endogenous compounds which are recognized to be photosensitizers capable of reducing the energy requirements for the production of singlet oxygen. TPPS is a synthetic porphyrin which does not occur in nature. The effects of TPPS administered intra-peritoneally (IP) on the energy requirements for induction of retinal light damage in the albino Sprague Dawley rat under 24 hour continuous light exposure were studied. Eight rats, four receiving TPPS at 5 mg/Kg and four receiving an equivalent volume of saline, were dosed IP on days 1, 2, and 3. The animals were dark adapted for 24 hours following dosing on day 2 and were placed under 24 hour continuous light exposure (800 mW) following dosing on day three. On day four the animals were placed in the dark for a 24 hour recovery period after which time the eyes were harvested and fixed with 2% glutaraldehyde/2% paraformaldehyde. Following fixation, the eyes were prepared and mounted in JB4. The eyes were examined using light microscopy (multiple stain) and retinal layers measured to assess difference in light damage from historical non-exposed controls. The results confirm that eyes of TPPS treated rats are more susceptible to continuous light damage than the eyes of saline treated rats. (Supported by Alcon Laboratories, Inc.)

Wound Healing Institute

34. S. Dan Dimitrijevich, Ph.D. Cutaneous Wound Healing Under Hyperbaric Conditions. Part I. Epidermalization of the Human Skin Equivalent

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CUTANEOUS WOUND HEALING UNDER HYPERBARIC CONDITIONS. PART I. EPIDERMALIZATION OF THE HUMAN SKIN EQUIVALENT

S. Dan Dimitrijevich*, J. Wilson, L. Cooper*, J. G. Mills *** and R. W. Gracy*, Depts. of *Biochem. & Mol. Biol.,**Physiol., UNTHSC af Ft. Worth, and ***Hyperbar. Med., Osteopathic Center of North Texas.**

A critical stage in cutaneous wound healing is the development and maturation of the epidermis. In the aged, as well as in some pathologies (e.g. diabetes), this repair process is compromised. *In vivo* studies with humans are difficult and often ethically unacceptable; however, the human Skin Equivalent (SE), provides an excellent opportunity for studying wound healing *in vitro*. Particularly helpful are the benefits resulting from the exclusion of the systemic complications encountered *in vivo*.

The goal of this project is to examine the effect of hyperbaric oxygen on epidermopoiesis of the SE. Toxic effect on human fibroblasts, as monolayers and in dermal equivalents, were observed with hyperoxia but not periodic hyperbaria (10 consecutive daily treatments of 90 min duration). In contrast oxygen pressures up to 3 At. were non-toxic to established monolayers of keratinocytes. The effects on proliferation and epidermal differentiation in the SE were explored over a wide range of oxygen concentrations. The results show a striking stimulation of differentiation and provide further support for oxygen requirements and utilization in peripheral human tissues.

Supported by grants from TIREA, Texas Adv. Tech Program (09768-008). and NIA (AGO1274).

Substance Abuse Institute of North Texas

35. Sue Gena Lurie, Ph.D.	Community Substance Abuse Intervention-Advocacy Planning
36. Douglas Lytle	Tolerance and Cross-Tolerance Patterns Between Ethanol and Diazepam in Rats Trained to Detect Ethanol
37. Beatriz de A. Rocha, Ph.D.	The 5-HT_3 Antagonists Ondansetron (GR 38032) and ICS 20593 Do Not Block the Discriminative Stimulus of Ethanol in Rats
38. Cynthia McCuistion Taylor	Seven Months of Ethanol Exposure Has Little Lasting Effect on Spatial Discrimination Learning and Sensorimotor Skills of Mice
39. S. Mehdi Rezazadeh, Ph.D.	Ethanol Inhibits Forskolin-Stimulated Cyclic AMP Formation in Human Neuroblastoma Cells
40. Rachel Peltier	CNS Stimulants Produce Cross-Tolerance to Cocaine in a Self-Administration Paradigm
41. Donghang Li	Some CNS Stimulants Produce Cross-Tolerance to Cocaine Under a Progressive Ration Paradigm
42. Michael J. Forster, Ph.D.	Predictive Validity of Locomotor Activity Analysis for Action of Dopamine Receptor Antagonists on the Cocaine Discriminative Stimulus
43. Cleatus J. Wallis, Ph.D.	1-(3-Chlorophenyl)-Piperazine (MCPP) Discrimination in Rats as an Animal Model of Anxiety
44. Y. Egilmez, Ph.D.	Enhanced *In Vitro* Sensitivity of Stearate-Modified Carbon Paste Electrodes Following 48 Hour Brain Implantation

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

COMMUNITY SUBSTANCE ABUSE INTERVENTION-ADVOCACY PLANNING

Sue Gena Lurie, Ph.D., Department of Medical Humanities,
Substance Abuse Institute of North Texas
UNT Health Science Center, Fort Worth, Texas 76107

(a) Purpose: The project purpose was to develop a five-year plan for substance abuse prevention, intervention and advocacy, to be implemented by community service providers, associations and policy makers. Strategic planning was coordinated with staff and community committee for Challenge, Incorporated, Tarrant County advocacy and planning organization for substance abuse intervention and education programs, and Robyn Weise, UTA social work intern. (b) Materials and methods: (1) The preliminary plan was drafted for the planning committee on: extent of problem, services, funding, coordination, effectiveness, local target areas, special groups, social issues, and policy. The plan integrated data from: Tarrant Council on Alcoholism, United Way Needs Assessment, Challenge, Arlington Substance Abuse Project Data Bank, Texas Christian University research, local service agencies, state and national reports. (2) Community meetings were conducted over a five-month period with committee and agency members to prioritize problems in: education, intervention, coordination, support, and policy. Sub-groups evaluated experts' and agencies' presentations on effective strategies for prevention, treatment, and control. The committee drafted planning goals for Tarrant County programs, policy and management. (3) Strategic Plan was developed from these goals, using the Health Promotion/Disease Prevention format: rationales, goals, strategies. (c) Results and conclusions: The community planning process resulted in analysis and evaluation of relevant substance abuse policy, prevention-education, service coordination, control strategies, and goal development.
(SAINT service project.)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: DOUGLAS LYTLE
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Signature: Lytle

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

TOLERANCE AND CROSS-TOLERANCE PATTERNS BETWEEN ETHANOL AND DIAZEPAM IN RATS TRAINED TO DETECT ETHANOL. D.A. Lytle and M.W. Emmett-Oglesby. Department of Pharmacology, University of North Texas HSC, S.A.I.N.T., Fort Worth, TX 76107-2699.

The present experiment tested the hypothesis that cross-tolerance between ethanol (EtOH) and diazepam (DZP) would occur in an EtOH discrimination. Rats were trained to detect EtOH (1.0 g/kg, i.p.) from saline using a two-lever choice procedure where food was available under a fixed-ratio 10 (FR10) schedule of reinforcement. After subjects had met training criteria, dose-effect curves for EtOH (0.1 - 1.78 g/kg) and DZP (0.32 - 10.0 mg/kg) substitution for EtOH were determined. Subsequently, chronic administration of EtOH (5.0 g/kg/12 hrs for 7 days) or DZP (20 mg/kg/8 hrs for 7 days) occurred. Following termination of these regimens, substitution patterns for EtOH or DZP were re-determined. Acutely, EtOH and DZP substituted fully for EtOH in a dose-dependent manner. Chronic administration of EtOH resulted in 3-fold tolerance to EtOH and a six-fold cross-tolerance to DZP substituting for EtOH. Conversely, chronic administration of DZP did not confer in tolerance to DZP substitution for EtOH, nor cross-tolerance to EtOH. The degrees of tolerance to EtOH and cross-tolerance to DZP following chronic administration of EtOH suggests that tolerance to EtOH is mediated, in part, by tolerance occurring at the GABA/BZD complex. Previous reports have demonstrated that the chronic dose of DZP used in this experiment confers tolerance to benzodiazepines (BZD) substituting for other BZDs (Pugh *et al.*, 1992). The lack of tolerance to DZP substituting for EtOH following this same regimen of DZP suggests that the mechanism of DZP substituting for EtOH is different than the mechanism that mediates DZP substitution for BZD.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Beatriz de A. Rocha
Department/Institute: Pharmacology
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Signature: _____

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

THE 5-HT_3 ANTAGONISTS ONDANSETRON (GR 38032) AND ICS 20593 DO NOT BLOCK THE DISCRIMINATIVE STIMULUS OF ETHANOL IN RATS.

B.A. Rocha, D.A. Lytle and M.W. Emmett-Oglesby.
Department of Pharmacology and S.A.I.N.T., University of North Texas Health Science Center, Fort Worth, TX 76107.

Using pigeons as subjects, Grant and Barrett (Psychopharmacology, 1991) have reported that 5-HT_3 receptor antagonists block the discriminative stimulus effects of ethanol (EtOH). Because drug discrimination in animals has high predictive validity for subjective effects of drugs in humans, those results suggest that 5-HT_3 receptor antagonists might be efficacious in the treatment of alcohol disorders. This experiment tested the hypothesis that the selective 5-HT_3 receptor antagonists ondansetron and ICS 205930 would block the detection of the discriminative stimulus produced by EtOH in rats. Male Long-Evans rats were first trained to discriminate an i.p. injection of EtOH (1g/Kg) in a two-lever choice procedure with food as a reinforcer. Subsequently, they were tested for the substitution of various doses of EtOH (0.1-1.78 g/kg) using a cumulative dosing procedure. In this procedure, a small dose of EtOH (0.1 mg/kg) was injected, and 10-min later rats were allowed to make a lever selection. This procedure was continued until EtOH-lever selection occurred for at least 80% of subjects. Subsequently, the effects of ondansetron (0.01 mg/kg) and ICS 205930 (0.3-1 mg/kg) on EtOH discrimination were evaluated. These compounds were given 5 min before the first injection of EtOH, after which EtOH dose-effect curves were redetermined as described above. No dose of ondansetron or ICS 205930 significantly antagonized the discriminative stimulus produced by EtOH; thus, rats and pigeons may differ in their usefulness for predicting the EtOH-blocking effects of 5-HT_3 receptor antagonists.

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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

SEVEN MONTHS OF ETHANOL EXPOSURE HAS LITTLE LASTING EFFECT ON SPATIAL DISCRIMINATION LEARNING AND SENSORIMOTOR SKILLS OF MICE. C.M.Taylor, D.E. Krug, H.Lal, M.J. Forster, Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107.

Chronic alcohol consumption has been shown to have a deleterious effects on the central nervous system and cognitive functions in rodents (Arendt, Neurosci., **33**, 3, 435-463, 1989; Freund, J. Pharmaco. Exp. Ther., **179**, 2, 284-292, 1971). Hippocampal damage has been associated with spatial memory deficits, and because ethanol is known to damage the hippocampus (Freund, Science, **168**, 1599-1601, 1970.), it was expected that a spatial discrimination deficit would be found in mice which chronically consume ethanol. To test this hypothesis, we examined the effect of chronic ethanol on place learning in a water maze test. In order to test the generality of ethanol effects, additional tests for cognitive and sensorimotor abilities were performed. Male C57Bl/6J mice, 3 months of age, were maintained on nutritionally complete liquid diet as the sole source of nutrients and water for seven months. The diet contained 42.2 mg/ml ethanol while control groups received either an equivalent liquid diet with dextrin/sucrose (2.4:1) in amounts isocaloric to the ethanol used in the test group or laboratory mouse chow and water. After seven months of ethanol exposure, the mice were stepped off ethanol gradually over a two week period. Testing began after an additional two weeks of lab chow feeding. In the water maze test, spatial discrimination performance was measured using a procedure which required the mouse to locate a hidden platform in a circular tank of opacified water. The sensorimotor testing series included tests for simple reflexive capacities, spontaneous activity, and coordinated running. Analysis of data failed to reveal significant differences between the ethanol and the dextrin/sucrose control groups. However, there was a significant effect seen due to liquid diet when compared to the chow controls. These findings suggest that diet exposure, but not ethanol per se, adversely affects spatial discrimination performance. (Supported by NIH grant AA09567 (H.L.).)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ETHANOL INHIBITS FORSKOLIN-STIMULATED CYCLIC AMP FORMATION IN HUMAN NEUROBLASTOMA CELLS.

S. Mehdi Rezazadeh, Harbans Lal, and Michael W. Martin. Department of Pharmacology, Univ. of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie, Ft. Worth Texas, 76107.

Previous reports have shown that ethanol (ETOH) can enhance adenylate cyclase (AC) activity and potentiate the cyclic AMP (cAMP) response to receptor stimulation. Acute ETOH also increases extracellular adenosine by blocking its reuptake. Thus, adenosine by stimulating A2 adenosine receptors, may be responsible for enhanced cAMP responses in the presence of ETOH. The studies described here were designed to clarify whether ETOH directly modifies the AC system independent of its effects on extracellular adenosine levels. The ATP pool of human SK-N-SH neuroblastoma cells was radioactively labeled by pre-incubating the cells with 3H-adenine for 2 hr in serum-free DMEM followed by a 10 min incubation in fresh medium containing the phosphodiesterase inhibitor, Ro 20-1724, and adenosine deaminase (2U/ml). Under these conditions, negligible extracellular adenosine is present. Intracellular cAMP accumulation was stimulated by 10 min drug challenges followed by Dowex-alumina column chromatography to isolate 3H-ATP and 3H-cAMP. Forskolin (FSK) stimulates AC activity by promoting Gs-catalytic subunit coupling. In SK-N-SH cells, FSK increased cAMP levels 30-40 fold with an EC50 of 6 μM. Ethanol (>80 mM) inhibited FSK-stimulated cAMP synthesis in a concentration-dependent manner. Cyclic AMP accumulation was significantly stimulated in SK-N-SH cells by the selective A2 adenosine agonist CGS 21680 (CGS) and the β-adrenergic receptor agonist isoproterenol (ISO). However, neither the potency nor the efficacy of these agonists was affected by the presence of 160 mM ETOH. In the presence of 1μM FSK, receptor-dependent stimulation of cAMP synthesis by either CGS or ISO was markedly potentiated. ETOH (160 mM) significantly inhibited the response to both CGS and ISO measured in the presence of 1 μM FSK. The inhibitory effect of ETOH was not attenuated after chronic exposure of cells to ETOH (80 or 160 mM) for 2-48 hrs., an indication that tolerance does not develop to this effect. Thus, ETOH inhibits FSK-dependent, but not receptor-dependent, AC activity in a model neural cell line and tolerance does not develop to this pharmacologic effect. These results suggest that ETOH may alter the interaction between Gs and the cyclase catalytic subunit. (Partially supported by NIAAA grants No. AA06890 and AA09567).

