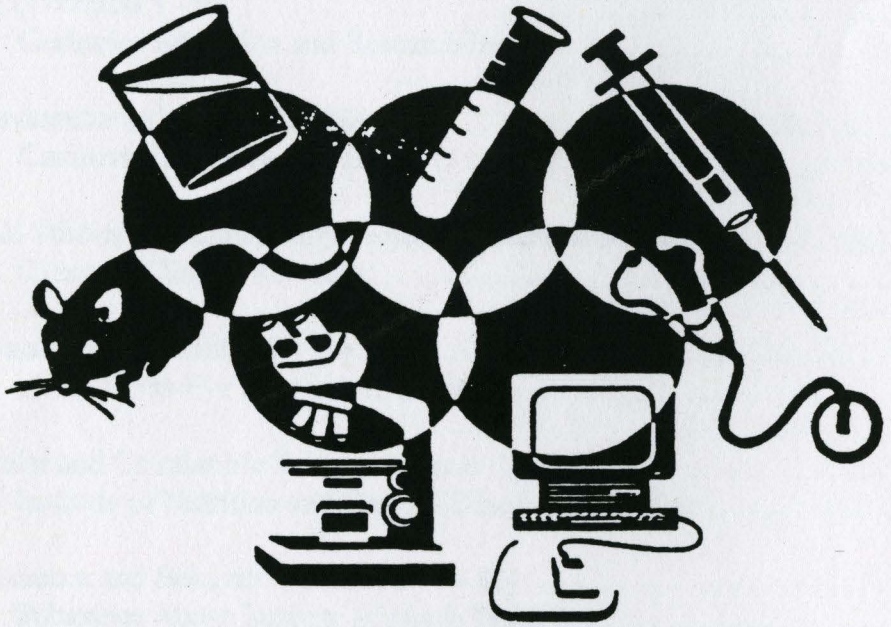
University of North Texas
Health Science Center at Fort Worth

Fifth Annual

Research Appreciation Day

March 26, 1997



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University of North Texas Health Science Center at Fort Worth

Research Appreciation Day

March 26, 1997

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RESEARCH APPRECIATION DAY 1997
REVISED AGENDA

8:00-9:00 am	Assemble Posters	Interdisciplinary Laboratory
9:00-10:00 am	Faculty/Non-Student Poster Session	Interdisciplinary Laboratory
10:00-11:30 am	Student Poster Competition	Interdisciplinary Laboratory
11:30 am-1:30 pm	Lunch and Keynote Speaker	Main Auditorium
	Welcome	David M. Richards, D.O. President
	Research at the HSC	Robert W. Gracy, Ph.D. Associate Dean Research and Biotechnology
	Overview of RAD '97 Activities	Thomas Yorio, Ph.D. Dean Graduate School of Biomedical Sciences
	Introduction of Keynote Speaker	Peter B. Raven, Ph.D. Chair Department of Integrative Physiology
	Cardiovascular Adaptations to Spaceflight: A Terrestrial Perspective	James A. Pawelczyk, Ph.D. Associate Professor Pennsylvania State University Noll Laboratory of Applied Physiology and Payload Specialist NASA Life Sciences Shuttle Mission Neurolab (To Launch in 1998)
1:45-4:30 pm	Student Oral Presentation Competition	Kiva Classroom
4:30-6:00 pm	Career Opportunities Forum	Panoramic Classroom
6:00 pm	Award Ceremony	Kiva Classroom
6:30 pm	Reception	Atrium
ALL DAY	Vendor Fair	Kiva Lounge/Hallway

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KEYNOTE SPEAKER

James A. Pawelczyk, Ph.D.
Assistant Professor of Physiology and Kinesiology
Noll Physiological Research Center
Pennsylvania State University

James A. Pawelczyk, Ph.D. is a specialist in the area of cardiovascular adaptation and regulation in unusual environments. His research focuses on neural and mechanical factors that affect rapidly-responding blood pressure regulatory mechanisms.

Unlike most quadrupedal animals, the cardiovascular system of humans must quickly adapt to large shifts in circulating blood volume resulting from changes in body posture with respect to gravity. With aging or disuse, changes in the control of vascular smooth muscle can lead to hypotension and orthostatic intolerance. Pawelczyk employs a variety of unusual models, ranging from chronic bedrest to long-term spaceflight to severe exercise, in order to study adaptations of neural control and mechanical properties of the circulation.

Currently, Dr. Pawelczyk is on leave from Pennsylvania State University to serve as Payload Specialist aboard the upcoming space shuttle flight "Neurolab" (STS-90) scheduled to launch in March of 1998. He is a co-investigator on the mission. Prior to joining Penn State, he was Assistant Professor of Cardiology at University of Texas Southwestern Medical Center, where he also performed his post-graduate training. He received his doctorate from University of North Texas under the direction of Peter B. Raven, Ph.D., Professor and Chair of the Department of Integrative Physiology at UNT Health Science Center.

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RESEARCH ACHIEVEMENT AWARDS

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Abbott's major businesses include pharmaceuticals, diagnostics, nutritionals, hospital products, and chemical and agricultural products. These businesses generated more than $9.2 billion in sales in 1994.

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The Abbott Laboratories Research Achievement Awards are given to the top three student poster presentations and the top three student oral presentations as determined by a panel of judges.

JUDGES

The 1997 Research Appreciation Day student poster presentation judges are: **Paul Chippindale, Ph.D.**, Department of Biology, University of Texas at Arlington; **Manfred H. Fleschar, Ph.D.**, Director of Product Development, Access Pharmaceuticals; **Norman Miner, Ph.D.**, Research Director, MicroChem Laboratory; **Ricardo E. Rodriguez, Ph.D.**; Associate Professor, Department of Chemistry, Texas Wesleyan University; **Arup Sen, Ph.D.**, Chairman and Chief Executive Officer, Health Tech Development, Inc.; **John W. Sheets, Jr., Ph.D.**, Senior Director of Development, Surgical IOL, R & D, Alcon Laboratories, Inc.; **Reginald Stilwell**, Scientific Manager, Johnson & Johnson Medical; and **Jill Van Wart Hood, Ph.D.**, Allied Health Coordinator, Department of Biology, University of Texas at Arlington.

The 1997 Research Appreciation Day student oral presentation judges are: **David G. Bernard, Ph.D.**, Assistant Professor, Department of Biology, University of Texas at Arlington; **Julia A. Nelson, M.S.**, Vice President for Scientific Affairs, Summa Laboratories, Inc.; and **John Segars, B.S., M.B.A.**, President, Electronic Monitors International, Inc.

VENDOR FAIR

The following companies have provided support for Research Appreciation Day 1997 by participating in our Vendor Fair. For your reference, a list of these vendors appears below. Please join us in thanking these companies for their support of our activities.