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Rachel Peltier
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Signature: Rachel Peltier

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CNS STIMULANTS PRODUCE CROSS-TOLERANCE TO COCAINE IN A SELF-ADMINISTRATION PARADIGM. R.L. Peltier and M.W. Emmett-Oglesby. Department of Pharmacology, S.A.I.N.T., University of North Texas HSC at Fort Worth, Fort Worth, TX 76107-2699.

The experiment determined whether the chronic administration of *d*-amphetamine or *meth*-amphetamine would result in cross-tolerance to cocaine in a self-administration paradigm. Rats were implanted with indwelling jugular catheters and were allowed to self-administer cocaine (0.25 mg/injection) on an FR2 schedule of reinforcement, 15 reinforcers each day, until stable baseline responding was observed. A dose-response curve for cocaine self-administration was then obtained for each rat using a multi-dose procedure. This procedure employees an FR2 schedule with a maximum of 24 reinforcers. The reinforcers are divided into three blocks of eight with each block of reinforcers providing a different dose of cocaine (i.e. reinforcers 1-8=0.5 mg/inj, 9-16=0.25 mg/inj, 17-24=0.125 mg/inj). After dose-response data was obtained, rats were randomly assigned to one of two groups. One group then received injections of *d*-amphetamine (0.32, 1.0 or 3.2 mg/kg, s.c.) two times daily for seven days, while the other group received injections of *meth*-amphetamine (0.32, 1.0 or 3.2 mg/kg, s.c.) two times daily for seven days. This was a random block design and each rat received all doses of one drug. During this chronic regimen, the rats were not allowed to self-administer cocaine. Twenty-four hours following the last chronic injection, a cocaine dose-response curve was then obtained. Following seven days without testing or training, all rats spontaneously returned to baseline rates of cocaine self-administration, after which time they were treated chronically with another dose of either *d*- or *meth*- amphetamine. There was a significant shift to the right of the post-chronic dose response curves for cocaine self-administration at the highest dose tested (3.2 mg/kg) for both *d*-amphetamine and meth-amphetamine. These data show that chronic treatment with a CNS stimulant of the amphetamine type produces dose dependent cross-tolerance to cocaine in a self-administration paradigm. Supported by NIDA RO1 4137.

University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

SOME CNS STIMULANTS PRODUCE CROSS-TOLERANCE TO COCAINE UNDER A PROGRESSIVE RATIO PARADIGM. D.-H. Li and M.W. Emmett-Oglesby. Dept. of Pharmacol., University of North Texas HSC, Fort Worth TX 76107-2699.

The purpose of this experiment was to determine whether chronic administration of *d*-amphetamine or methamphetamine would result in cross-tolerance to cocaine under a progressive ratio (PR) schedule of reinforcement. Rats were implanted with indwelling jugular catheters and were trained to self-administer cocaine, 0.25 mg/infusion, under a PR schedule. Under the PR schedule, an increasing number of responses was required to obtain each subsequent cocaine infusion. The required ratio needed to be completed within 1 hr, and the last cocaine injection that was received was termed the breaking point. When the breaking point for each subject was stable, a cocaine dose-response curve was determined. The subjects were then randomly assigned to two groups. One group received injections of *d*-amphetamine (3.2 mg/kg, s.c.) or saline three times a day for seven days, while the other group received injections of methamphetamine (3.2 mg/kg, s.c.) or saline twice a day for seven days. This was a random block design and each rat received all doses of each treatment. During this chronic regimen, the rats were not allowed to self-administer cocaine. Twenty-four hours following the last chronic injection, cocaine dose-effect data were redetermined in both treatment groups. Both the *d*-amphetamine and the methamphetamine group showed a significant shift to the right of the post-chronic dose response curves of breaking points for cocaine self-administration. These data support the hypothesis that chronic treatment with a CNS stimulant of the amphetamine type will produce cross-tolerance to the reinforcing efficacy of cocaine. Supported by DA RO1 4137.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Michael J. Forster
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

PREDICTIVE VALIDITY OF LOCOMOTOR ACTIVITY ANALYSIS FOR ACTION OF DOPAMINE RECEPTOR ANTAGONISTS ON THE COCAINE DISCRIMINATIVE STIMULUS

M.J. Forster, R.H. Handley, C.M. Taylor, M.E. Stokely and M.W. Emmett-Oglesby. Department of Pharmacology, S.A.I.N.T., University of North Texas HSC, Fort Worth, Tx 76107-2699.

The D_1/D_2 receptor antagonist flupentixol, the D_1 antagonist, SCH 23390, and the D_2 antagonist, sulpiride were tested for their ability to antagonize spontaneous and cocaine-induced locomotion of mice using a Digiscan apparatus. A ratio of the potency for reduction of cocaine-induced locomotor stimulation (AD_{50}) versus potency for reduction of spontaneous activity (ID_{50}) was calculated for each drug. These ratios were compared with the ability of each compound to produce a shift in the cumulative dose-effect for substitution of cocaine in a group of rats trained for cocaine discrimination. Flupentixol and SCH 23390 had ID_{50}/AD_{50} ratios of 2.8, and 3.1, respectively, whereas sulpiride had a ratio approaching unity. In discrimination studies, sulpiride had only a marginal influence upon the cocaine stimulus, whereas SCH 23390 and flupentixol produced a 2-3-fold shift to the right in the cocaine dose-effect curve. These findings suggest that low ID_{50}/AD_{50} ratios may identify compounds with low efficacy for modification of the discriminative stimulus produced by cocaine.
[Supported by U.S.D.H.H.S.P.H.S. grant RO1-DA-4137 and contract NO1-DA-2-9305.]

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Cleatus J. Wallis
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

1-(3-CHLOROPHENYL)-PIPERAZINE (MCPP) DISCRIMINATION IN RATS AS AN ANIMAL MODEL OF ANXIETY. C. J. Wallis, M. Selvig, and H. Lal Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107.

mCPP is a serotonin (5HT) receptor binding ligand that acts as a potent agonist at $5HT_{1b/1c}$ receptors and weakly at $5HT_2$ receptors. In humans, mCPP has been reported to produce an interoceptive state described as "anxiety" by normal subjects. In rats, mCPP produces "anxiety-like" behaviors in the elevated plus-maze (EPM), the dark-light box (DLB) and in a social interaction test. To date the only animal model which tests for an interoceptive "anxiety" state is a two lever discrimination task in which rats are trained to respond on one lever after a saline injection and on another lever after injection with the anxiety producing drug, pentylenetetrazol (PTZ). mCPP substitutes for PTZ (16 mg/kg) with an ED_{50} of 0.8 mg/kg. This agrees well with the ED_{50} for mCPP to reduce time spent on the open arms of the EPM (1.02 mg/kg) and to reduce time spent in the light side of the DLB (0.8 mg/kg). Because the PTZ stimulus is resistant to serotonergic anxiolytic agents, it has not proven to be a good model to screen these medications, therefore, we have trained rats in a two lever discrimination between saline and mCPP (1.4 mg/kg) to test their potential for screening serotonergic anxiolytic agents. In rats trained to discriminate mCPP, N-(3 trifluoromethylphenyl)-piperazine (TFMPP), a serotonin agonist, fully substitutes for mCPP, while 2,5-dimethoxy-4-iodoamphetamine (DOI), a $5HT_{2/1c}$ agonist partially substitutes for mCPP. 1-naphthyl-piperazine (1-NP), a $5HT_{1b/1c}$ agonist and $5HT_2$ antagonist, does not substitute for mCPP. PTZ (16 mg/kg) fully substitutes for the mCPP stimulus, supporting the "anxiety-like" character of the mCPP stimulus. The mCPP stimulus is blocked in a dose related manner by methysergide, a $5HT_{2/1c}$ antagonist, that also blocks "anxiety-like" behavior in the EPM and DLB. These data indicate that mCPP discrimination in rats may prove to be a useful animal model for anxiety. Supported by NIAAA grants AA06890 and AA09567.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ENHANCED *IN VITRO* SENSITIVITY OF STEARATE-MODIFIED CARBON PASTE ELECTRODES FOLLOWING 48 HOUR BRAIN IMPLANTATION.

Y. Egilmez, M.W. Emmett-Oglesby and J.D. Lane Department of Pharmacology and S.A.I.N.T., University of North Texas Health Science Center, Fort Worth, TX 76107

This study tested the hypothesis that the sensitivity to dopamine (DA) of stearate-modified carbon paste electrodes (SGE) would increase following implantation in the rat brain. Semiderivative voltammetry (at 100 mV/sec scan rate) and chronoamperometry (1 sec pulse duration) were used to examine the electrochemical response characteristics of SGEs to dopamine (DA) in neutral phosphate buffered solutions before and after 48 hour insertion in the rat brain. The extent of electrocatalytic effect of ascorbate (AA) as well as the contribution of dihydroxyphenylacetic acid (DOPAC) on the electrochemical measurement of DA were also examined. SGEs showed a linear response to DA from 0.05 to 2 μM. Before implantation 1 μM DA produced 0.11 nA increase in the baseline current. The presence of 20 μM DOPAC had no effect on the linearity or the sensitivity of the electrodes to DA. Forty eight hour exposure to the rat brain produced an approximately 3-fold increase in the sensitivity of the SGEs to DA (0.41 nA/1 μM DA). While DOPAC (20 μM) produced a slight increase in the response of SGEs to DA (0.59 nA/1μM DA), the presence of AA (200 μM) significantly increased the DA signal resulting in a 4.4 nA change in the current to 1 μM DA. The present results show that exposure to brain tissue of SGEs enhances their sensitivity to DA . The results also suggest that the amount of DA signal amplification due to the electrocatalytic effect of physiological concentrations AA may help in detecting low *in vivo* concentrations of DA using SGEs.

Supported by NIDA grant RO1-DA-4137 and Texas Advanced Technology Award 3711.

Cardiovascular

45. Song-Jung Kim	α_1-Adrenergic Vasoconstriction Does Not Limit Coronary Vasodilation and Cardiac Output During Maximal Exercise
46. Song-Jung Kim	Intracoronary EDRF Blockade Limits Coronary Vasodilation and dP/dt_{max} During Submaximal Exercise
47. Rita M. Welch-O'Connor	Detraining Augments Cardiopulmonary Baroreflex Function
48. Kevin Gallagher	Cardiopulmonary Baroreflex Function Is Not Altered with Aging
49. Xiangrong Shi, Ph.D.	Carotid Baroreflex Function Is Not Enhanced with Unloading of the Cardiopulmonary Baroreceptors in Elderly Humans
50. Patricia A. Gwirtz, Ph.D.	Existence of a Coronary α_1-Adrenergic Constrictor Tone in Conscious Dogs After Development of Hypertension
51. Dan Manor, Ph.D.	Coronary Vascular Compression Increases with Elevated Venous Pressures
52. James B. Parker	Endothelial Derived Nitric Oxide Production Determines the Responses of Canine Coronary Resistance Arteries to Norepinephrine
53. Xiao-Juan Bai, M.D.	Mechanisms Affecting Coronary Blood Flow in Systematic Hypoxemia
54. Arthur Williams, Jr., Ph.D.	Adrenergic Blockade Has Minimal Effect on Coronary Vasodilation During Systemic Hypoxia
55. Toshihiro Iwamoto, M.D.	Alteration of Oxygen Supply/Demand Balance and Autoregulation of Right Heart
56. H. Fred Downey, Ph.D.	Perfusion-Related Increases in Myocardial Contractile Force and Systolic Myocardial Stiffness
57. Kristin Bryant	Contributions of Coronary Arteries to Coronary Sinus Drainage
58. Walter J. McConathy, Ph.D.	High Frequency of Autoantibodies to Phosphatidyl Serine in Hyperlipemia
59. Jay Shores, Ph.D.	Lipoprotein Lipids as Metabolic Indeces of the Efficacy of Gemfibrozil Therapy for Raising High Density Lipoprotein (HDL) Levels During Lopid Therapy
60. Andrew Reason, Ph.D.	Characterization of Plasma and Recombinant Lecithin: Cholesterol Acyltransferase (LCAT): I. Carbohydrate Structure
61. C. Nuckols	Characterization of Plasma and Recombinant Lecithin: Cholesterol Acyltransferase (LCAT): II. Catalytic Properties
62. L. Armstrong	The Effect of *Captopril* on the Oxidation of Plasma Lipoproteins
63. Thomas V. Fungwe, Ph.D.	Regulation of Secretion of Lecithin:Cholesterol Acyltransferase: Effect of Agents Influencing Hepatocyte Lipoprotein Secretion

64.	Thomas V. Fungwe, Ph.D.	Synthesis and Secretion of Apolipoprotein E in Primary Cultures of Hepatocytes: Effect of Oleic Acid
65.	Vuong N. Trieu, Ph.D.	Identification of an Apolipoprotein (a)/Lipoprotein (a) Binding Site on Apolipoprotein B
66.	James L. Caffrey, Ph.D.	Intrinsic Cardiac Enkephalins Inhibit Vagal Control of Cardiac Function in the Dog
67.	Zaira Mateo	Extraction and Measurement of Circulating and Myocardial Enkephalins in the Dog
68.	Barbara A. Barron, Ph.D.	Open Chest Sham-Operation Depresses Myocardial Catecholamines
69.	Wen-Lin Fan	Pyruvate Accelerates Diastolic Relaxation in Working Guinea-Pig Left Venricle
70.	Miao-Xiang He, M.D.	Disassociation of Cardiac Function from Cytosolic Phosphorylation Potential (log [ATP]/[ADP][Pi], PP, M^{-1}) During 2-Deoxyglucose (2DG) Infusion in Guinea Pig Hearts
71.	Zhiping Gao	Decreased Energy Demand in Ischemic Guinea Pig Myocardium
72.	A.J. Mia	SEM Studies of Comparative Membrane Remodeling by Endocytosis in Toad Urinary Bladders Following Withdrawal of ADH and MZ

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

α_1-ADRENERGIC VASOCONSTRICTION DOES NOT LIMIT CORONARY VASODILATION AND CARDIAC OUTPUT DURING MAXIMAL EXERCISE.