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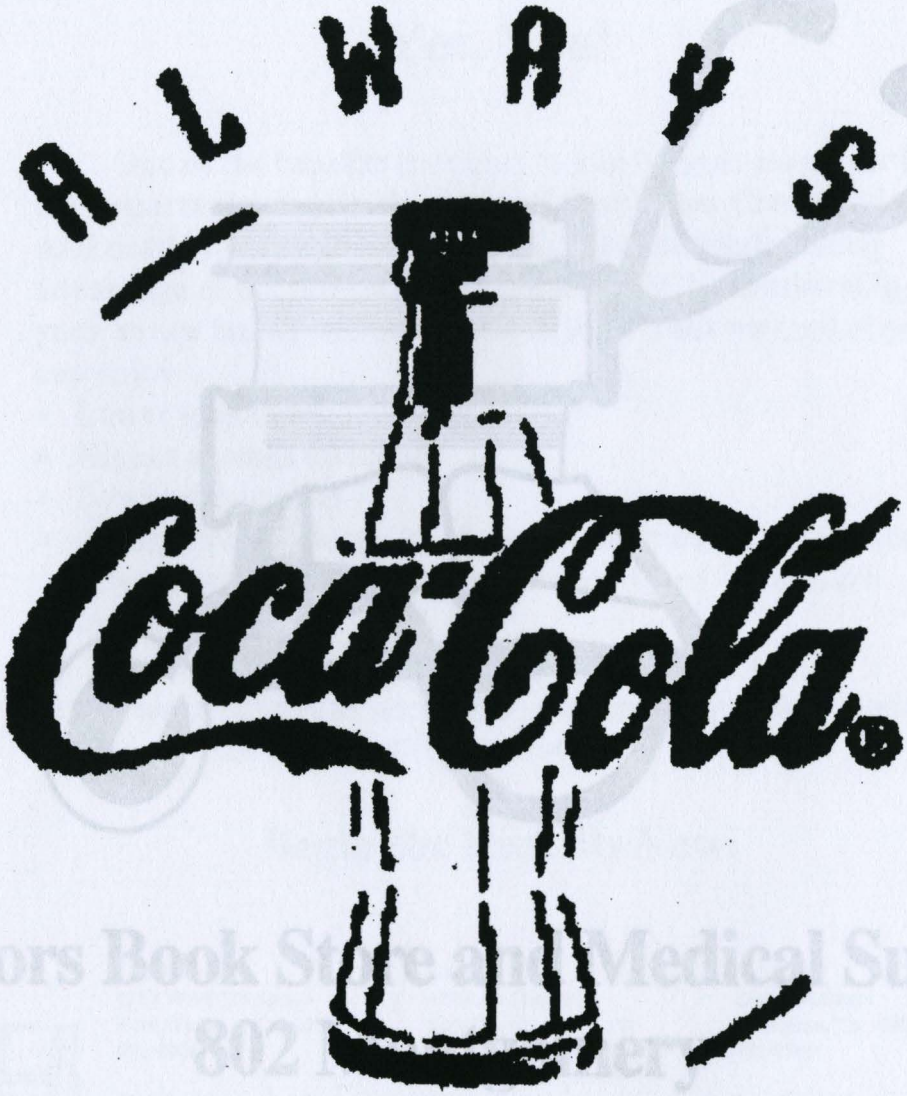
Coca-Cola Bottling Company of North Texas has generously provided the soft drink fountain for our break this afternoon. Please join us in thanking Coca-Cola Bottling Company of North Texas for its support of our activities.



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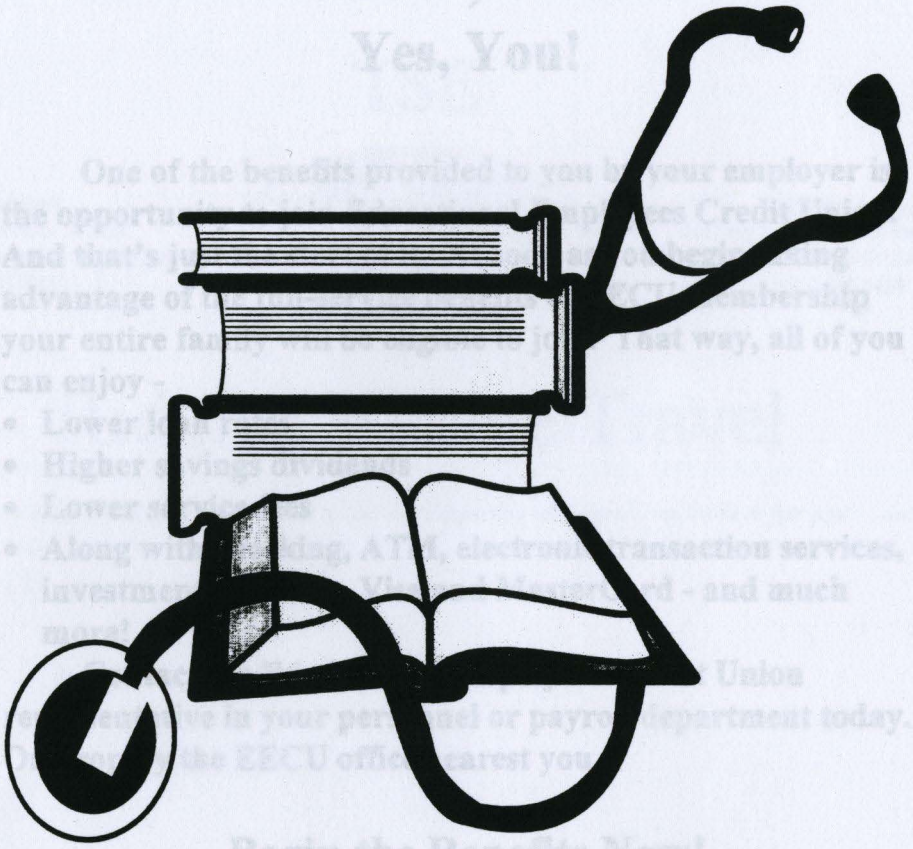

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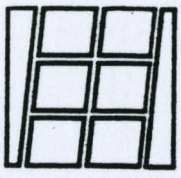

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TSE

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CAREER OPPORTUNITIES FORUM

For Research Appreciation Day 1997, the Graduate Student Association (GSA) is sponsoring a forum with selected professionals on career opportunities in academics and industry.

Erma Johnson, M.B.A., is the Vice Chancellor for Administration of the Tarrant County Junior College (TCJC) system. TCJC offers three kinds of college credit programs at each of four campuses. These programs include academic (for transfer to a senior college), occupational (for entry into or upgrading of a career) and remedial.

Ricardo E. Rodriguez, Ph.D., is an Associate Professor of Chemistry at Texas Wesleyan University. Texas Wesleyan University is a small, private, four-year undergraduate institution.

Glenn Dillon, Ph.D., is an Assistant Professor of Pharmacology at UNT Health Science Center. He performed his postdoctoral work at The Upjohn Company in Kalamazoo, Michigan.

David Gibson, Ph.D. (UNT '80), is the director of the Office of Clinical Research at UNT Health Science Center. Prior to taking his current position, Dr. Gibson managed clinical research and business development departments in the private sector.

Edward M. Brotman, Assistant Director of Human Resources for Research and Development at Alcon Laboratories, Inc. Alcon is a global leader in the research, development, manufacture and marketing of ophthalmic products, including prescription drugs, contact lens care solutions, surgical instrumentation and accessory products, intraocular lenses and disposable products, and office systems for eye care specialists and other medical offices. Alcon is a wholly-owned subsidiary of Nestlé S.A.

AGING

1	Karen S. Godwin, Ph.D.	GERIATRICS EDUCATION AND RESEARCH INSTITUTE
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3	Naveendra Korivi*	RELATIONSHIP AMONG MOOD, EXERCISE, AND HEALTH IN ELDERLY CAREGIVERS OF DEMENTIA PATIENTS
4	Stephanie Stafford*	IN-HOME HEALTH SCREEN AND NEEDS ASSESSMENT: HOME-BOUND LOW INCOME ELDERLY
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7	John Talent	ORAL POLYMERIC N-ACETYL-D-GLUCOSAMINE AS A POTENTIAL TREATMENT FOR PATIENTS WITH OSTEOARTHRITIS: PILOT STUDY
8	Stephen J. Moorman	MATURING OLIGODENDROCYTES INHIBIT NEURONAL GROWTH CONE MOTILITY IN DIFFERENT WAYS
9	Brian K. Koller	THE EFFECT OF DIETARY RESTRICTION ON COGNITIVE AND MOTOR PERFORMANCE IN 37-MONTH OLD B6D2F$_1$ MICE

*Student Presenter

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Karen S. Godwin, Ph.D.

Department: Geriatrics Education and Research Institute

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

GERIATRICS EDUCATION AND RESEARCH INSTITUTE.

Michael Forster, Ph.D., Co-Director; Karen Godwin, Ph.D., Co-Director.
University of North Texas Health Science Center 76107

Purpose: To create an "introduction" to the Geriatrics Education and Research Institute for viewers and to create a "lead" poster presentation preceding other posters/abstracts on aging supported or endorsed by GERI.
Methodology: The mission of GERI, a general focus on aging demonstrating a national focus and GERI's focus, and a "food for thought" section constitute the totality of the poster display.
Conclusions: GERI is an inter-disciplinary and multi-disciplinary Institute that focuses on the phenomenon of aging. Its Executive Council reflects most general areas surrounding the study of aging.

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Janelle House, DO

Department: Geriatrics Fellowship Program

Graduate Student___ Medical Student___ Postdoctoral Fellow_X_ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

GERIATRICIANS AND NSAID TREATMENT: BALANCING THE ODDS J. House, DO*; J. Knebl, DO*; R. Pertussi, DO; K. Godwin, PhD; J. Alexander, PhD; University of North Texas Health Science Center, Fort Worth, TX 76107

Purpose: This study looked at the prescribing patterns of physicians for elderly patients who present with a healed peptic ulcer and require that NSAID therapy be resumed.
Methods: 821 surveys were sent to physicians of the American Geriatric Society; 229 were returned. The survey allowed descriptive analyses. Its design determined collateral prescribing patterns for the elderly requiring NSAID treatment. Groups studied included Fellowship Trained Physicians, Medical Directors of Long Term Care Facilities, and Physicians with Certificates of Added Qualifications in Geriatric Medicine. The "preferred pharmaceutical treatment" selected was Misoprostol. Prescribing for the NSAID ulcer factors were also studied by groups
Conclusions: There appeared to be no significant differences among the groups studied for the preferred pharmaceutical treatment for the elderly who presented with a healed peptic ulcer and required continuation of NSAID therapy. Of the four "risk factors" articulated as guidelines in the *Annals of Internal Medicine, Vol 123,4; August, 1995,* only a GI related complication seems to be considered by all groups.

(This study was supported by Searle Pharmaceuticals, the Geriatrics Education and Research Institute, and the Bureau of Health Professions through the Geriatric Fellowship Program.)

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: S/D Naveendra Korivi

Department: Geriatrics Education and Research Institute

Graduate Student___ Medical Student_X__ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

RELATIONSHIP AMONG MOOD, EXERCISE, AND HEALTH IN ELDERLY CAREGIVERS OF DEMENTIA PATIENTS. N. Korivi, BS; S Castle, MD; S Wilkins, PhD; N Harada, PhD; UCLA School of Medicine and West Los Angeles VA Medical Center, Los Angeles CA 90024

Purpose: This project studies the relationship between depression, burden, physical activity, and baseline health of caregivers.
Methods: 13 wives recruited from a GRECC clinic and dementia support group. Instruments used in this study included the Geriatric Depression Scale (GDS), Folstein Mini-Mental Status Exam (MMSE), Zarit Burden Scale, Physical Activity Scale for Elderly (PASE) and the General Health Subscale of the SF-36. Subjects were processed individually or in groups of five or less. Rank correlation was used to demonstrate relationships among GDS, burden, PASE, and SF-36. Caregivers were determined to be at risk if they were 1) mildly depressed, GDS > 10; 2) moderately burdened, Zarit > 41; 3) PASE below mean for age group; 4) weight of 20% over IBW; 5) low SF-36 score; and 6) hypertensive.
Summary and Conclusions: Studies have shown that both aerobic and non-aerobic exercise significantly reduces depression. The benefits of physical activity on caregiver health are numerous and justify the development of a targeted treatment intervention involving exercise of low intensity, low impact, and gradual progression. The data collected support the hypothesis that burdened caregivers will exhibit worse health profiles than non-burdened caregivers. Weight, blood pressure, alcohol and tobacco use were not related to burden. When the self-reported physical activity (PASE) was compared to the readings obtained by a pedometer (Digiwalker), no relationship could be found. Exercising caregivers had lower burden, lower depression, higher general health scores (SF-36) and were closer to their ideal body weight.

(This study was supported by the John A. Hartford Foundation, the American Federation for Aging Research and the Geriatrics Educational and Research Institute.)

Abstract #3

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: S/D Stephanie Stafford

Department: Geriatrics Education and Research Institute

Graduate Student___ Medical Student_X_ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

"IN-HOME HEALTH SCREEN AND NEEDS ASSESSMENT: Home-Bound Low Income Elderly" S. Stafford, Student; W. Zaloga, Student; J.A. Knebl, DO; K. Godwin, Ph.D.; University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107

Purpose: The purpose of this project was to provide in-home health screening and needs assessment to low-income, home-bound elderly to enable continuation of independent living.

Methodology: The model was designed at the request of the Tarrant County Area Agency on Aging. 40 clients were eligible for Meals-On-Wheels because of low-income status; case workers identified a strong need for medical/dental screening and assessments. Medical students were trained by a geriatrician and a dentist. Data elements for analysis include demographic information; medical history; current conditions, health and dental assessments; and scores on mini-mental, ADL/IADL, and nutritional assessments. Analyses show that the demographic profile of elderly in Tarrant County is changing relative to independent living and life-style patterns.

Conclusions: Proactive services through collaboration with the social service agencies in the spirit of health promotion and disease prevention formed the basis of this health service model. The model allows the elderly to maintain independence without jeopardizing the integrity of primary care physicians and social service agencies.

(Funding Source: Area Agency on Aging of Tarrant County and the Bureau of Health Professions through the Geriatric Fellowship Program.)

Abstract #4

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Sue Gena Lurie, Ph.D.

Department: Geriatric Education and Research Institute

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty XX Staff___

Read instructions and fit abstract inside the space given below:

AGING IN HEALTHY COMMUNITIES AND NEIGHBORHOODS. S.G.Lurie,PhD. Geriatric Education and Research Institute, University of North Texas Health Science Center, Fort Worth, Texas 76107

Purpose: To compare "Healthy Communities"with "Healthy Neighborhoods" projects in: (a) identifying community resources and problems for aging residents; (b)participatory health planning methods; (c) community mobilization; (d) application of state, national, international project models. Methods: Projects are compared from participant observation in "Healthy Communities" aging committee and qualitative interviews of elderly residents in the "Healthy Neighborhoods" project.Results are evaluated based on goals and methods of each project. Summary and Conclusion: Local "Healthy Communities" and "Healthy Neighborhoods" projects apply various methods to develop and implement community planning goals, from state, national and World Health Organization models. (1) "Healthy Communities" aging committee is defining goals and methods to maximize local resources and resolve problems through coalition of hospital,public health, social service, business, educational professionals (with committees on children, housing, transportation, education, violence). (2)"Healthy Neighborhoods" project elicits residents' perception of problems and resources in household interviews by VISTA staff and volunteers, and local meetings. Elderly residents report problems of disability, safety from crime; resources are neighbors, seniors' groups, city services, home health, schools, churches. These responses, with those from surveys and meetings in four ethnically-diverse neighborhoods, provide bases for participatory planning.

Abstract #5

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Raymond Pertusi, DO

Department: Internal Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

NSAID GASTROPATHY: SELF-REPORTED PRESCRIBING PATTERNS AMONG GERIATRICIANS. R. Pertusi, DO; J. Knebl, DO; K. Godwin, Ph.D.; J. House, DO; J. Alexander, Ph.D.; B. Rubin, DO; and M. Forman, DO. Departments of Internal Medicine and Information Technology Services, University of North Texas Health Science Center, Fort Worth, TX 76107.

Purpose: The study examines the prescribing patterns for GI-protective agents when NSAID treatment is started for elderly patients. ***Methods:*** Surveys were sent to 817 physician members of the American Geriatrics Society; 229 responded to the following questions. Assuming.....

1. A well elderly patient requires treatment with an NSAID, would you prescribe...?
2. A nursing home patient requires treatment with an NSAID, would you prescribe...?
3. A well elderly patient on an NSAID develops dyspepsia, would you prescribe...?
4. A well elderly patient has developed a paptic ulcer requiring discontinuation of an NSAID, would you prescribe...?
5. That the ulcer has healed an an NSAID is restarted, would you prescribe...?

Each questions was followed by 5 choices in the following order: antacids, H_2 anatagonists, misoprostol, omeprazole, or sucralfate. Combinations were permissable. ***Summary and Conclusions:***

% Likely to treat with 1+agents					
Question	**1**	**2**	**3**	**4**	**5**
TX	56	67	98	99	99
No TX	44	33	02	01	01

Choices among those who treat (%)					
Question	**1**	**2**	**3**	**4**	**5**
Antacids	29	26	25	19	16
H_2 Antagonists	27	29	32	33	26
Misoprostol	26	28	19	07	29
Omezprazole	07	06	13	24	15
Sucralfate	10	11	11	17	13

We conclude that physicians may be underutilizing GI-protective agents in populations at risk for serious NSAID-induced GI complications

Abstract #6

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: John Talent

Department: Biochemistry and Molecular Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff ✓

Read instructions and fit abstract inside the space given below:

ORAL POLYMERIC N-ACETYL-D-GLUCOSAMINE AS A POTENTIAL TREATMENT FOR PATIENTS WITH OSTEOARTHRITIS: PILOT STUDY

J.M. Talent,M.S. and R. W. Gracy, Ph.D. University of North Texas Health Science Center at Fort Worth, Molecular Aging Unit, Dept. of Biochemistry and Molecular Biology, Ft. Worth, TX 76107.