Song-Jung Kim and Patricia A. Gwirtz
UNT Health Science Center at Fort Worth, Ft. Worth, TX 76107

It has been shown that a coronary α_1-adrenergic vasoconstriction opposes local metabolic vasodilation during sympathetic stimulation, such as exercise. As a result, myocardial O_2 supply and contractile function are impeded. The present study tests the hypothesis that during maximal exercise (ME), a state of high oxygen demand, an α_1-constrictor tone still prevents attainment of maximal coronary dilation and, thus, limits cardiac output (CO). Four healthy mongrel dogs were chronically instrumented to measure left ventricular pressure (LVP), dP/dt_{max}, heart rate (HR), mean aortic pressure (AOP), circumflex blood flow (CBF), and cardiac output (CO) at rest and during ME. Bolus injection of prazosin (0.5 mg), a selective α_1-adrenergic antagonist, and adenosine (2.0 mg) were administered into the circumflex artery during ME. ME alone significantly increased all measured variables, including CBF (0.85 ± 0.09 to 2.4 ± 0.38 ml/min/g), dP/dt_{max} (2905 ± 168 to 8621 ± 432 mmHg/sec), HR (82 ± 8 to 267 ± 14 bpm) and CO (2.0 ± 0.3 to 10.1 ± 1.2 l/min). Surprisingly, prazosin did not result in any additional increases in CBF or CO during ME. However, adenosine increased CBF to 3.0 ± 0.2 ml/min/g. These results indicate that unlike submaximal level of exercise, a coronary α_1-adrenergic constrictor tone does not mobilize a coronary flow reserve during maximal level of exercise in the dogs. (Supported by NIH HL-34172)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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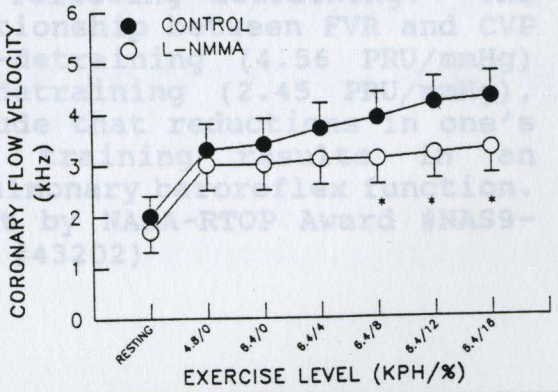
Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

INTRACORONARY EDRF BLOCKADE LIMITS CORONARY VASODILATION AND dP/dt_{max} DURING SUBMAXIMAL EXERCISE.

Song-Jung Kim and Patricia A. Gwirtz
UNT Health Science Center at Fort Worth, Ft. Worth, TX 76107

This study evaluates the role of endothelium-derived relaxing factor (EDRF) induced by shear stress in coronary circulation by selective intracoronary infusion (i.c.) of N^G-monomethyl-L-arginine (L-NMMA) during submaximal exercise (SME). Four healthy mongrel dogs were chronically instrumented to measure left ventricular pressure, dP/dt_{max}, heart rate (HR), mean aortic pressure (MAP), and coronary flow velocity (CFV) at rest and during SME. Each animal was subjected to the standardized treadmill SME regimen (control) and with L-NMMA (35 mg, i.c.) before recording resting and exercise data. Control and blockade experiments were conducted on separate days. During rest and running at 4.8 kph/0 % incline, MAP, HR, dP/dt, and CFV were not different between control and L-NMMA conditions. CFV and dP/dt_{max} increased as intensity of exercise increased in the control condition. After EDRF blockade with L-NMMA, CFV increased to control values at 4.8 kph/0 % incline, but did not increase any further despite increases in the exercise workload (Figure). During blockade at higher than 4.8 kph/0 % incline, CFV and dP/dt_{max} were significantly reduced compared to respective control values. These data indicate that EDRF, produced by flow-induced shear stress, plays a significant role in the regulation of coronary flow during submaximal exercise. (Supported by HL-34172)



SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

DETRAINING AUGMENTS CARDIOPULMONARY BAROREFLEX FUNCTION. R.M. Welch-O'Connor, X. Shi, K.M. Gallagher, B.H. Foresman, F. Schaller, L. Walder, and P.B. Raven, FACSM. Dept. of Physiology, UNT Health Science Center, Fort Worth, TX 76107

Six men and women (aged 22 to 40 years) volunteered to stop all forms of aerobic exercise training for a period of eight weeks (detraining). Prior to and following detraining cardiopulmonary baroreflex function, changes in forearm vascular resistance (FVR) per unit change in central venous pressure (CVP), was assessed during lower body negative pressure (LBNP). Detraining decreased maximal oxygen uptake 6 ± 1% (47.1 ± 2.6 to 44.6 ± 2.7 ml/kg/min, $P<0.05$). However, heart rate (HR), mean arterial pressure (MAP by Finapress), CVP, forearm blood flow (FBF by Whitney strain gauge) and FVR (ratio of MAP/FBF) at rest were unchanged pre- and post-detraining. Detraining did not affect the subjects' physiologic responses to -20torr LBNP, yet the change in FVR per unit change in CVP was greater ($P<0.05$) following detraining. The slope of the relationship between FVR and CVP was greater post-detraining (4.56 PRU/mmHg) compared to pre-detraining (2.45 PRU/mmHg), $P<0.05$. We conclude that reductions in one's aerobic exercise training results in an augmented cardiopulmonary baroreflex function.
(Supported in part by NASA-RTOP Award #NAS9-611 and NIH Grant #43202)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Kevin Gallagher
Department/Institute: Physiology
Graduate Student X Medical Student ____ Fellow ____ Intern ____ Faculty ____
Signature: _____

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CARDIOPULMONARY BAROREFLEX FUNCTION IS NOT ALTERED WITH AGING. K.M. Gallagher, X. Shi, S.A. Smith, R.M. Welch-O'Connor, and P.B. Raven, FACSM. Dept. of Physiology, UNT Health Science Center, Ft. Worth, TX 76107

Cardiopulmonary baroreflex function was assessed in eleven young (aged 25 to 38 yrs) and thirteen old (aged 60 to 69 yrs) men and women volunteers using forearm vascular resistance (FVR) response to the changes in central venous pressure (CVP, directly or indirectly measured) elicited by lower body negative pressure (LBNP) to -20torr. Baseline heart rate (HR), mean arterial pressure (MAP by Finapress), CVP, forearm blood flow (FBF, by Whitney strain gauge) and FVR (ratio of MAP/FBF) were not statistically different between the two groups, see table below:

	HR (bpm)	MAP (mmHg)	CVP (mmHg)	FBF (ml/100ml/min)	FVR (PRU)
Young	61±2	87±3	6.9±0.5	2.6±0.2	37±5
Old	56±3	90±3	8.3±0.4	2.7±0.2	36±3

LBNP -20torr did not alter HR and MAP, but significantly decreased CVP (young: 2.9±0.2 and old: 4.0±0.6 mmHg) and increased FVR (young: 48±5 and old: 53±5 PRU). The slope of changes in FVR to CVP was 3.7±1.2 PRU/mmHg of the young subjects and 3.8±0.7 PRU/mmHg of the old subjects (p=n.s.). We concluded that cardiopulmonary baroreflex control of forearm vasoconstriction was not altered with aging.

(Supported in part by a grant from the UNT Texas Institute for Research and Education on Aging)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Xiangrong Shi, Ph.D.
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CAROTID BAROREFLEX FUNCTION IS NOT ENHANCED WITH UNLOADING OF THE CARDIOPULMONARY BARORECEPTORS IN ELDERLY HUMANS. **X.Shi, K.M. Gallagher, and P.B.Raven.** Dept. of Physiology, UNT Health Science Center, Fort Worth, TX 76107.

Cardiopulmonary baroreceptors exert tonic inhibitory influence on the cardiovascular center. It has been demonstrated that in young individuals, the carotid baroreflex responsiveness is potentiated when this inhibitory influence is diminished. To investigate the aging effect on the interaction between cardiopulmonary baroreceptors with carotid baroreflex, eleven men and women ($\geq$60yrs) were evaluated using heart rate (HR) and mean arterial pressure (MAP, Finapres) responses to rapid pulsatile neck pressure and suction during control and lower body negative pressure (LBNP) of -15 torr. The sensitivity of carotid baroreflex (CBR) was assessed from the slopes of HR and MAP to carotid sinus pressure (CSP estimated from the difference between MAP and neck chamber pressure applied). LBNP significantly decreased the peripheral venous pressure from control 7.95$\pm$0.43 to 3.84$\pm$0.54mmHg at -15torr, whereas HR and MAP were not altered (60.1$\pm$3.5 vs. 60.2$\pm$3.8bpm and 97.8$\pm$2.8 vs. 93.6$\pm$2.6mmHg), $p > 0.05$. Furthermore, there was no significant difference between the control and LBNP in either HR-CSP slope (-0.125$\pm$0.027 vs. -0.124$\pm$0.029 bpm/mmHg) or MAP-CSP slope (-0.142$\pm$0.019 vs. -0.168$\pm$0.020 mmHg/mmHg). We conclude that the expected interaction between cardiopulmonary baroreceptors and CBR function is absent in elderly humans.

(Supported in part by a grant from the UNT Texas Institutefor Research and Education on Aging)

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Patricia A. Gwirtz, Ph.D.
Department/Institute: Physiology/UNT Health Science Center
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

EXISTENCE OF A CORONARY α_1-ADRENERGIC CONSTRICTOR TONE IN CONSCIOUS DOGS AFTER DEVELOPMENT OF HYPERTENSION.

Patricia A. Gwirtz, Song-Jung Kim, Abraham Heymann, and Linda Howard
UNT Health Science Center at Fort Worth, Ft. Worth, TX 76107

It has been shown that a coronary α_1-adrenergic coronary constrictor tone exists under conditions associated with increased sympathetic stimulation of the heart, but not during resting conditions in the normal heart. In hypertension associated with renovascular dysfunction, an elevation of circulation angiotensin II may enhance sympathetic stimulation of the heart even while at rest. This study was performed to test the hypothesis that an α-adrenergic constrictor tone imposes limitations on coronary blood flow in resting dogs after induction of renovascular hypertension. Left circumflex coronary artery blood flow velocity (CFV), aortic pressure (AoP), and heart rate (HR) were examined in five (5) quietly resting dogs during control conditions and after selective α_1-adrenergic blockade with intracoronary injection of 0.5 mg prazosin. In the normotensive state, mean AoP was 87 ± 7 (SD) mmHg, heart rate was 105 ± 25 bpm, and CFV was 28 ± 6 cm/sec. These values were not affected by prazosin. After induction of renovascular hypertension, plasma renin levels were increased two-fold, mean AoP was increased to 114 ± 7 mmHg, heart rate was 111 ± 28 bpm, and CFV was 21 ± 8 cm/sec. In contrast to the normotensive state, intracoronary prazosin caused a further 28 ± 6 % ($P < 0.05$) increase in CFV in the hypertensive dogs. Thus, it appears that renovascular hypertension results in an enhanced α-adrenergic coronary constriction in the resting dog.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Dan Manor
Department/Institute: Physiology / UNTHSC at Ft Worth
Graduate Student ___ Medical Student ___ Fellow ✓ Intern ___ Faculty ___

Signature: Dan Manor

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CORONARY VASCULAR COMPRESSION INCREASES WITH ELEVATED VENOUS PRESSURES.

Dan Manor, Sue Williams, Robert Ator, Kristin Bryant and Konrad W. Scheel
Department of Physiology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas, 76107.

The purpose of this study was to determine whether changes in coronary venous pressure affect coronary vascular compression. The study was conducted on isolated, blood perfused, dog heart preparation during autoregulation and maximal vasodilation with adenosine. Coronary venous pressure (P_V) was controlled via a column inserted into the right atrial/ventricular chamber, while the left ventricle was vented to atmospheric pressure. During maximal vasodilation an increase in P_V from 7±1 (control) to 15±1 and 24±1 mmHg resulted in a left circumflex artery (Circ) oscillatory flow amplitude increase of 11±4 and 25±7% relative to control, Circ systolic to diastolic flow ratio decrease of 12±5 and 31±6%, and mean Circ flow decrease of 6±1 and 16±2%, respectively ($p<0.05$, $n=8$). A Similar response was obtained for the left anterior descending artery at elevated P_V. Left ventricular (LV) function, as determined by changes in systolic LV pressure and maximal rate of change of LV pressure, changed significantly. Similar results were obtained during autoregulation. An elevation in right atrial pressure such as commonly seen during heart failure, raises coronary venous pressure and may result in coronary venous engorgement. This, in turn, may raise interstitial fluid pressure, resulting in an increase in the compressive pressure applied to the coronary vasculature. We conclude that elevated coronary venous pressures change the phasic nature of arterial inflow, indicating an increase in coronary vascular compression. (Supported by NIH Grant HL 35030).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: James B. Parker
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Signature: James B Parker

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

Endothelial derived nitric oxide production determines the responses of canine coronary resistance arteries to norepinephrine.

Parker, J.B., P.A. Gwirtz, C.E. Jones, and J.L. Caffrey. Dept. of Physiology, University of North Texas Health Science Center, Fort Worth, Tx. 76107.