Glucosamine and its derivatives such as glucosamine sulfate and N-acetylglucosamine (NAG) have been shown to be effective in the treatment of osteoarthritis. Unfortunately, the half life of glucosamine in the blood is relatively short; therefore, a sustained-release of the material would be highly desirable. The purpose of this pilot study was to determine if the polymeric form of NAG (POLY-Nag®) could provide a longer-lasting oral source for serum NAG. Ten healthy subjects each ingested 1 g/d of either NAG or POLY-Nag for 3 days. After a 4-day washout period, each subject was crossed over to receive the other compound for 3 days. Serum samples were collected and analyzed using high-performance liquid chromatography. Results show that orally ingested NAG and POLY-Nag are absorbed resulting in increased serum levels of NAG, and POLY-Nag appears to be at least as effective as NAG. Serum levels of NAG had decreased by 48 hours after cessation of ingestion of NAG or POLY-Nag but were still above baseline levels. Increases in serum glucosamine levels indicate that NAG or POLY-Nag are converted to glucosamine *in vivo*. In conclusion, POLY-Nag may provide a source of serum glucosamine for treatment of patients with osteoarthritis. Longer and more rigorous pharmacokinetic and clinical studies need to be done. (Supported by Lescarden, Inc., New York, New York)

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Stephen J. Moorman

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

MATURING OLIGODENDROCYTES INHIBIT NEURONAL GROWTH CONE MOTILITY IN DIFFERENT WAYS. S.J. Moorman* and R.M. Gould[1]. Dept. of Anat. and Cell Bio. UNT Health Science Center at Fort Worth, TX 76107; [1]NYS Inst. for Basic Res. in Devel. Dis., Staten Island, NY 10314

Among the many molecules produced by oligodendrocytes are proteins that might limit regeneration by inhibiting neuronal growth cone motility. Maturing oligodendrocytes can be divided into 5 specific stages from O2A (Stage 1) precursors to mature (Stage 5) myelinating cells based on stage-specific antigen expression and characteristic morphology. Each Stage has a predictable array of surface molecules which, for certain stages, includes inhibitory molecules. We tested whether growth cones respond to contact with each stage of maturing oligodendrocytes the way they respond to contact with the purified oligodendrocyte-specific inhibitory proteins known to be expressed at specific stages.

Cells from 3-day-old neonatal rats were grown on poly-lysine for 3 to 7 days. Oligodendrocytes were physically removed from the substratum using a patch-clamp electrode and a micromanipulator. Each 'manipulated' oligodendrocyte was then placed into contact with a neuronal growth cone for 30 minutes. Growth cone surface area was compared before and after contact with an oligodendrocyte. Surface area decrease of 50% or more = collapse.

As oligodendrocytes matured from Stage 1 to Stage 4, they developed an increasing ability to cause neuronal growth cones to collapse, peaking at 95% for Stage 4. This increasing ability to inhibit the neuronal growth cone coincides with the onset of expression of NI35 by Stage 2 oligodendrocytes. Stage 5 oligodendrocytes only induced 15% of neuronal growth cones to collapse on contact. This result is surprising considering NI35 and MAG are both expressed by Stage 5 oligodendrocytes. These results suggest that growth cone response to contact depended on the developmental state of the oligodendrocyte, independent of the presence of inhibitory molecules. Therefore, growth cone response is likely to be dependent on the context in which inhibitory molecules are presented.

Supported by the American Paralysis Association.

Abstract #8

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Brian K. Koller

Department: Department of Pharmacology, Geriatrics Ed & Research Inst

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

THE EFFECT OF DIETARY RESTRICTION ON COGNITIVE AND MOTOR PERFORMANCE IN 37-MONTH OLD B6D2F$_1$ MICE.

Koller, B.K., Forster, M.J., Morris, P., and Lal, H. Department of Pharmacology, Geriatrics Education and Research Institute, University of North Texas Health Science Center, Fort Worth TX 76107

It is well known that dietary restriction prevents the decline of cognitive and motor function normally observed in mice aged up to 22 months (*Dubey,* et al., *Arch. Biochem, Biophys:* 33,189,1996). In order to determine if these protective effects persist at later ages, the present study re-examined this issue in 37-month-old mice. A total of 24 naive 37-month old B6D2F$_1$ mice were used, 12 of which were maintained under 40% diet restriction (DR) from four months of age, whereas the remaining 12 had ad libitum (AL) access to food. Mice were tested in a variety of cognitive and motor tasks. In the T-maze test, a cognitive test which assesses discriminative and active avoidance, DR animals had a greater number of correct turns and avoidances than AL mice in the first five trials. Additionally, DR mice left the stem area of the maze faster than the AL animals. Motor tests included the wire suspension test, which assess muscle strength and agility, and the accelerating rotorod test, which assess balance and coordination. In the wire suspension test DR animals were able to pull their hind legs up faster (treading) and had a higher latency to fall than their AL counterparts. In addition, DR animals had a larger latency to fall and obtained greater maximum levels of performance than AL mice in the accelerating rotorod test. It was concluded that the preventive effects of dietary restriction against age related cognitive and motor dysfunctions persists even into the latter stages of life. (Supported by NIH - NIA grant AG07692)

Abstract #9

CARDIOVASCULAR

10	Peter B. Raven, Ph.D.	CARDIOVASCULAR RESEARCH INSTITUTE
11	Patrick Hayes	BLOOD PRESSURE (BP) RESPONSE TO THE ONSET OF LOWER BODY NEGATIVE PRESSURE (LBNP): AGING EFFECT IN HUMANS
12	Hong-Wei Wang*	CARDIAC AUTONOMIC RESPONSE TO CENTRAL HYPOVOLEMIA: EFFECT OF AGE
13	Kevin M. Gallagher*	STROKE VOLUME CONTINUOUSLY INCREASES DURING DYNAMIC EXERCISE TO MAXIMUM OXYGEN UPTAKE (VO_{2max}) IN ENDURANCE ATHLETES
14	Steven Stuewe*	AEROBIC EXERCISE TRAINING INCREASES EXPRESSION OF GLYCOLYTIC AND OXIDATIVE ENZYMES IN CANINE MYOCARDIUM
15	Ross G. Querry*	EFFECTS OF EXERCISE TRAINING ON RECOVERY FROM PERCUTANEOUS MITRAL BALLOON VALVULOPLASTY
16	Scott Alan Smith*	ROLE OF INTRAMUSCULAR AFFERENT FIBERS IN THE VENTILATORY RESPONSE TO DYNAMIC EXERCISE IN HUMANS
17	James S. Shirley*	CHANGES IN HUMAN BETA-ADRENERGIC RECEPTOR DENSITY IN PERIPHERAL MONONUCLEAR LEUKOCYTES IN RESPONSE TO CHRONIC EXERCISE
18	Wayne W. Loney*	THE EFFECTS OF PROGRESSIVE MARATHON TRAINING ON HIGH DENSITY LIPOPROTEINS AND PREDICTIVE RESPONSE WITH LIPOPROTEIN SUBFRACTIONS
19	Kristin Bryant*	CAROTID ARTERIAL BAROREFLEX FUNCTION IN RELATION TO ACTIVE SKELETAL MUSCLE MASS

20	Stephen L. Wasmund*	IMPORTANCE OF ARTERIAL BAROREFLEX CONTROL OF SYMPATHETIC NERVE ACTIVITY DURING RAPID VENTRICULAR PACING IN HUMANS
21	Geoffrey Kline*	ENDOTHELIUM-DEPENDENT CORONARY DILATION IN RESPONSE TO NOREPINEPHRINE IN CONSCIOUS DOGS
22	Ying Yu*	PRESSURE-INDUCED CHANGES IN RIGHT CORONARY VASCULAR VOLUME
23	Isabel Tejero-Taldo*	ß-ADRENERGIC STIMULATION AND PYRUVATE RESTORE POST-ISCHEMIC CARDIAC FUNCTION BY DIFFERENT MECHANISMS
24	Amber A. Stanfill*	ACUTE MORPHINE AND VAGAL ACTIVITY IN DOGS
25	Leslie D. Napier*	REDUCED CARDIAC PARASYMPATHETIC CONTROL IN MORPHINE-ADDICTED DOGS
26	Keith E. Jackson*	MEASURING PROENKEPHALIN IN ISOLATED WHITE BLOOD CELLS
27	Miao-Xiang He, M.D.	DOWNREGULATION OF CARDIAC CONTRACTION BY INORGANIC PHOSPHATE (Pi)
28	Edward L. Orr, Ph.D.	CORONARY OCCULSION INCREASES HISTAMINE LEVELS IN CORONARY VENOUS AND COLLATERAL CIRCULATIONS OF THE DOG
29	G. M. Rocha, M.D., Ph.D.	HYPEROSMOTIC STRESS INDUCES AN ATTENUATED VASOCONSTRICTIVE EFFECT ON AGED MICE ARTERY
30	Jami R. Kern*	HUMAN VASCULAR TISSUE EQUIVALENT
31	Juan Wang*	REGULATION OF CARDIAC PHOSPHOINOSITIDE-SPECIFIC PHOSPHOLIPASE C ISOENZYMES