Responses of isolated canine coronary resistance arteries (lumen diameter 121±23μm) to norepinephrine were evaluated after mechanical or pharmacological interruption of endothelial derived relaxing activity. Blockade of relaxing activity was verified by the absence of acetylcholine-mediated relaxation. Isolated arteries were mounted in a vessel chamber (Halpern), the lumens were pressurized to 40 mmHg at zero flow and the vessel diameters were determined with a video dimension analyzer (Living Systems). Maximal vessel lumen diameter (D_{max}) was determined in a Ca^{++} free medium and a reference vessel diameter (84±5.3% of D_{max}) was established by equilibration of vessels in a medium containing 2.0 mM Ca^{++}. Increasing norepinephrine concentrations added to the chamber produced a slight decrease in diameter (81±2.1% of D_{max}) in two vessels and a slight increase (89±4.2% of D_{max}) in four others. The overall changes were not significantly different from control (n = 6). Following blockade of endothelial derived nitric oxide production with 10^{-5} M N-Nitro-L-Arginine Methylester (L-NAME), norepinephrine produced a reduction in diameter to 56.6±2.3% of D_{max} ($p<0.05$) with an ED_{50} of 0.249±0.023 μM. After mechanical removal of the endothelial cell layer, norepinephrine produced a nearly identical reduction in diameter to 61.4±2.3% ($p<0.05$) with an indistinguishable ED_{50} (0.245±0.021 μM). These data suggest that the weak and equivocal response of coronary resistance arteries to norepinephrine results from the competitive dilatory influence of endothelial derived nitric oxide production and not to the absence of norepinephrine receptors.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Xiao-Juan Bai, M.D.
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Signature: H. F. Downey for X J Bai (Dr. Bai is in China)

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

MECHANISMS AFFECTING CORONARY BLOOD FLOW IN SYSTEMIC HYPOXEMIA. X.-J. Bai, G. J. Crystal, A. G. Williams, Jr., T. Iwamoto, and H. F. Downey. Univ. N. Tex. Hlth. Scien. Ctr., Ft. Worth, TX 76107 and Univ. Ill. Col. of Med., Chicago, IL 60657.

Studies were conducted in six anesthetized, atropinized, open-chest dogs in whom LAD was perfused with normoxic (CaO_2 = 18.4 - 20.4 ml O_2/100 ml) or moderately hypoxic (CaO_2 = 7.8 - 8.1 ml O_2/100 ml) blood with a constant pressure system. Mean aortic pressure and heart rate were held constant, and LV dP/dt_{max} (mmHg/s) was measured. In LAD bed, blood flow (CBF, ml/min/100 g) and O_2 consumption (MVO_2, ml/min/100 g) were measured before (Pre-B) and after alpha (phenoxybenzamine) and beta (propranolol) adrenergic blockade (Post-B) during normoxia (N), local hypoxemia (LH), and LH + systemic hypoxemia (LH + SH). Results are Mean ± SE; $P < 0.05$, * vs. N; § vs LH; + vs Pre-B.

		Normoxia	LH	LH + SH
CBF	Pre-B	73±5	239±25*	336±38*§
	Post-B	83±5	241±31*	290±31*§
MVO_2	Pre-B	10.3±1.0	9.7±0.6	13.1±1.0*§
	Post-B	7.5±0.4+	8.2±0.5	8.4±0.8+
dP/dt_{max}	Pre-B	2400±180	2500±200	3170±180*§
	Post-B	1720±120+	1800±120+	2170±200*

Conclusions: 1) Metabolic vasodilation secondary to increased contractility via adrenergic pathway contributes significantly to increased CBF during SH. 2) Local vasodilator effects of hypoxic blood account for much of the increase in CBF during SH. This mechanism is independent of local activation of adrenergic receptors. 3) An additional mechanism may contribute to coronary dilation during SH. (Supported by NIH grants HL35027 and HL47629)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Arthur G. Williams, Jr.
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Signature: Arthur G. Williams, Jr.

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ADRENERGIC BLOCKADE HAS MINIMAL EFFECT ON CORONARY VASODILATION DURING SYSTEMIC HYPOXIA. A. G. Williams, Jr., X.-J. Bai, T. Iwamoto, and H. F. Downey. University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

The role of hypoxia per se in the coronary vasodilatory response to systemic hypoxia is controversial. Studies were conducted in five anesthetized, atropinized, open-chest dogs ventilated with air containing normal (N) or reduced O_2 (H). Coronary blood flow (ml/min/100 g) was measured by electromagnetic flowmeter on LAD before (pre-B) and after alpha (phenoxybenzamine) and beta (propranolol) adrenergic blockade (Post-B). Systemic arterial blood pressure and heart rate were held constant, and LV dP/dt_{max} (mmHg/s) and regional MVO_2 (ml O_2/min/100 g) were measured. Arterial O_2 content was 19.2-21.2 ml O_2/100 ml during N and 8.6-8.7 ml O_2/100 ml during H. Results, mean and (SE):

	Pre-B, N	Pre-B, H	Post-B, N	Post-B, H
CBF	59.6 (7.8)	157[a] (24)	61.8 (7.4)	139[a] (16)
dP/dt_{max}	2800 (90)	3920[a] (240)	1840[b] (230)	2590[a,b] (410)
MVO_2	8.82 (1.02)	11.3[a] (1.06)	7.13 (0.99)	8.88[b] (0.78)

[a] $P < 0.05$ vs respective N; [b] $P < 0.05$ vs respective Pre-B

Although adrenergic blockade attenuated the hypoxia-induced increase in contractile function and MVO_2, coronary blood flow was not significantly reduced.

(Supported by NIH grant 35027)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Toshihiro Iwamoto, M.D.
Department/Institute: Physiology/UNT Health Science Center
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ALTERATION OF OXYGEN SUPPLY/DEMAND BALANCE AND AUTOREGULATION OF RIGHT HEART. T. Iwamoto, X.-J. Bai, A. G. Williams, Jr., and H. F. Downey. University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

Myocardial oxygen tension has been suggested to be a determinant of coronary vascular resistance (CVR) and autoregulation in left heart. The effects of the alterations in oxygen supply or demand on coronary perfusion pressure (CPP)-induced changes in CVR, i.e., autoregulation, were studied in right coronary artery (RCA). In pentobarbital anesthetized dogs, RCA blood flow (CBF; $ml \cdot min \cdot g^{-1}$) was measured, CVR ($mmHg \cdot min \cdot g \cdot ml^{-1}$) was calculated, and autoregulation was examined at CPP 60-160 mmHg. CBF and CVR were analyzed as logarithmic values and expressed as geometric means. When oxygen demand was increased by changing heart rate (60 to 180 bpm, Protocol 1, n = 8) and when oxygen supply was lowered by hypoxic perfusion (PAO_2 = 35.9 mmHg, Protocol 2, n = 8), CVR decreased, but autoregulation was not significantly affected. CVR was linearly correlated with PVO_2 when CPP was below 100 mmHg in both protocols, but the threshold for autoregulatiion was independent of $PVO._2$.

	HR = 60						HR = 180					
CPP	**60**	**80**	**100**	**120**	**140**	**160**	**60**	**80**	**100**	**120**	**140**	**160**
CBF	**0.33**	**0.40**	**0.47**	**0.57**	**0.68**	**0.84**	**0.49**	**0.59**	**0.69**	**0.81**	**0.97**	**1.18**
CVR	**127**	**157**	**175**	**180**	**180**	**169**	**87**	**106**	**119**	**126**	**126**	**121**
PVO_2	**28.1**	**30.0**	**32.9**	**37.5**	**40.2**	**46.1**	**24.5**	**26.5**	**28.6**	**32.0**	**36.1**	**38.4**
	Normoxia						**Hypoxia**					
CPP	**60**	**80**	**100**	**120**	**140**	**160**	**60**	**80**	**100**	**120**	**140**	**160**
CBF	**0.34**	**0.42**	**0.50**	**0.63**	**0.79**	**0.99**	**0.58**	**0.72**	**0.88**	**1.08**	**1.32**	**1.55**
CVR	**120**	**144**	**161**	**162**	**153**	**142**	**71**	**85**	**92**	**93**	**92**	**91**
PVO_2	**25.2**	**27.2**	**29.3**	**33.0**	**37.3**	**41.2**	**19.8**	**21.2**	**21.8**	**23.5**	**25.2**	**26.8**

In right heart, 1) changing oxygen supply/demand balance does not modify autoregulation, 2) myocardial oxygen tension does not directly determine autoregulatory range. (Supported by NIH grant 35027)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: H. Fred Downey, Ph.D.
Department/Institute: Physiology/UNT Health Science Center
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

PERFUSION-RELATED INCREASES IN MYOCARDIAL CONTRACTILE FORCE AND SYSTOLIC MYOCARDIAL STIFFNESS. H. F. Downey, T. Iwamoto, X.-J. Bai, and A. G. Williams, Jr. University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

The mechanism by which changes in coronary perfusion alter myocardial oxygen consumption (MVO_2; Gregg Phenomenon) is controversial. To test the effect of coronary perfusion on systolic myocardial stiffness, the loop inscribed by regional myocardial segment length (SL, piezoelectric crystals) and developed force (F, miniature force transducer) was analyzed during selective perfusion of the LAD. $\Delta F/\Delta SL$ during the ejection period was used as a index of internal resistance, which reflects systolic myocardial stiffness. MVO_2 was calculated from arteriovenous O_2 difference and LAD flow. When coronary perfusion pressure (CPP) was varied from 60 to 180 mmHg (protocol 1, n = 11), coronary blood flow (CBF), maximal developed force (F_{max}), $\Delta F/\Delta SL$, and MVO_2 significantly increased with CPP ($P<0.05$), whereas end-diastolic (EDL) and end-systolic length (ESL), segmental shortening (SS), and other systemic hemodynamic parameters stayed constant. In protocol 2 (n = 8), after the baseline measurement (COND A), CBF was doubled by low dose adenosine infusion (COND B), and then maximally dilated by increasing adenosine (COND C). F_{max}, $\Delta/\Delta SL$, and MVO_2 increased by adenosine infusion ($P<0.05$), whereas the other parameters stayed constant. The present study showed that 1) increased CBF with or without increase in CPP enhances myocardial contractile force, systolic myocardial stiffness, and MVO_2 in intact, ejecting hearts, 2) CBF-induced changes in myocardial contractile force and systolic myocardial stiffness, but not EDL, are probably responsible for CBF-related changes in MVO_2. (Supported by NIH grant 35027)

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Kristin Bryant
Department/Institute: Physiology
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Signature: Kristi Bryant

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CONTRIBUTIONS OF CORONARY ARTERIES TO CORONARY SINUS DRAINAGE

Kristin Bryant, James Parker, Sue Williams, and Konrad Scheel
University of North Texas Health Science Center
Fort Worth, Texas 76107

The purpose of this study was to determine the percentage of total coronary sinus (CS) effluent that is contributed by each of the major canine coronary arteries, the left anterior descending (LAD), left circumflex (LCX), right (RT), and septal (SEP) arteries. Also, the percentage of each coronary artery's inflow which eventually drains via the coronary sinus into the right atrium was determined. To achieve these measurements, inflow to the myocardium was occluded in all but one artery and coronary sinus effluent was collected for 30 seconds in a graduated cylinder. Dividing the value for CS effluent due to one artery by the value for CS effluent when all arteries are open yields the percentage contribution of each artery to CS drainage. One must also take into account, however, that an individual artery's flow may be increased by the occlusion of another artery. Therefor, the following data for the contribution of each artery to CS effluent may be taken as an upper limit. When all four coronary arteries were perfused simultaneously, CS effluent accounted for 58.7 ±7.4 % of the combined total inflow. Of the total outflow from the CS cannula, the LCX and LAD contributed the majority of the flow at 47.5 ±7.6 % and 45.8 ±4.4 %, respectively. To further illustrate this point, the LCX and LAD arteries were kept open simultaneously with the right and septal artery flow lines occluded. In this case, the combined LCX and LAD inflows accounted for 85.7 ±9 % of total CS outflow. The septal artery alone contributed 5.5 ±2.2 % of total CS outflow and the right artery alone contributed only 2.9 ±1 %. The percent of each artery's inflow which drains via the coronary sinus was also determined. Of LCX inflow, 82.2 ±13 % drained via the CS as measured in 30 second timed collections of CS effluent. Of LAD inflow, 76.2 ±16.9 % drained via the CS. Of right coronary artery inflow and septal artery inflow, 12.2 ±6.4 % and 16.7 ±7.8 % respectively drained via the CS.

Supported by NIH Grant HL35030.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Walter J. McConathy, Ph.D.
Department/Institute: Medicine, UNT Health Science Center at Fort Worth
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

HIGH FREQUENCY OF AUTOANTIBODIES TO PHOSPHATIDYL SERINE IN HYPERLIPEMIA. Walter McConathy, Zhong Chen, Raymond Pertusi, Daniel M. Lane, Stephen Weis, Michael Clearfield, and Andras Lacko. Departments of Medicine and Biochemistry, University of North Texas Health Sciences Center at Fort Worth, Fort Worth, Texas 76107.

Occurrence of anti-phospholipid autoantibodies (aPL) has been associated with increased risk of stroke, myocardial infarction, and other vascular thrombotic events. Following a microELISA method for detecting anti-phospholipid antibodies, we examined a panel of plasma samples (n=42) expecting a frequency of 4-8 % of detectable autoantibodies to phosphatidyl serine. However, 19 % (n=8) were positive for IgG autoantibodies to phosphatidyl serine (aPS). In a subset, 7 of 30 hyperlipidemic (HL) samples (TC>240 mg/dl and/or TG>150 mg/dl) were positive for anti-phosphatidyl serine (23.3 % incidence). A group of hyperlipemic patients receiving heparin induced extracorporeal LDL precipitation (H.E.L.P.) treatment for hypercholesterolemia also had increased incidence of anti-phosphatidyl serine autoantibodies (9 of 21 subjects positive, 42.9 %). In examining apoE phenotypes, the frequency of the apoE3 allele was decreased ($p < 0.05$) in hyperlipemics positive for aPS. Sera samples from a local blood bank (n=193) had the expected low incidence of autoantibody to phosphatidyl serine (6.3 %). The difference in incidence of autoantibodies to PS in hyperlipidemia when compared to controls was significant by Fisher's exact test ($p < 0.001$). The occurrence of aPL in hyperlipidemia may reflect inflammatory and/or thrombotic components of atherosclerosis. (Support, NIH HL46967).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Jay Shores
Department/Institute: Medical Education/UNTHSC
Graduate Student ___ Medical Student ___ Fellow ___ Intern ___ Faculty X
Signature: _____

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

LIPOPROTEIN LIPIDS AS METABOLIC INDECES OF THE EFFICACY OF GEMFIBROZIL THERAPY FOR RAISING HIGH DENSITY LIPOPROTEIN (HDL) LEVELS DURING LOPID THERAPY. Jay Shores, Bhalchandra J. Kudchodkar, Stephen Weis, Michael B. Clearfield and Andras G. Lacko, Departments of Medical Education, Medicine and Biochemistry and Molecular Biology, University of North Texas Health Science Center, Fort Worth Texas.