*Student Presenter

32	Lara Keyser*	CHARACTERIZATION OF A NOVEL INOSITOL TETRAPHOSPHATE STIMULATED Ca^{2+} TRANSPORTER IN CARDIAC MEMBRANES
33	Victoria L. Rudick, Ph.D.	THE HUMAN APOA-I GENE IN MDCK CELLS: CONSEQUENCES OF ITS EXPRESSION

*Student Presenter

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Cardiovascular Research Institute

Department:

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

The Cardiovascular Research Institute, established in 1995, was developed from the multidisciplinary research efforts of molecular biologists, pharmacologists, physiologists, and physicians in the internal medicine subspecialties of cardiology and pulmonary. A key role of the institute is to integrate basic research findings with the clinical therapeutic problems associated with over 50 million Americans who suffer from cardiovascular diseases.

Institute studies focus on heart disease, with special emphasis on understanding the role of exercise in the prevention of and rehabilitation from heart disease. Research is conducted into the fundamental molecular biologic and cellular mechanisms associate with the improved cardiovascular function, cardio-protection from heart attacks and longer life of those people who have moderate- to high- activity lifestyles. Both the basic science and clinical divisions of the institute collaborate with pharmaceutical and biotechnology corporations in order to validate new diagnostic, preventive, therapeutic and corrective procedures. Institute activities involve local, national and international partnerships.

The institute also provides educational and research training opportunities for graduate and medical students, and postdoctoral and clinical fellows.

Abstract #10

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Patrick Hayes

Department: Integrative Physiology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff XX

Read instructions and fit abstract inside the space given below:

BLOOD PRESSURE (BP) RESPONSE TO THE ONSET OF LOWER BODY NEGATIVE PRESSURE (LBNP): AGING EFFECT IN HUMANS.

P.Hayes, H.Wang, R.Welch-O'Connor, P.Reese, F.Schaller and X.Shi.
Department of Integrative Physiology, the Cardiovascular Research Institute, UNT Health Science Center, Fort Worth, TX 76107.

To test the hypothesis that hypotension would be transiently present at the onset of low level of LBNP with aging, we compared the changes in BP (Finapres) and heart rate (HR) in 6 young (26±2yr) and 5 older (62±3yr) healthy subjects between LBNP -15 and -40 torr. The *initial* response, an average of the first 10 beats of BP and HR after the intended LBNP was achieved (~4 to 5 *sec*), and the *later* response, an average of the following 1-min beat-to-beat data, were compared to the baseline (averaged 1-min data prior to the application of LBNP), see table.

LBNP Torr		Young			Older		
		Baseline	Initial	Later	Baseline	Initial	Later
HR bpm	-15	60±3	63±2	60±3	56±3	59±3	59±3
	-40	59±4	73±2*	68±3*	57±3	62±2*	62±3*
SP mmHg	-15	123±7	123±7	124±8	130±8	133±12	129±11
	-40	123±6	110±7*	122±7	134±10	111±9*	123±11
MP mmHg	-15	84±4	82±5	82±4	93±5	93±8	91±7
	-40	88±4	76±5*	85±5	98±8	80±7*	91±8
DP mmHg	-15	68±2	66±3	67±3	70±4	70±6	70±5
	-40	71±3	62±4*	70±4	75±6	63±6*	73±7

Conclusion: Though there was an *initial* hypotension associated with a tachycardia observed at -40 torr, LBNP -15 torr did not change BP or HR in both groups, indicating an absence of the perturbation of the arterial baroreceptors at low level LBNP. The older subjects showed a greater hypotension with a less tachycardia in the *initial* responses, suggesting that the cardiovascular reflex was compromised with aging in response to the onset of orthostatic challenge.

(Supported by NIH AG14219)

Abstract #11

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Hong-Wei Wang

Department: Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CARDIAC AUTONOMIC RESPONSE TO CENTRAL HYPOVOLEMIA: EFFECT OF AGE. H.Wang, R.Zhang, P.Hayes, P.Reese, K.Bryant, P.Raven and X.Shi. Department of Integrative Physiology & the Cardiovascular Research Institute, UNT Health Science Center, Fort Worth, TX 76107.

To test the hypothesis that cardiac autonomic function was compromised with aging, we compared the heart rate variability (HRV) during lower body negative pressure (LBNP) induced central hypovolemia in 12 young (27±1yr) and 12 older (65±2yr) healthy subjects. Frequency domain of HRV (correlogram) was assessed from 8-min record of beat-to-beat HR during breathing at 0.25Hz. The power at low frequency (LF, 0.04-0.15Hz) and high frequency (HF, 0.15-0.40Hz) was extracted and the normalized changes in HF (HF/(LF+HF)) and LF (LF/HF) were calculated as the index of the cardiac parasympathetic (Rp) and sympathetic (Rs) responses, respectively. There was no age-related difference in the baseline HR (young vs. older: 58±2 vs. 60±2bpm) or blood pressure (Finapres), nor in the normalized HF (0.32±0.06 vs. 0.28±0.06) or LF (5.1±1.7 vs. 6.9±2.3). LBNP significantly decreased pulse pressure (PP, $P<0.001$) in both young and older subjects (baseline: 59±3 and 65±4mmHg), and this decreased PP appeared to be greater ($P<0.04$) in the older group (see table). However, the significant changes in the cardiac autonomic responses at high levels of

		Young			Older		
$LBNP_{torr}$	0	-15	-30	-40	-15	-30	-40
Rp (%)	100	101±16	70±9	45±7*	90±14	95±13	72±12
Rs (%)	0	132±28	202±34*	279±53*	161±33	140±2	160±28
ΔHR_{bpm}	0	+0.9±2	+7.2±2**	+9.3±2**	+1.2±1	+3.1±1	+8.7±4*
ΔPPmmHg	0	-5±2	-7±2*	-9±7*	-8±3*	-13±2*	-15±5*

* & ** indicate significant different from the baseline & baseline and -15 torr. N=5 at -40 torr in both groups.

LBNP were observed only in young group. We concluded that aging compromised both cardiac sympathetic activation and vagal withdrawal during LBNP induced central hypovolemia. (Supported by NIH AG14219 and HL45547)

Abstract #12

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Kevin M. Gallagher

Department: Integrative Physiology

Graduate Student X Medical Student X Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

STROKE VOLUME CONTINUOUSLY INCREASES DURING DYNAMIC EXERCISE TO MAXIMUM OXYGEN UPTAKE (VO_{2max}) IN ENDURANCE ATHLETES

K.M. Gallagher, S.A. Smith, R.G. Querry and P.B. Raven

Department of Integrative Physiology and Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107

Five endurance trained (ET; 62 VO_{2max}; 4 men/1 women) and five untrained (UT; 36 VO_{2max}; 3 men/2 women) young adults performed incremental exercise on a cycle ergometer to volitional fatigue. Heart rate (HR; beats•min^{-1}), cardiac output (Q_c; L•min^{-1}), VO_2 (ml•kg^{-1}•min^{-1}) were measured and stroke volume (SV; ml•beat^{-1}) was calculated (mean ± SEM).

Percent of VO_{2max}

	20%	40%	60%	80%	100%
ET SV	106.9±11	128.8±12	136.5±12	144.5±14	155.2±14
UT SV	69.0±14	87.0±14	94.3±15	97.5±16	103.2±18

ET subjects had similar maximal HRs and significantly higher maximal Q_cs and SVs compared to the UT subjects ($p < 0.05$). Regression slopes of SV from 40% to 100% VO_{2max} were significantly higher in ET (0.43 ± 0.06) than UT (0.24 ± 0.08) subjects ($p < 0.05$). Since the UT subjects SV is known to plateau, we conclude that stroke volume continuously increases during dynamic exercise to VO_{2max} in ET subjects.