Thirty subjects were treated with gemfibrozil(600 mg BID) for 12 weeks in order to achieve elevation of their HDL. Plasma lipoproteins were isolated from pre and post treatment samples by density gradient ultracentrifugation to yield ten fractions (designated as 1-10). The lipid component of these fractions (free cholesterol, esterified cholesterol, triglycerides and phospholipids) were subsequently determined by enzymatic reagent kits.

Considering the change in HDL levels during therapy as the dependent variable, stepwise correlational analysis of the data reduced the 80 original parameters to six and eventually to two. These components (pretreatment triglyceride levels in fraction 8 and pretreatment cholesteryl esters in fraction 9) predicted inclusion into the low responder group with 78% efficiency and into the high responder group with 92% efficiency. The potential diagnostic and metabolic implications of these data will be discussed.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

First Author: Andrew Reason, Ph.D.
Department/Institute: Columbia University, Dept. of Chemistry
Graduate Student ___ Medical Student ___ Fellow X Intern ___ Faculty ___
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CHARACTERIZATION OF PLASMA AND RECOMBINANT LECITHIN: CHOLESTEROL ACYLTRANSFERASE (LCAT): I. Carbohydrate structure

Andrew Reason, H.R. Morris, Anne Dell, P.,S. Paranjape, G. Sundarrajan,P. Haydn Pritchard and Andras G. Lacko. Department of Biochemistry and Molecular Biology, University of North Texas Health Science Center, Fort Worth, TX Department of Biochemistry, Imperial College of Science , Technology and Medicine, London, U.K. and Department of Pathology, University of British Columbia, Vancouver, CANADA.

The carbohydrate structures in plasma and recombinant LCAT were analysed following reduction and carboxymethylation, trypsin digestion, proline specific protease digestion and the release of glycans by PNGase F treatment. The N-glycans were separated from the proteolytically cleaved peptides using a C18 Sep-pak® column. Following permethylation,a portion was analysed using FAB mass spectrometry.

The data, show that the major structures on r-LCAT are bi-, tri- and tetra-antennary glycans and that a proportion of these are core fucosylated. The plasma LCAT, on the other hand, appears to have a considerably simpler structure. The FAB and linkage data taken together show that all the detectable glycans present on human plasma LCAT are biantennary.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CHARACTERIZATION OF PLASMA AND RECOMBINANT LECITHIN: CHOLESTEROL ACYLTRANSFERASE (LCAT): II. Catalytic properties

C. Nuckols, B.J. Kudchodkar, P.S. Paranjape, G. Sundarrajan, P. Haydn Pritchard and Andras G. Lacko.
Department of Biochemistry and Molecular Biology, University of North Texas Health Science Center, Fort Worth, TX and Department of Pathology, University of British Columbia, Vancouver, CANADA.

Utilizing the the fluorescent substrate 1,2 bis[4-(1-pyreno)-butanoyl-sn-glycero-3-phosphatidylcholine, the Km values of the recombinant and plasma enzymes were very similar and desialylation of both enzymes gave rise to a nearly three fold increase in Km. During the phospholipase activity studies, with the fluorescent substrate, apo AI was found to enhance the activity of LCAT at low substrate concentrations. When labeled high density lipoprotein was used as the substrate, the reactivity of both rLCAT and pLCAT with lipoprotein substrates. The Vmax and Km values are essentially the same for the pLCAT and the sialylated rLCAT preparations while the Km was 2-3 times higher for the desialylated recombinant enzyme.

Comparison of the catalytic parameters of the recombinant and plasma enzymes show that their functional properties are essentially indistiguishable. These findings provide the foundation for additional studies where the recombinant enzyme forms will be used to elucidate the functional consequences of structural modifications in LCAT.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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THE EFFECT OF *CAPTOPRIL* ON THE OXIDATION OF PLASMA LIPOPROTEINS. L. Armstrong, Michael B. Clearfield, Stephen Weis, Nam Ly, Peggy DeFazio, B.J. Kudchodkar and A.G. Lacko. Department of Medicine and Biochemistry and Molecular Biology, University of North Texas Health Science Center, Ft. Worth TX. 76107

This study was designed to determine the potential beneficial effects of the drug *Captopril* in the prevention the of the oxidation of low density lipoproteins (LDL) by evaluating lipid peroxide levels in LDL following oxidation *in vitro*. Lipoproteins were isolated by ultracentrifugation from fasing plasma to yield VLDL, LDL, HDL. Determination of lipid peroxides in lipoproteins was carried out by the $CuCl_2$ oxidation method in the presence and absence of *Captopril* During these *in vitro* srudies up to 65% inhibition of oxidation of LDL was observed at the concentration of 100 µg/ml of *Captopril*. However, during subsequent studies hypertensive patients with *Captopril* vs. *Enalapril*, no significant differences were seen in the oxidation patterns of lipoproteins between the two agents.

Captopril was most effective protecting LDL compared to protecting HDL and VLDL against oxidation *in vitro*. These findings, however, were not substantiated by studies with patients. Although the protection by *Captopril* was apparent even at low levels (20 mg/mL) *in vitro* this is a much higher dose than the expected circulating level of the drug (~1 µg/ml). Perhaps lower than these amounts are physiologically effective only over longer periods of time (months or years of treatment). Alternatively, other methods may have to be employed to demonstrate reduction of atherosclerotic risk by *Captopril* including association of low density lipoproteins with macrophages, delay of the coagulation process or enhancement of fibrinolysis.

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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

REGULATION OF SECRETION OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE:EFFECT OF AGENTS INFLUENCING HEPATOCYTE LIPOPROTEIN SECRETION. Thomas V. Fungwe Andras G. Lacko, Ladislav Dory and Bhalchandra J. Kudchodkar. Department of Biochemistry and Molecular Biology. University of North Texas HSC at Fort Worth.

Lecithin-cholesterol acyltransferase (LCAT) participates in reverse cholesterol transport by catalyzing the formation of cholesterol ester and lysolecithin through the transfer of fatty acids (FA) at the 2-position of lecithin to unesterified cholesterol in plasma. LCAT is primarily secreted by the liver but at present little is known about the factors influencing its synthesis and secretion. In this study we investigated whether primary cultures of hepatocytes secrete LCAT and if LCAT activity is influenced by FA species.

Hepatocytes were isolated from normal adult Sprague-Dawley rat livers following collagenase perfusion. The cells were seeded for 4 hr during which a nearly confluent monolayer is established. The plating media was replaced with William's E media (control), or the same, supplemented with 1 mM oleic acid or linoleic acid and cultured for 24 hr. LCAT activity was assayed using a liposome substrate containing apolipoprotein AI.

Secretion of LCAT, determined by activity measurements, inceased by 3 and 4-fold over control levels when hepatocytes were incubated with 1 mM oleate and linoleate. LCAT activity under these conditions was 56.0, 171.2 and 199.0 *pmol* CE hr^{-1} mg cell protein for control, oleate or linoleate, respectively. Both oleate and linoleate markedly increased the secretion of very low, and high density lipoproteins by the hepatocyte. These experiments indicate that factors increasing lipoprotein secretion, that may in turn increase secretion of LCAT.

University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

SYNTHESIS AND SECRETION OF APOLIPOPROTEIN E IN PRIMARY CULTURES OF HEPATOCYTES:EFFECT OF OLEIC ACID. Thomas V. Fungwe and Ladislav Dory, Department of Biochemistry and Molecular Biology. University of North Texas HSC at Fort Worth.

Apolipoprotein E (apo E) functions as a ligand for removal of cholesterol in plasma from peripheral cells to the liver. The major site of apo E expression (synthesis and secretion) is the liver, however, little is known about regulation of this process in that organ.

Hepatocytes were isolated from normal adult rat livers after collagenase perfusions. The cells were seeded for 4 hr to establish a confluent monolayer. Plating media was replaced with William's E media (control), or the same, supplemented with 1 mM oleic acid and cultured for 24 hr. Synthesis and secretion of apo E , relative to other secretory proteins such as apo AI and albumin, was examined by pulse or pulse-chase experiments, followed by specific immunoprecipitation, SDS-PAGE, scintillation counting and flourography.

These studies suggest that specific apoE secretion was diminished in cells cultured for more than 2 days due to continuos down regulation of apoE mRNA. Kinetic studies show that in day 1 cells, pulsed for up to 4 hr, secretion of total proteins, including apoE, A-I and albumin remained linear. The apparent rates of synthesis for apoE and albumin were similar while apoA-I was synthesized at a slower rate. To further examine protein secretion and degradation, 24 hr cultured cells were pulsed for 60 min. and chased for 0, 30, 60 and 120 min., and the extent of secretion of total protein, apoE, A-I and albumin was quantified based on cell-associated levels at zero time chase. These experiments suggest that a significant portion of newly synthesized apoE is degraded while 20% or less is secreted. On the other hand, essentially all of albumin and most of apoA-I are secreted with limited degradation. Hepatocytes incubated with oleic acid, synthesize higher amounts of total proteins and secrete ^{35}S-apoE distributed between VLDL and HDL while in controls, apoE was associated mostly with HDL or in a non lipid form. These results suggest that apoE processing by hepatocytes during secretion is significantly different from that of apoA-I or albumin.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

IDENTIFICATION OF AN APOLIPOPROTEIN (a)/LIPOPROTEIN(a) BINDING SITE ON APOLIPOPROTEIN B.

Vuong N. Trieu, Eric Mills, Urban Olsson[a], and Walter J. McConathy. Departments of Medicine and Biochemistry, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas, 76107, and [a]Wallenberg Laboratory, Göteborgs University, Göteborg, Sweden

Lipoprotein(a) [Lp(a)] is a risk factor for coronary artery disease. It is composed of apolipoprotein(a) [apo(a)] disulfide linked to apolipoprotein B (apoB) and lipids. The formation of Lp(a) probably occurred in two steps: apo(a) binds apoB-Lp followed by formation of a disulfide linkage between kringle-36 cysteine and apoB-Lp cysteine. Affinity of apo(a)/Lp(a) for apoB-Lp is mediated by one of its kringles and is important in the formation of Lp(a). In this report, the initial binding site for apo(a)/Lp(a) on apoB is localized to residues 3284-3318. The binding site was found by its homology to the plasminogen kringle-4 binding site on α_2-antiplasmin. A synthetic peptide ($apoB_{3304-3317}$), corresponding to a portion of this binding site, inhibited the binding of ApoB-Lp to apo(a)/Lp(a) competitively (K_i = 1.5 $\pm$ 0.7 x 10^{-4} M, n= 5). Inhibition was due to the direct binding of the peptide to apo(a) as $ApoB_{3304-3317}$ covalently linked to Sepharose specifically removed Lp(a) from plasma and separated Lp(a) from a mixture of apoB-cointaining lipoproteins (apoB-Lp). Human Lp(a) and recombinant apo(a) also bound mouse apoB-Lp (K_D of 5.4 $\pm$ 4.3 X 10^{-8} M), which does not form covalent complex with apo(a). Binding was due to a different mechanism, however, as it was not inhibited by the peptide $apoB_{3304-3317}$. These studies provide a clearer indication of the domains on apoB and apo(a) responsible for the formation of Lp(a). (Support, NIH HL46967).

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ABSTRACT FORM

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James L Caffrey

Intrinsic Cardiac Enkephalins Inhibit Vagal Control of Cardiac Function in the Dog

James Caffrey, Zaira Mateo, Leslie Napier, Melissa Hamrick, Darice Yoshishige, John Gaugl, Barbara Barron
Department of Physiology
University of North Texas Health Science Center Fort Worth Texas

Met-enkephalin-arg-phe (MEAP) has been identified in acid extracts of canine heart tissue. The effects of synthetic MEAP on the vagal control of heart rate and atrial contractility were investigated in anesthetized dogs. The arterial infusion of MEAP (3 nmol/min/kg) inhibited the bradycardia observed during electrical stimulation of the right vagus nerve by 75%. After stopping the infusion, the responsiveness to vagal stimulation returned to normal with a half-time of approximately 2 minutes. The inhibition by MEAP was reversed by the high affinity opiate antagonist, diprenorphine (100 μg/kg). MEAP did not alter the negative chronotropic effect of arterial methacholine. This observation suggested MEAP exerts its effect at a site in the efferent vagal tract proximal to nodal muscarinic receptors. During left vagal stimulation, left atrial contractile activity declines sharply. This negative inotropic effect of the vagus was also suppressed by arterial MEAP infusion suggesting that both chronotropic and inotropic effects are modified. Increasing MEAP infusions (0.09 - 3.00 nmol/min/kg) produced a graded suppression of vagal bradycardia with a half-maximal effect near 0.3 nmol/min/kg. Methionine-enkephalin (ME) produced responses very similar to those obtained with MEAP. The effects of ME were also blocked by prior administration of diprenorphine. Dose responses to ME were shifted to the right of those for MEAP and half-maximal responses for ME were obtained at 2-4 times the dose required for MEAP. The data suggest that the intrinsic cardiac enkephalin, MEAP can regulate vagal control of heart rate at physiologically achievable concentrations and may serve as a local regulator of the parasympathetic/myocardial interface.

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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5x7 inches) in box given below.

EXTRACTION AND MEASUREMENT OF CIRCULATING AND MYOCARDIAL ENKEPHALINS IN THE DOG. Z. Mateo, L.D. Napier, M. Hamrick and D. Yoshishige. Department of Physiology, University of North Texas Health Science Center, Fort Worth, Texas 76107.

Pro-enkephalin (PE) like immunoreactivity has been identified in canine myocardium. The following experiments were conducted to extract and measure small and intermediate sized products of the PE gene in the heart and circulation of the dog. Blood samples were preserved with iced citrate, centrifuged at 15,000 rpm for 10 minutes and the supernatants boiled and centrifuged again. Myocardial tissue samples were extracted in five volumes of 1N acetic acid /0.2N HCL/0.1% 2-mercaptoethanol. The supernatants were separated by gel filtration chromatography on Bio-gel P-10 columns. Fractions corresponding to authentic Peptide-B and met-enkephalin-arg-phe (MEAP) were concentrated on Porapak Q columns and subjected to radioimmunoassay. Preliminary data from plasma samples indicate immunoreactivity corresponding to MEAP predominates compared to immunoreactivity which elutes with the larger Peptide-B. Data from extracted cardiac tissue indicate that the relative distribution of the activity is reversed and immunoreactivity corresponding to the precursor, Peptide-B, is greater compared with MEAP in the same samples. These results suggest that the heart may be processing precursor to product prior to its secretion. Studies are currently being conducted to compare circulating and myocardial tissue enkephalins in altered physiological conditions.

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ABSTRACT FORM

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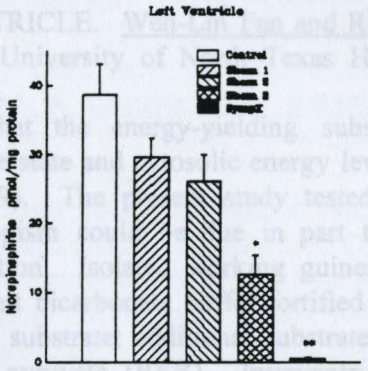
Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

OPEN CHEST SHAM-OPERATION DEPRESSES MYOCARDIAL CATECHOLAMINES. B.A. Barron, M.X. He, M. Hamrick, A. Heymann, M. Bina, C.E. Jones. Dept. of Physiology, University of North Texas Health Science Center, Fort Worth, TX 76107



We observed differences in myocardial catecholamines between control dogs and sham-operated dogs as controls for ventricular sympathectomy (SympX, n=13). Thus, we subdivided sham-operated animals into groups to discover the cause for this myocardial catecholamine depression. The groups included sham-operation without opening the pericardium (SHAM1, n=3), sham-operation with opened and sutured pericardium (SHAM3, n=7) and sham-operation with pericardium remaining open (SHAM2, n=4). Tissue was collected four weeks after recovery from surgery except control dogs. The heart was collected immediately from naive controls without recovery from anesthesia (CTL, n=8). There were similar changes in myocardial norepinephrine (figure) and epinephrine. Other heart sections had less of a decrement in tissue catecholamines. It appears that the mechanical friction of the sutured pericardium affects the tissue catecholamines. This emphasizes the need for careful attention to proper controls for animals undergoing surgery. This data implies that pericardial suturing should be placed toward the ventricular apex, away from neuronal tracts which run more on the surface at the junction between atria and ventricles.

Supported in part by NIH HL 29232 and HL 48076.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

PYRUVATE ACCELERATES DIASTOLIC RELAXATION IN WORKING GUINEA-PIG LEFT VENTRICLE. Wen-Lin Fan and Robert T. Mallet, Department of Physiology, University of North Texas Health Science Center, Fort Worth, TX 76107.

We have previously shown that the energy-yielding substrate pyruvate increases myocardial contractile state and cytosolic energy level in parallel (*Eur J Biochem* 180:221, 1989). The present study tested our proposal that pyruvate-enhanced inotropism could be due in part to an increased rate of left ventricular relaxation. Isolated working guinea-pig hearts were perfused with Krebs-Henseleit bicarbonate buffer fortified with 5 mM glucose (+ 5 U/l insulin) as basal substrate; additional substrate was either 5 mM lactate (LAC) or 5 mM pyruvate (PYR). Intraventricular pressure was measured with a 3F Millar catheter and dP/dt obtained by electronic differentiation. In the presence of each substrate combination, aortic overflow pressure (P_a) was held at 90 and 110 cm H_2O for 20 min each step at 12 cm H_2O left atrial filling pressure; 3 hearts received LAC, then PYR, another 3 hearts *vice-versa*. Data (Table) are left ventricular end diastolic pressure (LVEDP; mmHg), dP/dt_{max} and dP/dt_{min} (mmHg/sec), stroke work (SW; mJ/g wet), and myocardial O_2 uptake (MVO_2; µmol/min/g wet); $^{*}P < 0.05$ *vs.* LAC (paired t test).

P_a	LVEDP	dP/dt_{max}	dP/dt_{min}	SW	MVO_2
90 LAC	6.2±0.3	1490±0.3	-1520±80	0.87±0.11	3.0±0.1
90 PYR	5.3±0.6*	1940±150*	-1740±80*	1.17±0.12*	3.6±0.2*
110 LAC	6.9±0.3	1510±80	-1450±110	0.56±0.12	3.2±0.2
110 PYR	5.5±0.5*	2140±190*	-1920±110*	1.19±0.18*	3.8±0.2*

Increased dP/dt_{min} at lower LVEDP indicates that, relative to lactate, pyruvate enhances the relaxation system in glucose-perfused left ventricle. These findings are consistent with an accelerated sarcoplasmic reticular Ca^{2+} uptake secondary to pyruvate energization. NIH HL 50441; AHA Texas Affiliate 92G-155.

University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

DISASSOCIATION OF CARDIAC FUNCTION FROM CYTOSOLIC PHOSPHORYLATION POTENTIAL (log [ATP]/[ADP][Pi], PP, M^{-1}) DURING 2-DEOXYGLUCOSE (2DG) INFUSION IN GUINEA PIG HEARTS. M. -X. He, H. F. Downey, M. W. Gorman, G. D. Romig, and H. V. Sparks. Univ. N. Tex. Hlth. Sci. Ctr. at Ft. Worth, Ft. Worth, TX 76107, and Mich. State Univ., E. Lansing, MI 48824.

To study the relationship between energy status and cardiac function, myocardial high energy phosphates were diminished by infusing 2DG (5mM) to isolated guinea pig hearts perfused at constant flow (5.0±0.1 ml/min/g). Cytosolic phosphorus compounds were monitored with ^{31}P-NMR spectroscopy, and left ventricular pressure (LVP, mmHg) and heart rate (HR, beats/min) were recorded simultaneously. Oxygen consumption (MVO_2, ul O_2/min/g) and adenosine release (Rado, pmol/min/g) were measured. Results:

	PP	LVPxHRx10^{-3}	Rado	MVO_2
Control	5.45±0.07	17.09±1.86	35±15	52.7±2.0
2DG 10 min	5.33±0.08	17.47±0.96	114±32**	52.2±0.9
2DG 20 min	5.38±0.07	14.96±0.86**	67±17*	50.7±1.4
2DG 40 min	5.43±0.11	13.91±0.72**	54±18	47.0±2.7**

* $P<0.05$ vs. control, ** $P<0.01$ vs. control

During 2DG infusion, cardiac function decreased by 20 minutes, but PP remained at control level, indicating disassociation of cardiac function from phosphorylation potential. The decrease in MVO_2 was accompanied by an increase in ADP. The early increase in adenosine formation may downregulate myocardial energy demand when energy supply is limited.

(Supported by USPHS grants HL 24232 and HL 35027)

University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

DECREASED ENERGY DEMAND IN ISCHEMIC GUINEA-PIG MYOCARDIUM. Z. Gao, W.-L. Fan, H. F. Downey, R. T. Mallet. Dept. Physiology, Univ. North Texas Health Sci. Ctr., Fort Worth, TX 76107

The purpose of this study was to characterize an isolated working heart model of acutely down-regulated energy demand. Hearts, perfused with Krebs-Henseleit fortified with 10 mM glucose + 5 U/l insulin, were subjected to the following protocol: 15 min preischemia (aortic pressure, Pa=90 cm H_2O), 15 min mild hypoperfusion (Pa=45 cm H_2O), 30 min ischemia (Pa=22.5 cm H_2O), 30 min reperfusion (Pa=90 cm H_2O). Left atrial filling pressure was held at 12 cm H_2O. Time controls were perfused 90 min at P_a = 90 cm H_2O. Coronary flow and pressure-volume work fell 75-80% during ischemia, but fully recovered during reperfusion. Hearts were stop-frozen at peak purine nucleoside release (10 min ischemia), 30 min ischemia, and 30 min reperfusion. Myocardial O_2 uptake (MVO_2; μmol/min/g wet), creatine phosphate potential ([CrP]/[Cr][Pi]; M^{-1}), lactate release (v_{lac}, μmol/min/g wet), and purine nucleoside release ($V_{ado+ino}$, nmol/min/g wet) were measured (*P < 0.05 vs. CONTROL).

GROUP	MVO_2	[CrP]/[Cr][Pi]	Vlac	Vado+ino
CONTROL	2.26±0.08	634±78	0.022±0.007	0.000±0.000
10' ISCH	0.92±0.16*	123±20*	0.763±0.090	3.692±0.433*
30' ISCH	0.89±0.11*	237±73*	0.486±0.091*	0.886±0.690*
30' REPERF	1.91±0.14	579±127	0.104±0.054	0.165±0.090*

Cytosolic energy level fell rapidly at ischemic onset, partially recovered despite continued ischemia, and fully recovered during reperfusion. A reduction in myocardial energy demand during limited energy supply aided recovery of energetics and function upon reperfusion. [Supported by NIH grants: HL 35027 (HFD), HL 50441 (RTM)]

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

SEM STUDIES OF COMPARATIVE MEMBRANE REMODELING BY ENDOCYTOSIS IN TOAD URINARY BLADDERS FOLLOWING WITHDRAWAL OF ADH AND MZ. A.J Mia*, A.D. Davidson*, C. Robinson*, L.X. Oakford** and T. Yorio**. *Jarvis Christian College, Hawkins, Texas, 75765. **UNTHSC at Fort Worth/TCOM, Fort Worth, Texas, 76107.

Vasopressin (ADH) and mezerein (MZ) induce transmembrane water flow through water channels inserted into the apical plasma membrane. The water channels are then recycled into the cytosol during retrieval following withdrawal of vasopressin or mezerein. Little is known about the apical membrane recycling process during prolonged periods of hormone stimulation. We currently report on surface membrane remodelling in toad urinary bladders following prolonged stimulation with ADH and subsequent retrieval following removal of hormone. Toads, *Bufo marinus*, were doubly pithed and the urinary bladders were removed and suspended as sacs with an imposed osmotic gradient (1/10 diluted Ringer's solution). Some tissues were retained in ADH 100 mU/ml for 60 min with no change in buffer and then fixed. While other tissues were exposed to ADH or MZ 10^{-6} M for 15 and 10 min, respectively, and then hormone and drug removed and allowed to continue for up to 60 min while maintaining the osmotic gradient by fresh buffer exchanges at 30 and 45 min before fixation at 60 min. Tissue fixation was carried out for 1 hr in 2% glutaraldehyde in PIPES with a post fixation in 1% osmium tetroxide for 1 hr. Tissues processed through graded acetone and Peldri II were critical point dried for gold coating prior to SEM studies. Control tissues, regardless of length of time during exo- and endocytic retrieval, showed little or no observable membrane internalization at the apical plasma membrane. Tissues retained in ADH for 60 min showed nearly complete membrane recovery with only 3.86% of cells showing signs of endocytosis. There was no observable difference in tissues which received stimulation with ADH for 15 min and intermittent buffer rinses at 15, 30 and 45 min as only 2.62% of the cells having surface changes. Similar tissue treatment with MZ for 10 min and buffer rinses resulted in apical membrane recovery and only 9.47% of cells showing endocytosis. Water flow measurement indicated a slower rate of water loss from tissues with continuous presence of ADH in contrast to an increased rate of water flow from tissues with removal of ADH and intermittent buffer changes. These observations suggest that membrane remodeling is a spontaneous process following hormonal stimulation, and that the remodeling process continues until the apical membrane undergoes a complete recovery.

Supported by grants from the US Army DAMD17-19-C-1096 and R15 DK46550.

Biochemistry and Molecular Biology

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SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

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ABSTRACT FORM

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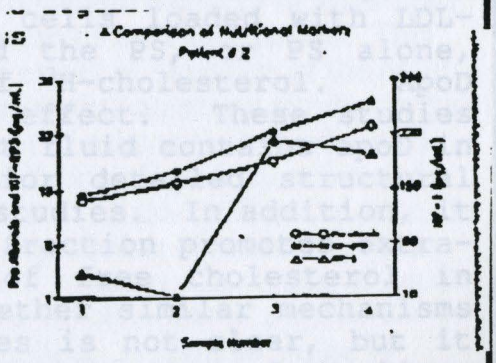
Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

NUTRITIONAL ASSESSMENT OF HOSPITALIZED PATIENTS - USING PREALBUMIN (PAB), RETINOL BINDING PROTEIN (RBP) AND INSULIN-LIKE GROWTH FACTORS (IGF) AS BIOCHEMICAL MARKERS, N.Y. Zachariah, J. T. O'Shea, (Osteopathic Medical Center of Texas, Depts. of Pathology & Nutrition, Ft. Worth, Texas) Robert Hagan Jr., John Pung (University of North Texas Health Science Center at Fort Worth)

With early detection of protein calorie malnutrition (PCM) enternal or total parenteral nutrition therapy (TPN) may be initiated and length of stay of at risk patients may be reduced. Literature supports a relationship between inpaired nutritional status and increased morbidity and mortality. Serum albumin, Transferrin, Somatomedin C, PAB and RBP have been unsed to assess the nutritional status. 72 patients (ages 32-89) yrs, 31 females and 41 males) were tested within 48 hous of admission for biochemical assessment of PCM. Laboratory studies included total protein, albumin, PAB, RBP, Ca, Mg, TIBC, creatinine, and other nephelometric method (Behring Nephelometer 100). A significant rise in PAB and RBP with improved nutritional status (PAB 12.4-25.8 mg/dl, RBP 3.6-6.1 mg/dl) was observed in 51.4% of the patients. Serum creat., tot. prot., or alb. did not show a relative increase. There was no relationship with either age or the sex. the IGFS are produced by multiple tissues under the control of growth hormone.