(Supported by NIH # HL45547)

Abstract #13

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Steven Stuewe

Department: Integrative Physiology

Graduate Student_X_ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

AEROBIC EXERCISE TRAINING INCREASES EXPRESSION OF GLYCOLYTIC AND OXIDATIVE ENZYMES IN CANINE MYOCARDIUM

Steven Stuewe, Neeraj Agarwal, Patricia A. Gwirtz, Robert T. Mallet. Depts. Integ. Physiol. and Anat. and Cell Biol., Univ. North Texas Hlth. Sci. Ctr., Fort Worth, Texas 76107-2699

Aerobic exercise training increases rate-controlling glycolytic and oxidative enzyme activities in canine myocardium. These metabolic adaptations could result from expression of isoforms with increased substrate affinity (lower K_m), or from increased expression of adult isoforms, where maximum activity (V_{max}) is increased but K_m is unchanged. To differentiate between these alternatives, we analyzed kinetics of training-enhanced (CS: citrate synthase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HADH: hydroxyacyl CoA dehydrogenase) and unaltered (LDH: lactate dehydrogenase) enzymes. Dogs completed a 9 wk running program or were cage-rested for 4 wk. Left ventricular myocardial enzymes were extracted and assayed at 38°C. K_m and V_{max} were determined from Lineweaver-Burk analyses. Data (K_m: μM; V_{max}: U/mg protein) are means ± SE, n = 6 (*: $P < 0.05$ *vs.* sedentary).

	Sedentary		Trained	
Enzyme	**K_m**	**V_{max}**	**K_m**	**V_{max}**
CS	60±5	0.80±0.06	100±21	1.57±0.22*
GAPDH	392±49	4.99±0.47	501±57	8.32±0.94*
HADH	7.0±1.0	1.64±0.10	7.0±1.0	2.18±0.15*
LDH	105±3	12.2±1.0	109±2	14.8±0.8

Increased V_{max} and unchanged K_m following training indicated increased contents of the adult CS, GAPDH, and HADH isoforms. Immunoblots revealed 2.2±0.4- and 3.2±0.4-fold increases in CS and GAPDH protein, respectively ($P < 0.05$). Thus, aerobic exercise stimulates constitutive expression of adult enzyme isoforms. NIH support: HL50441, HL34172.

Abstract #14

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Ross G. Querry

Department: Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EFFECTS OF EXERCISE TRAINING ON RECOVERY FROM PERCUTANEOUS MITRAL BALLOON VALVULOPLASTY

R.G. Querry[a], J. Ramadan[b], N. Hayatt[b], H. Salman[b], A. Abul[b], P.B. Raven[a], [a]Dept. of Integrative Physiology & Cardiovascular Research, Ft. Worth, TX, [b] Kuwait University Hospital, Kuwait

The purpose of this case study was to investigate the effects of exercise training on peak exercise performance following percutaneous mitral balloon valvuloplasty (PMBV). Fourteen subjects (age = 33.6 ±1.7 yrs., NYHA class of 3.2 ± 0.1) with symptomatic mitral stenosis underwent PMBV surgery. Thirteen of the subjects were allowed to recover without exercise training (NT). One subject with a history of aerobic exercise had a recovery that included a prescribed exercise training program (ET). All subjects were administered a graded exercise test prior to surgery and 1 week, 1 month, and 3 months post surgery to determine peak exercise performance capabilities. Maximal exercise performance data for NT and ET subjects follows:

	HR (bt/min)		VO2 (ml/kg)		O2 pulse (ml/bt)		Exer. Dur. (s)	
	NT	ET	NT	ET	NT	ET	NT	ET
Pre	160±5	155	17.1±1.6	28.9	7.30±.69	14.71	261±37	522
1wk	166±6	172	22.2±1.6*	34.6	9.13±.72*	15.82	425±35†	730
1mo	175±4*	176	23.4±1.9*	36.2	8.99±.69*	16.15	500±46†	836
3mo	173±4*	182	23.4±1.6*	41.5	9.36±.76*	17.94	494±39†	1022

Significant difference between pre and post valvuloplasty; *p<0.05; †p<0.01

The effects of ET post-PMBV compared to NT patients were evaluated. Significant increases in exercise performance and tolerance data are seen in the non-exercising group at 1 week and plateaued at 1 and 3 months. The exercised trained individual markedly increased exercise performance at 1 and 3 months of recovery. This data indicates that exercise training during recovery from PMBV provides additional increases to functional performance and may have marked effects on daily activity.

Abstract #15

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Scott Alan Smith (Poster Competition)

Department: Integrative Physiology, Cardiovascular Research Inst.

Graduate Student X Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

ROLE OF INTRAMUSCULAR AFFERENT FIBERS IN THE VENTILATORY RESPONSE TO DYNAMIC EXERCISE IN HUMANS. S.A. Smith, K.M. Gallagher, K.H. Bryant, R.G. Querry, and P.B. Raven. Dept. Of Integ. Physiol. & Cardiovascular Research Inst., UNTHSC, Fort Worth, TX 76107.

Eight subjects, aged 26.5 ± 3.7 yrs., performed incremental workload cycling to investigate the interaction of skeletal muscle ergoreceptors in eliciting a ventilatory stimulus response to exercise. Each subject performed three bouts of exercise: control (exercise - no intervention); exercise with thigh cuff inflation to 90 mmHg (to reduce venous outflow and stimulate metaboreceptors); and exercise with application of lower-body positive pressure (LBPP) to 45 mmHg (to stimulate mechanoreceptors). Measurements of ventilation ($\dot{V}_E$, $L \cdot min^{-1}$), change in intramuscular pressure (ΔIMP, mmHg), and pH were collected.

Watts	CONTROL			CUFFS			LBPP		
	$\dot{V}_E$	pH	ΔIMP	$\dot{V}_E$	pH	ΔIMP	$\dot{V}_E$	pH	ΔIMP
Rest▪	14.3	7.38	0.0	13.9	7.38	0.0	13.9	7.38	0.0
67W	30.4	7.37	1.1	29.2	7.37	0.4	37.4	7.37	44.8*
137W	48.7	7.36	1.7	51.0	7.37	1.3	57.7	7.36	48.9*
200W	67.8	7.33	2.0	76.9†	7.36*	1.8	90.9*	7.36*	50.1*

(*Significance from control at p<0.05; †Significance from control, p=0.08; Rest▪ - no interventions. Mean data is presented.)

From these data, we conclude that stimulation of ergoreceptors generates a ventilatory stimulus to exercise. (Supported by NIH HL45547)

Abstract #16

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: James S. Shirley

Department: Integrative Physiology

Graduate Student_X_ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CHANGES IN HUMAN BETA-ADRENERGIC RECEPTOR DENSITY IN PERIPHERAL MONONUCLEAR LEUKOCYTES IN RESPONSE TO CHRONIC EXERCISE.

J. S. Shirley, M. Martin, S. Grant, R.W. O'Connor, P.B. Raven.

Dept. Of Integrative Physiology and Cardiovascular Research Institute. UNTHSC at Fort Worth, TX 76107.

To test the hypothesis that endurance exercise training influences β-adrenergic receptor (β-AR) density we compared β-AR densities on mononuclear leukocytes (MNL) obtained from whole blood in eight high fit (HF) male subjects (19-33 yrs) with average VO_{2max} of 66.8 $ml/kg^{-1}/min^{-1}$ and seven average fit (AF) male individuals (21-34 yrs) with VO_{2max} of 40.4 $ml/kg^{-1}/min^{-1}$ (determined via treadmill or bicycle). After an overnight fast and 24 hr. abstinence from heavy exercise and alcohol; the subjects arrived at the lab, a 40 ml venous blood sample from an antecubital vein was withdrawn in the presence of EDTA following 30 in supine rest. MNL fractions were obtained and counted in a hemacytometer to obtain cell number. The remaining MNL's were suspended in buffer, homogenized, centrifuged and the washed membrane preparations subjected to radioligand binding with [^{125}I]-(-) Iodocyanopindolol. The β-AR densities calculated and normalized with respect to either cell number or protein concentration. Our data indicate that β-AR density in MNLs obtained from HF, chronically exercised individuals is significantly decreased ($p<0.05$) compared to the density observed in AF MNLs.

(Supported in part by NIH Grant #HL 45547)

Abstract #17

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

ORAL

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: WAYNE W. LONEY

Department: BIOMEDICAL SCIENCE

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE EFFECTS OF PROGRESSIVE MARATHON TRAINING ON HIGH DENSITY LIPOPROTEINS AND PREDICTIVE RESPONSE WITH LIPOPROTEIN SUBFRACTIONS.