They act as regulators of cell growth and may be sensitive markers to assess the nutritional status. Specimens from 8 patients on TPN were tested for IGF-1 and IGF-binding protein 3 (IGF-BP3) by RIA method (Diagnostic Systems Lab., Webster, TX) - Results of a patient summarized in the graph indicated relative changes in PAB, IGF-1 and IGF-BP3. Six (6) patients demonstrated proporetional changes bweteen PAB and IGF-BP3 levels. Five (5) patients showed correlation between the PAB and IGF-1 levels. PCM was the common factor for these patients. Primary diagnosis or treatment did not seem to influence PAB/IGF correlations.



SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

STUDIES ON ROLE OF BREAST CYST FLUID POLYSACCHARIDE AND APOLIPOPROTEIN D IN CELLULAR CHOLESTEROL CONTENT. Walter J. McConathy, Dale Freeman[a], and Pam Brett, Departments of Medicine and Biochemistry, UNTHSC, Fort Worth, TX and U. Oklahoma HSC[a], Oklahoma City, OK.

Because of the elevated levels apoD in cyst fluid, we purified apoD to pursue its structural and functional role. In addition to apoD, breast cyst fluid contained high levels of unesterified cholesterol not associated with apoD but with a carbohydrate-rich fraction. By Schiff's stain, this carbohydrate-rich fraction is uncharged on gradient gel electrophoresis and eluted in the void volume of a 6% agarose column. Delipidization of this fraction followed by rechromatography did not change its properties on the Sepharose 6B-CL column and indicated the polysaccharide nature of this fraction (PS). In initial functional studies, we monitored the effects of apoD and PS on reverse cholesterol transport using the mouse Leydig tumor cell line, MA-10. The crude preparation containing both apoD and PS was effective in reducing the cholesterol content of cells loaded with LDL-cholesterol. ApoD and the PS, or PS alone, enhanced the afflux of ^{3}H-cholesterol. ApoD alone did not have an effect. These studies confirm that breast cyst fluid contains apoD in sufficient quantities for detailed structural studies and functional studies. In addition, it demonstrates that a PS fraction promotes extracellular accumulation of free cholesterol in breast cyst fluid. Whether similar mechanisms operate in other tissues is not clear, but it would appear that apoD, as a member of the lipocalin family, may play a role as cholesterol shuttle between the cell and appropriate acceptors such as the cyst fluid polysaccharide(s).

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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PH- DEPENDENT SORTING AND CHLOROQUINE-SENSITIVE SECRETION OF NEWLY SYNTHESIZED APOE IN MACROPHAGES. Jiatai Deng* and Ladislav Dory#, *Dept. Pharmacology, University of Tennessee, Memphis and #Dept. Biochemistry & Molec. Biol., University of North Texas HSC at Fort Worth.

Past studies in our laboratory demonstrated that in the absence of HDL_3 up to 50% of newly synthesized apoE is degraded prior to secretion. In the present experiments, using a pulse-chase design and quantitative immunoprecipitation techniques, we report that the extent of intracellular apoE degradation can be inhibited in a dose dependent manner by up to 50% in macrophages incubated in the presence of NH_4Cl (0.1-5 mM). The inhibition of apoE degradation is accompanied by a proportional increase in its secretion. The pH-mediated effect of NH_4Cl appears to be non-specific, as total protein secretion is also stimulated. Treatment of cells with chloroquine (CQ; 10-100 μM) inhibits apoE degradation by >80%, but also leads to a >80% inhibition of apoE secretion. Treatment of macrophages with CQ thus results in nearly complete recovery of sialo-apoE associated with the cells at the end of the chase period. This effect is specific for apoE; the extent of secretion of the other major secretory proteins is increased to an extent similar to that observed with NH_4Cl. Treatment of cells with Brefeldin A (5 μg/ml) results in a complete inhibition of apoE secretion and degradation and an accumulation of asialo-apoE in the cells. In summary: 1) the intracellular degradation of nascent apoE in macrophages takes place in the lysosomes; 2) sorting of apoE to lysosomes is pH sensitive; the involvement of a specific receptors for apoE sorting is implied; 3) apoE secretion is selectively blocked by CQ (between the trans-Golgi network and the plasma membrane) in an apparently pH-independent manner.

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University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

AMINO ACID ANALYSIS OF PHOSPHOPEPTIDES: ABRF-93 AAA.
K.Ü. Yüksel, T.T. Andersen, I. Apostol, J.W. Fox, J.W. Crabb, R.J. Paxton, and D.J. Strydom. Biopolymer Analysis Laboratory, Dept. Biochem. & Mol. Biol., U. North Texas Health Science Center and the Association of Biotechnology Resource Facilities.

In response to the increasing need for identification and quantitation of posttranslationally modified amino acids, ABRF has provided its members with peptides containing phosphorylated and hydroxylated amino acids. ABRF-93AAA was a sample containing hydroxyproline and phosphorylated and unmodified Ser, Thr and Tyr. Fifty facilities participated in this study which produced 49 sets of useable data. These investigators have utilized diverse chemistries (AQC, DABSYL, ninhydrin, OPA, OPA/FMOC, PITC) and chromatographic conditions (ion exchange, reversed phase, and capillary electrophoresis). The study has provided a useful comparison of phosphoamino acid identification methods, although the precise amount of phosphate in the sample was not determined. Resolution of phosphorylated amino acids is more readily achieved by pre-column techniques, and these sites were more successful in their identification of phosphoamino acids than post-column sites (77% vs. 56%). The fact that 60% of the participants were able to identify the phosphoamino acids in the sample emphasizes the feasibility and screening value of phosphoamino acid analysis. The results indicate that identification of common amino acids and phosporylated amino acids must be made on separate hydrolyzates. Short hydrolysis times (e.g. 2 h at 110°C) provide a better yield of the phosphorylated amino acids. Longer hydrolyses (≥ 24 h) are required to obtain accurate values for common amino acids, especially for slow cleaving bonds like Ile-Ile. Hydroxyproline was equally well identified by pre- and post-column techniques. Hydroxyproline was less frequently identified than the phosphoamino acids, in part due to its co-elution with phosphotyrosine and/or Glu in some PTC-sites, as well as its unannounced presence.

This work was supported in part by NSF grant DIR 9003100 (to JWC) on behalf of the ABRF.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

ROUTING OF A SECRETORY PROTEIN TO THE LYSOSOMAL COMPARTMENT IN TRANSFECTED MDCK CELLS. V.L. Rudick*, M.J. Rudick†, D.A. Munoz-Medellin†, A.-M. Brun-Zinkernagel*, I-Fen Chang*.
*Dept. of Anatomy & Cell Biology, Univ. North Tx. Hlth. Sci. Ctr. at Ft. Worth TX 76107; †Dept. of Biol., Tx. Woman's Univ., Denton, TX 76204.

A clone (3A) of transfected MDCK cells expressing human growth hormone (hGH) and containing twice as many Golgi stacks as untransfected cells has been described (Rudick et al. 1993 *J. Cell Sci.* **104**, 509). Since hGH constituted only 10% of total secreted proteins, it was not apparent why there was a need for Golgi amplification. Thus, studies were undertaken to determine the fate of hGH. Because 3A dimensions are identical to those of untransfected cells, any increase in vesicular traffic to the PM would have to be balanced by a compensatory increase in endocytosis. This was shown not to be so using horseradish peroxidase, because 3A and untransfected cells have identical rates of endocytosis, recycling, and transcytosis. This suggested that a portion of the hGH might enter a post Golgi, non-secretory compartment. Cells were treated with 20mM cycloheximide which inhibited protein synthesis 98% after 0.5 h. Samples of cells and media were assayed for hGH and the results indicated that approximately 30% remained within the cells. This was corroborated by pulse labeling the cells, immunoprecipitating hGH at various times during the chase, and analyzing the precipitates by SDS-PAGE and fluorography. The question was where does that portion of the hGH go? Since it must be a post Golgi compartment, an obvious candidate was the lysosome. Thus, immunoelectron microscopy was performed using antibodies against hGH, clathrin, and cathepsin D. Clathrin and hGH colocalized, as did hGH and cathepsin D, at the trans Golgi network. It was also observed that hGH and cathepsin D were found together in very large vesicles characteristic only of 3A cells. No hGH was evident in mature lysosomes. The data suggest that some hGH enters clathrin coated vesicles that also contain cathepsin D, which presumably are pre-lysosomes. These may fuse giving rise to the larger vesicles. However, it seems that hGH is degraded during or shortly after completion of lysosomal maturation. Immunogold analysis of an isolated lysosome/endosome fraction revealed relatively small numbers of vesicles containing both hGH and cathepsin D but many more vesicles containing only cathepsin D. Thus, Golgi amplification in 3A cells seems to have resulted in an enlarged lysosomal compartment to accommodate increased production of secretory protein.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

REGULATION OF LYSOZYME EXPRESSION DURING MONOCYTE DIFFERENTIATION INDUCED BY A NOVEL LEUKEMIA DIFFERENTIATION FACTOR Wang, S.Y.[1], Su, D.[1], Wu, M.-C.[1] and Irwin, D.M.[2], Dept. of Biochem. and Mol. Biol. UNT-HSC, Fort Worth, Tx and Dept. of Clin. Biochem. Banting Inst. U. of Ontario Canada[2].

A rat myelomonocytic leukemia cell line MIA C-51 has been used to study the biochemical mechanism of monocyte differentiation. MIA C-51 cells can be induced to undergo terminal monocytic differentiation by a newly purified leukemia differentiation factor (LDF). Previous studies have shown that cAMP is the possible signal since it elicits similar reactions as LDF when incubated with MIA C-51 cells. Among the enzyme markers elevated during the LDF-induced differentiation, lysozyme expression is of particular interest since it is one of the marker enzyme for phagocyte activation. Attempt has been made to elucidate the molecular mechanism of LDF-induced monocyte differentiation by studying the regulation of lysozyme gene expression. A plasmid containing 2.8 kb PstI fragment of the 5' flanking region and exon one of the rat lysozyme gene in front of a CAT reporter gene was constructed. The 2.8 kb PstI fragment was isolated from the PstI digest of p11B22 which is a plasmid genomic subclone containing 5' flanking region and exon one of the rat lysozyme gene. The pCAT-basic that carries a bacterial CAT reporter gene but lacks eukaryotic promoter and enchancer sequences was used as the vector. The constructed recombinant plasmid which has the 5' flanking sequence upstream of the CAT reporter gene was named pCAT-LYZ. Three plasmids were used in the transfection study. pCAT-LYZ is to study the cAMP effect on the production of CAT activity. The pCAT-basic is the negative control. Another plasmid pCAT-control, which contains SV40 promoter and enhancer sequences, was used as positive control. The plasmids used for transfection were purified by two rounds of cesium chloride ultracentrifugation. The purified plasmids were used to transfect into the MIA C-51 cells by the method of DEAE-Dextran transfection. After transfection, 0.4 mM cAMP was added 24 hours before harvesting. The cell extracts were prepared by three rounds of freeze-thaw and CAT enzyme assay of either liquid scintillation counting (LSC) or thin layer chromatography (TLC) were performed according to the protocol of enzyme assay Kit. Currently, various concentrations of DNA and DEAE-Dextran are being tested to determine the optimizing condition for transfection and this system will be used to study the regulation of lysozyme expression induced by LDF.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
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ABSTRACT FORM

First Author: Robby Dickerman
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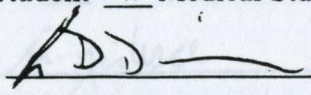
Signature: 

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

CREATINE KINASE AND LACTATE DEHYDROGENASE ISOENZYME MEASUREMENTS IN THE MALE ASIAN ELEPHANT (ELEPHAS MAXIMUS) DURING NONMUSTH AND MUSTH, Robby D. Dickerman, Doug Pernikoff, N.Y. Zachariah, P.B. Raven, R.W. Gracy, W.M. McConathy, University of North Texas Health Science Center, 3500 Camp Bowie Blvd. Fort Worth, Tx 76107

Testosterone related organ tissue damage has been documented in humans abusing anabolic steroids by elevated levels of Creatine Kinase (CK) and Lactate Dehydrogenase (LDH). Asian male elephants are known to have elevated free testosterone, total testosterone, and dihydrotestosterone during the period of Musth. Therefore CK and LDH measurements were compared during Nonmusth (eutestosterone) and Musth (high testosterone) in five Asian bull elephants, ranging in age 15-30 years. The elephant serum was obtained from blood banks at Texas A&M University and the Fort Worth Zoo. All animals had olfactory and visual contact with Asian female elephants and were fed similar rations of hay and grain. Blood (1-10ml) was collected from an ear or leg vein with 15ml vacutainer tubes. Blood was subsequently centrifuged and serum decanted into storage vials. Testosterone levels were measured by RIA. CK and LDH isoenzyme measurements were determined by electrophoresis on a Helena Cliniscan II Work Center.The total CK and LDH were increased during Musth. The CK isoenzymes did not express significantly different variability during Nonmusth and Musth, however the CK-BB isoenzyme comprised 37% of the total CK in the serum during Musth. In humans this large percentage is indicative of chronic renal failure and prostatic hypertrophy. LDH-3 was significantly higher ($P < 0.05$) during Musth compared to Nonmusth. LDH-3 is elevated in humans after pulmonary or renal infarctions. The data suggest that,like human anabolic steroid abusers,the Asian Bull elephants may suffer transient reproductive and urinary system tissue damage during Musth. (Fort Worth Zoo & UNTHSC).

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ABSTRACT FORM

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Signature: A. Yüksel

Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

TERMINAL MARKING OF TRIOSEPHOSPHATE ISOMERASE: CONSEQUENCES OF DEAMIDATION. A.-Q. Sun , K.Ü. Yüksel, & R.W. Gracy. Molecular Aging Unit, Dept .Biochem. & Mol. Biol., U. North Texas Health Science Center, Fort Worth, TX, 76107 USA.

Mammalian triosephosphate isomerase (EC 5.3.1.1; TPI) spontaneously deamidates at Asn71 and Asn15 located at the subunit interface of the isologous dimer. These deamidations constitute the terminal marking event of the enzyme. The structure of rabbit TPI is substantially altered by this deamidation, as indicated by a 30% lower secondary structure content and blue shifted ellipticity minimum of the far-UV CD spectra. Furthermore, increased fluorescence (10-22%) and red-shifted emission maximum (8.7-15.6 nm) indicate exposure of tryptophans to a polar environment. Increased binding of the fluorescent hydrophobic probe 1,1'-bis(4-anilino)-naphthalene-5,5'-disulfonic acid (bis-ANS) to the deamidated enzyme corroborates these observations and suggests that more hydrophobic residues are exposed at the subunit interface as a result of deamidation. Decreased covalent cross-linking (80% *vs.* 20%) of the deamidated enzyme by the bifunctional reagent ethylene glycol*bis*(succinimidylsuccinate) (EGS) also suggests separation of the two subunits at the interface. These structural changes are accompanied by a decreased thermal stability (3.1 °C lower *Tm*) and increased dissociation in urea. Deamidation results in generation of new proteolytic sites and increases susceptibility to proteolysis. These studies establish that the specific deamidation at the subunit interface of TPI is indeed the terminal marking event and causes significant structural changes which lead to rapid degradation of the protein.

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

PURIFICATION AND CHARACTERIZATION OF TURKEY TRIOSEPHOSPHATE ISOMERASE. Yin Zhang, Ümit Yüksel, and R. W. Gracy, Molecular Aging Unit, Dept. of Biochemistry and Molecular Biology, University of North Texas Health Science Center, Fort Worth, TX 76107

Triosephosphate isomerase (TPI, EC 5.3.1.1) which catalyzes the interconversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate, has been extensively studied as an aging model. TPI from mammals such as rabbit, pig, dog, and human undergoes deamidation at two specific asparagine-glycine sites (Asn 71 and Asn 15). Chicken TPI, which contains a lysine instead of asparagine at position 71, undergoes specific oxidation of Cys 126 rather than deamidation at Asn 15. Thus both deamidation and oxidation can provide the terminal marking event leading to the proteolysis of the enzyme from different species. TPI from turkey breast was purified to determine if all avian TPI in general is terminally marked via deamidation or oxidation. Ammonium sulfate precipitation followed by two hydrophobic interaction and ion exchange chromatography steps provided homogeneous enzyme. The amino acid composition of turkey and chicken TPI were virtually the same, except for the presence of cysteic acid in the turkey TPI. The sequence of amino terminal 23 residues and of two internal peptides (13 and 8 residues long) of the two enzymes were identical. The isoelectric point (pI), enzyme kinetics, and proteinase susceptibility of turkey TPI were also similar to the chicken TPI. However, oxidation and deamidation experiments showed that turkey TPI was more resistant to oxidants and alkaline conditions than the chicken enzyme. The relationship between the stability and structure of turkey TPI relative to that of chicken enzyme is currently under investigation. (This work was supported by NIH AG01274, the R.A. Welch Foundation B0502 , and the Texas Advanced Technology Program (#2147)).

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

INHIBITION OF THE ADP-RIBOSE POLYMERIZATION REACTION CATALYZED BY POLY(ADP-RIBOSE)POLYMERASE WITH AGMATINE-(ADP-RIBOSE). G. Pacheco-Rodriguez and R. Alvarez-Gonzalez. Depts. of Microbiol. & Immunol. and Biochem. & Mol. Biol. University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699

Poly(ADP-ribose) polymerase (PARP) [E.C. 2.4.2.30], a DNA-dependent enzyme poly(ADP-ribosyl)ates several chromatin proteins in eukaryotes, including itself (automodification) as well as histone proteins (heterologous modification). The enzyme mechanisms of ADP-ribose (ADPR) transfer to a growing ADPR polymer have been studied here with mono(ADP-ribosyl)ated-molecules. We have synthesized agmatine-(ADPR) with cholera toxin utilizing agmatine and β-NAD as the substrates. Chromatographically pure agmatine-(ADPR) inhibited the polymerization activity of PARP in a concentration-dependent manner at 2 μM NAD both in the presence or absence of histones. At 200 μM agmatine-(ADPR), the size distribution of ADPR polymers decreased significantly in the automodification and heterologous poly(ADP-ribosyl)ation reactions as observed by high resolution polyacrylamide gel electrophoresis. Addition of agmatine or ADPR alone did not inhibit the ADPR polymerization activity of this enzyme. Thus, our data are consistent with the conclusion that the inhibitory effect is due to the ADPR covalently linked to the guanidinium group of agmatine. Our data also suggest that the inhibition of ADPR polymerization is a competition between agmatine-(ADP-ribose) and the protein-distal ADPR residue of the growing polymer.

Acknowledgment is made to the donors of The Petroleum Research Fund administered by the American Chemical Society.

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University of North Texas Health Science Center
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ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

MOLECULAR MECHANISM OF POLY(ADP-RIBOSE) POLYMERASE H. Mendoza-Alvarez and R. Alvarez-Gonzalez. Dept. of Microbiology & Immunology. University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699.

We have determined the molecular mechanism of the automodification reaction of poly(ADP-ribose) polymerase (PARP) [E.C. 2.4.2.30]. While PARP-mono(ADP-ribose) conjugates were the predominant products of automodification at 200 nM NAD, highly branched polymers were preferentially synthesized at 200 μM NAD. Thus, the initiation, elongation, and branching reactions catalyzed by PARP appear to be dependent on the concentration of NAD. Initial rates of automodification increased with second order kinetics as a function of the concentration of PARP at both 200 nM and 200 μM NAD. Furthermore, the initial rates of auto-mono(ADP-ribosyl)ation with 3'-deoxyNAD as a substrate also increased with second order kinetics. Therefore, two molecules of PARP, i.e., a catalytic dimer, are required for both the auto-mono(ADP-ribosyl)ation and the auto-poly(ADP-ribosyl)ation reactions of this enzyme. Interestingly, the initial rates of automodification also increased with second order kinetics at low NAD concentrations. Therefore, the catalytic dimer also requires two molecules of NAD. These results are consistent with the conclusion that the automodification reaction of PARP is intermolecular and that the two monomeric units of PARP may simultaneously function as catalyst and acceptor molecules.

Acknowledgment is made to the donors of The Petroleum Research Fund administered by the American Chemical Society.

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University of North Texas Health Science Center
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ABSTRACT FORM

First Author: Yubo Sun
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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

SEPARATION AND QUANTITATION OF SPORE PHOTOPRODUCT AND OTHER THYMINE-CONTAINING DNA PHOTOPRODUCTS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY. Yubo Sun, Kampan Palasingam, and Wayne L. Nicholson, Dept. of Microbiology and Immunology, Univ. of North Texas Health Sci. Ctr., Fort Worth, TX 76107

In contrast to cyclobutyl pyrimidine dimers, the major DNA photoproduct of UV-irradiated *B. subtilis* spores is the thymine dimer 5-thyminyl-5,6-dihydrothymine, or spore photoproduct (SP). Methods for detection of SP and cyclobutyl dimers by high pressure liquid chromatography (HPLC) were developed to replace traditional paper chromatography. First, tritiated thymine-containing photoproducts from TFA-hydrolyzed DNA samples were subjected to HPLC on a Microsorb phenyl-5 μm column using water as the mobile phase and detected by scintillation counting of collected fractions. At a flow rate of 1.2 ml per min, thymine-containing compounds eluted in the order: thymine monomers (T; 7.5 min), *cis-syn* cyclobutyl thymine-thymine dimers (csTT; 10 min), cyclobutyl cytosine-thymine dimers (CT; 13 min), and SP (17 min). This method was used to monitor SP repair by SP lyase during spore germination. Second, an enzymatic DNA hydrolysis method, combined with HPLC and scintillation counting, was also developed to pursue rapid SP isolation and quantitation. DNA purified from UV-irradiated spores was completely hydrolyzed with P1 nuclease and the digestion products run on a Microsorb C18-5 μm column using 10 mM phosphate buffer, pH 7, as the mobile phase. At a flow rate of 1.8 ml per min, dTMP eluted at 2 min and SP dinucleotide at 5.5 min.

Supported by NIH and Tex. Advanced Res. Program.

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University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

INVOLVEMENT OF THE SCHIFF BASE LYSINE IN THE REACTION CATALYZED BY *O*-ACETYLSERINE SULFHHYDRYLASE FROM *SALMONELLA TYPHIMURIUM*.

Vaishali D. Rege*, William E. Karsten#, Nicholas M. Kredich+, Klaus D. Schnackerz°, and Paul F. Cook*#. Departments of *Biochem. and Mol. Biol., and #Microbiology and Immunology, UNT-Health Science Center, Fort Worth, Texas 71607, USA; +Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27707, USA; and °Physiologische Chemie Institut, Biozentrum der Universitaet Wuerzburg, Am Hubland, Wuerzburg D97074, Germany.

The final step in the biosynthesis of cysteine in *Salmonella typhimurium* is the reaction catalyzed by O-acetylserine sulfhydrylase (OASS-A), that is the conversion of O-acetyl-L-serine (OAS) and sulfide to L-cysteine and acetate. OASS-A is a pyridoxal 5'-phosphate-dependent enzyme in which the PLP is in Schiff base linkage with lysine-42 (K42) of the protein, and the enzyme has maximal absorbance in the visible at 412 nm. Site directed mutagenesis was used to change K42 to alanine in order to investigate the involvement of the lysine in catalysis. The K42A mutant protein has no activity when 5-thio-2-nitrobenzoate (TNB) is used as the nucleophilic substrate. As isolated, K42A shows a λ_{max} at 424 nm corresponding to an external aldimine, and cannot be reduced with $NaBH_4$, unlike the wild type (wt) enzyme, unless treated with 3 M GnHCl. Apo-K42A can be reconstituted with PLP to give a protein with a λ_{max} at about 398 nm. Addition of OAS to the reconstituted mutant protein generates absorbance maxima at about 320 and 420 nm, interpreted as a mixture of species including the *gem*-diamine and external aldimine. Chemical mutagenesis of cysteine-43 (43) in the K42A mutant with bromoethylamine gives an enzyme (K42A/C43EAC) with absorbance maxima at 330 and 412 nm. The K42A/C43EAC enzyme is active, but only about 10^{-4}-fold compared to wt using TNB as the nucleophilic substrate. Activity and the absorbance at 412 nm are lost upon reduction with borohydride. These data show: 1) the importance of lysine-42 in catalysis acting as the general base to remove the α–proton in the β-elimination of acetate; and 2) the flexibility of the active site portion of the protein in accommodating change. This work was supported by grants to PFC from the Robert A. Welch Foundation (B-11031) and NSF (DMB 8912053), to KDS from DFG (Schn 139/11-2), and a grant from NAATO (CRG 900519) to PFC and KDS.

SUBMIT TO CARLA LEE, GRADUATE OFFICE DEADLINE IS MARCH 1, 1994

University of North Texas Health Science Center
1994 RESEARCH APPRECIATION DAY

ABSTRACT FORM

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Read instructions and fit abstract in a rectangle (5 x 7 inches) in box given below.

Molecular cloning of cDNA encoding proteins immunologically cross-reactive with surfaces of Candida albicans. Sundstrom, P., and Woods, C. L. Department of Microbiology and Immunology, University of North Texas Health Sciences Center at Fort Worth, Fort Worth, Texas 76107.

The purpose of this research was to obtain cDNA clones that encode surface proteins of the endogenous, pathogenic yeast, Candida albicans. Surface proteins are important because of their interaction with host cells and because of their differential expression during fungal dimorphism. A C. albicans cDNA library in the expression vector lambda ZAP was screened with polyvalent rabbit antibody generated against formalin-killed C. albicans cells. Of 140 clones that reacted with the antiserum on the primary screening, 20 were purified. To identify clones that encoded surface proteins , IPTG-induced proteins from confluent plaques were used to affinity-purify specific antibodies from the polyvalent serum. The affinity-purified antibodies were then tested for their ability to react with surfaces of C. albicans in whole cell immunofluorescence assays. Four clones were found to bind antibodies that also reacted with surfaces of C. albicans in immunofluorescence. In a control experiment, a clone encoding the cytoplasmic protein (enolase) bound antibodies that reacted with enolase in Western blot assays, but did not react with surfaces of C. albicans in indirect immunofluorescence assays. Preliminary sequence analyses showed that the four clones were related, but were not similar to other proteins in the data bases. Southern blot analyses using the radiolabeled cDNA's encoding surface proteins suggested the presence of a gene family. These clones will be useful for studying the regulation of expression of surface proteins by C. albicans.
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ABSTRACT FORM

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REGULATION OF CARBON METABOLISM IN *ESCHERICHIA COLI* VIA THE PLEIOTROPIC GENE *csrA*. Honghui Yang and Tony Romeo. Department of Immunology and Microbiology. University of North Texas Health Science Center at Fort Worth, TX. 76107.

One metabolic pathway that is transcriptionally activated as *Escherichia coli* enters the stationary phase of growth is that involving glycogen biosynthesis. The gene *csrA* encodes a 61 amino acid polypeptide that potently inhibits biosynthesis of glycogen in *E. coli* and also affects gluconeogenesis. By measuring the expression of *lacZ* fusions in *csrA+* and *csrA :: kanR* strains, we have obtained evidence that *csrA* negatively regulates the expression of at least four genes involved in glycogen synthesis, *glgA* (glycogen synthase), *glgB* (glycogen branching enzyme), *glgC* (ADPglucose pyrophosphorylase), and *glgS* (a novel gene involved in glycogen synthesis), which are constituted in three different operons. In addition, *csrA* exhibits negative effects on *glgY* expression (glycogen phosphorylase) which is involved in glycogen degradation. No effects were observed on the expression of *zwf* (glucose-6-phosphate dehydrogenase) and *gnd* (6-phosphogluconate dehydrogenase) which participate in the pentose phosphate pathway. By assaying native enzyme activities from cell extracts, the expression of the glycolytic gene *tpi* (triosephosphate isomerase) was found to be positively affected. In vitro expression of *glgB, glgC* and *glgA* was specifically inhibited by cell extracts containing the *csrA* gene product (CsrA). This study provides evidence that *csrA* encodes an important regulator of intermediary carbon metabolism in *Escherichia coli.*

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