Wayne W. Loney and Andras G. Lacko. Department of Biomedical Science, University of North Texas Health Science Center, Fort Worth, TX 76107

Moderately trained subjects with elevated levels of high density lipoprotein cholesterol (HDL-C) concentrations (55±9.6 mg/dl) were selected for this study to determine if progressive marathon training can influence HDL-C levels and cardiovascular risk. Changes in plasma HDL-C were examined to better understand the variability of training responses and to gain a better understanding of the metabolic events during progressive marathon training. Serum samples were analyzed at the outset and following the conclusion of eight and sixteen weeks of progressive marathon training. Following repeated measures analysis there was a significant difference between the three periods of marathon training ($p=0.043$). Further analysis revealed that HDL-C may have a maximum capacity to become elevated with no further significant increases. HDL-C levels were significantly increased between the initial and first eight weeks of training ($p=0.003$) but showed a decline to previous concentrations in HDL-C levels following the conclusion of sixteen weeks. Regression analysis was used to predict the outcome response to estimate the HDL-C levels following marathon training with significant results ($R^2 = .74$). Differences in the changes in lipoprotein lipids suggest that hepatic triglyceride lipase (HTGL) and other enzyme activity may also have limited capacity to increase HDL-C.

Abstract #18

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Kristin Bryant

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CAROTID ARTERIAL BAROREFLEX FUNCTION IN RELATION TO ACTIVE SKELETAL MUSCLE MASS. K.H. Bryant, R. Boushel, S. Strange, B. Saltin and P.B. Raven. Cardiovascular Res. Inst. - UNTHSC, Ft. Worth, TX; Inst. Exerc. & Envir. Med, UTSWMSC, Dallas, Tx.; CMRC, Copenhagen, DK.

The purpose of this investigation was to test the hypothesis that the resetting of the carotid arterial baroreflex (CBR) function curve with exercise occurs in relation to the amount of actively exercising skeletal muscle mass. In 5 healthy men, CBR function was determined using the random order neck pressure / neck suction protocol previously developed by Potts et al (1) at rest, during leg cycling (Lg) exercise producing 50% of maximal oxygen uptake (VO_{2max}) and during combined leg cycling exercise at 50% VO_{2max} and arm cranking exercise (Lg /A) such that the total VO_2 = 75% VO_{2max}. The heart rate responses to the various levels of neck pressure and neck suction (ranging from -80 to +40 torr) were analyzed and modelled using the logistic funtion described by Potts et al (1). The threshold, saturation and gain (mean ± S.E.) of the resultant CBR function curves are below:

Condition	Threshold (mmHg)	Saturation (mmHg)	Gain (mmHg/mmHg)
Rest	70.05 ± 8.89	128.09 ± 12.39	-.39 ± .083
50% Leg only	95.12 ± 7.75 *	145.08 ± 7.75 p=.07	-.31 ± .070
75% Leg+Arm	113.04 ± 11.55 *†	163.40 ± 8.13 *†	-.28 ± .053

(*indicates significance from rest, † significance from 50% VO_{2max}; $p<.05$)

These results indicate that the CBR is classically reset from rest to the onset of 50% VO_{2max} exercise and is further reset by exercise with a larger active muscle mass, i.e. leg exercise plus arm exercise eliciting a total VO_2 of 75% VO_{2max}.

1. Potts, J.T., X. Shi, and P.B. Raven. Carotid baroreflex responsiveness during dynamic exercise in humans. *Am.J.Physiol.* 265:H1928-1938, 1993.

(supported in part by NASA #NAGW-3582 and a grant from the CMRC)

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Stephen L. Wasmund

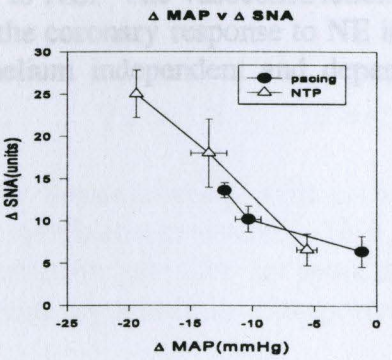
Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

IMPORTANCE OF ARTERIAL BAROREFLEX CONTROL OF SYMPATHETIC NERVE ACTIVITY DURING RAPID VENTRICULAR PACING IN HUMANS. S. Wasmund, *R. Page, M.L. Smith, Dept of Integrative Physiology, Univ. Of North Texas Health Science Center at Ft. Worth, TX 76107 and *Div. of Cardiology UT-Southwestern Med. Ctr., Dallas, TX.

The purpose of this study was to assess the contributions of the arterial (ABR) and cardiopulmonary baroreflexes (CPBR) in control of sympathetic nerve activity (SNA) and arterial pressures during ventricular pacing (VP). Ventricular pacing, used to simulate ventricular tachycardia, causes an increase in central venous pressure (CVP). This results in stimulation of CPBR which function to decrease in SNA. Pacing also causes a decrease in mean arterial pressure (MAP) which results in removal of stimulation to ABR, resulting in increase of SNA. **METHODS:** We measured SNA, CVP and MAP in 5 patients referred for electrophysiologic testing. VP was initiated at cycle lengths of 450 to 300 msec. Nitroprusside (NTP) was then administered in doses (ranging from 0.2-0.8μM/kg/min) sufficient to cause decreases in MAP comparable to those seen during pacing. The NTP was given to decrease stimulation of ABR without significant changes in the stimulus of CPBR. This is done by decreasing MAP while not effecting CVP. **RESULTS:** All values are mean±SEM. VP and NTP both produced graded increases in SNA with decreases in MAP. Mean data are summarized in the figure. The slopes of the ΔSNA/ΔMAP were significantly ($p<0.03$) greater for NTP (-1.44±0.23) than for VP (-0.5±0.26). These data suggest that ABR play the predominant role in mediating the increase in SNA, but that CPBR also play a modulatory role by inhibiting the SNA increase.



Abstract #20

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Geoffrey Kline

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

Endothelium-dependent Coronary Dilation in Response to Norepinephrine in Conscious Dogs. Geoffrey P. Kline and Patricia A. Gwirtz, University of North Texas Health Science Center, Dept. of Integrative Physiology, Fort Worth, TX 76107.

The coronary vascular response to norepinephrine (NE) in conscious dogs is biphasic: a functional hyperemia followed by an α-receptor mediated vasoconstriction. This study examined the role of nitric oxide (NO) in mediating the dilatory response to NE. Four dogs were chronically instrumented to measure left ventricular systolic and diastolic pressures (LVSP, LVEDP), maximal rate of rise of LVP (dP/dt_{max}), heart rate (HR), mean aortic pressure (MAP), and circumflex flow velocity (CFV). Resting control values were: LVSP 118±1.5 (SEM) mmHg, LVEDP 6±1.5 mmHg, dP/dt_{max} 2550±428 mmHg/sec, MAP 83±6 mmHg, HR 80±5 bpm, and CFV 3.4±1.4 kHz. Intracoronary (i.c.) injection of 0.3 μg NE caused a 53±17% increase in CFV followed by a 15±4% decrease. After blockade of NO synthesis using Nω-nitro-L-arginine (L-NA, 75 mg, i.c.), resting hemodynamic values were unchanged, but the coronary dilatory response to 20 μg acetylcholine, i.c., was reduced by 58±4%. After NO blockade the coronary flow increased only 28±11% in response to NE. The vasoconstriction was unchanged. These data indicate that the coronary response to NE in the conscious dog involves both endothelium independent and dependent components.

Abstract #21

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Ying Yu

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

PRESSURE-INDUCED CHANGES IN RIGHT CORONARY VASCULAR VOLUME

Ying Yu & H. Fred Downey
Department of Integrative Physiology, University of North Texas Health Science Center, Ft. Worth, TX 76107, USA

We previously reported poor autoregulation in the pump perfused right coronary circulation of the anesthetized canine (*Circ. Res.* 60:133, 1987). These pressure induced changes in flow [RCF (ml/min/100g)] were concomitant with a pronounced parallel effect on myocardial oxygen consumption. We also demonstrated that pressure induced changes in myocardial oxygen consumption in the canine left ventricle were associated with poor autoregulation and marked changes in coronary vascular volume [CVV(ml/100g), *Am. J. Physiol.* 271:H320, 1996]. To date, the effects of changes in coronary perfusion pressure on coronary vascular volume in the canine right ventricle have not been reported. We measured coronary vascular volume by an indicator dilution method during varied coronary perfusion pressure [CPP(mmHg) 60, 100, 140, and 180 mmHg] in 6 anesthetized dogs with perfused right coronary circulations. All preparations had poor autoregulation. Results:

CPP	58±2	102±2	141±1	177±3
*RCF**	54.1±8.6	93.6±10.7	164±20	226±41
CVV	7.0±1.0	10.0±1.2	12.9±1.5	18.8±2.1

Coronary vascular volume of poorly autoregulating right coronary circulations changes with coronary perfusion pressure. This may account the effects of coronary perfusion pressure on myocardial oxygen consumption of right ventricular myocardium. (Supported by NIH Grant HL35027.)

Abstract #22

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Isabel Tejero-Taldo (Poster Presentation)

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ß-ADRENERGIC STIMULATION AND PYRUVATE RESTORE POST-ISCHEMIC CARDIAC FUNCTION BY DIFFERENT MECHANISMS.

Isabel Tejero-Taldo, Jie Sun, James L. Caffrey, Robert T. Mallet. Dept. Integ. Physiol., Univ. North Texas Hlth. Sci. Ctr., Fort Worth, TX 76107- 2699

Cyclic AMP (cAMP) mediates ß-adrenergic stimulation of sarcoplasmic reticular (SR) Ca^{2+} turnover and cardiac inotropism. Having demonstrated that pyruvate (PYR) also increases cardiac inotropism and SR Ca^{2+} turnover (*Biochim Biophys Acta* 1994; **1224:** 22-32), we examined the role of cAMP in mediating PYR's effects in post-ischemic stunned myocardium. Isolated working guinea-pig hearts, perfused with 10 mM glucose-fortified Krebs-Henseleit, were reversibly injured by 45 min hypo-perfusion and 35 min reperfusion. **Table:** left ventricular stroke work (mJ/g wet), power (mJ/g wet/min), cytosolic energetics (~P: [phosphocreatine]/ [creatine][P_i], M^{-1}) and cAMP (nmol/g dry) were measured in time controls (TC), stunned hearts (STN), and stunned hearts treated at 15-35 min reperfusion with 50 nM isoproterenol (ISO), 5 mM PYR, or both interventions (COMB). Means ± SE. *: $P < 0.05$ *vs* TC; †: $P < 0.05$ *vs* STN; ‡: $P < 0.05$ *vs.* ISO.

	n	Stroke Work	Power	~P	{cAMP}
TC	6	0.59±0.05	112±9	256±25	2.24±0.23
STN	6	0.08±0.01*	15±3*	454±100*	2.55±0.19
ISO	9	0.64±0.09†	236±34*†	83±10*†	5.47±0.36*
PYR	6	0.53±0.06†	125±17†‡	515±78*‡	3.09±0.35‡
COMB	7	0.69±0.02†	244±6*†	228±15†‡	6.08±0.44*

Conclusion: Unlike ISO, PYR restores function by cAMP-independent mechanisms, and lessens ISO depletion of cytosolic energetics. However, PYR does not potentiate the effects of maximal β-adrenergic stimulation.. NIH support: HL50441.

Abstract #23

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Amber A. Stanfill

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ACUTE MORPHINE AND VAGAL ACTIVITY IN DOGS. A.A. Stanfill, L.D. Napier, B.A. Barron, and J.L. Caffrey. UNTHSC, Dept. of Integrative Physiology, Fort Worth, TX, 76107

Healthy vagal function is associated with highly variable heart rates. Patients who regain this heart rate variability soon after myocardial infarctions are much more likely to survive. Morphine, which is commonly administered to these patients, slows the heart by increasing vagal activity. It is unclear if morphine produces a more or less responsive vagus. We propose the hypothesis that morphine reduces heart rate by increasing vagal tone while reducing heart rate variability. The study in progress is designed to determine if the reduced heart rate following acute morphine correlates with increases or decreases in heart rate variability as measured by heart rate power spectral analysis (PSA). The high and low frequency components of the heart rate power spectrum reflect vagal and combined vagosympathetic contributions, respectively. Conscious animals will rest quietly in a sling while the heart rate power spectrum is determined from surface electrocardiograms. A fast Fourier transform is used to record the heart rate power spectrum continuously for four hours. Venous blood samples are obtained at intervals to correlate spectral changes with circulating morphine. Each animal will receive four treatments in random order at least 96 hours apart: vehicle (saline), morphine, naloxone (morphine antagonist), and morphine + naloxone. The study will require 6-8 subjects, two of which have been completed. These preliminary data indicate that morphine reduces both the heart rate and heart rate variability (high frequency power). The changes observed were correlated with circulating morphine concentrations and when naloxone was administered the PSA quickly returned to normal. These observations indicate the responses are mediated through opiate receptors. The limited number of subjects precludes even preliminary speculation about results from the low frequency spectrum. The data thus far support the hypothesis as proposed but firm conclusions will require the collection of more data. (Supported by NIDA and local funds)

Abstract #24

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Leslie D. Napier

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

REDUCED CARDIAC PARASYMPATHETIC CONTROL IN MORPHINE-ADDICTED DOGS. L.D. Napier, B.A. Barron, K.E. Jackson, A.A. Stanfill, D.A. Yoshishige, J.L. Caffrey. UNTHSC, Dept. of Integrative Physiology, Fort Worth, TX 76107.

Opiate abuse is a growing public health concern and opiates are commonly prescribed for chronic pain. Myocardial abnormalities are common post-mortem findings in opiate addicts and studies have shown altered cardiovascular autonomic control in heroin addicts and in related animal models. We recently demonstrated impaired vagal control of heart rate (HR) in dogs treated chronically with morphine. Since acute morphine is vagotonic, we hypothesized that chronic morphine reduces parasympathetic control of the heart by down-regulating muscarinic receptors, their coupling mechanisms and/or their second messenger systems. Dogs were treated with subcutaneous morphine via ambulatory infusion pumps for 14 days. Plasma morphine (PM) reached 50% of the target concentration (80-120 ng/ml) within three hours. Concentrations were maintained on subsequent days by adjusting the rate of morphine infusion. Average PM concentrations were 109, 140, 75, 116 and 79 ng/ml on Days 2, 4, 7, 10 and 14, respectively. After 14 days, dogs were anesthetized and the cervical vagus was exposed. Vagal stimulation generated reproducible, frequency-dependent decreases in HR in control animals. HR responses to vagal stimulation in morphine-treated animals were highly variable and included some unexpected increases at lower frequencies. When afferent vagal traffic was interrupted by ligating the nerves prior to stimulation, the HR responses were amplified in both saline- and morphine-treated dogs. Responses in treated animals, however, remained attenuated at all frequencies compared to controls. HR responses to vagal stimulation in a separate group of animals given morphine acutely (PM=223) were indistinguishable from those in animals treated chronically with saline. Thus circulating morphine was not responsible for attenuated responses in dogs treated chronically with morphine. Muscarinic receptor density and affinity were similar in left ventricular (B_{max}= 602 fmol/mg, K_D = 56.54 pM) and right atrial (B_{max}= 699 fmol/mg, K_D = 61.53 pM) microsomal membranes from control animals. Thus far, chronic morphine has had no apparent effect on muscarinic receptor number or affinity. Basal adenylate cyclase (AC) activity was higher in the left ventricle than in the right atria across treatment groups. Chronic morphine had no effect on maximal ($MnCl_2$-stimulated) AC activity or on its responses to isoproterenol (stimulatory) or carbachol (inhibitory). Basal AC activity in the right atria was reduced by both acute and chronic morphine. Chronic morphine did not increase atrial catecholamines indicating that increased catecholamines were not responsible for the decrease in vagal function. Further studies will be required to determine the mechanisms involved in the reduced vagal function in dogs treated chronically with morphine. Since efficient vagal control of the heart is widely accepted as cardioprotective, a reduction in vagal influence could have significant consequences for anyone chronically exposed to morphine. (Support: NIDA, local funds.)

Abstract #25

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Keith E. Jackson

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

MEASURING PROENKEPHALIN IN ISOLATED WHITE BLOOD CELLS. K.E. Jackson, D. Yoshishige, L.D. Napier, B.A. Barron and J.L. Caffrey. Integrative Physiology, UNTHSC-FW, Fort Worth TX 76107.

Opiates are essential clinical tools for managing chronic pain. They also pose a societal liability due to their potential for abuse. Data from other laboratories suggest that morphine may alter immune functions. Since white blood cells produce both opiate receptors and endogenous opiates, we have begun to evaluate the relationship between morphine and proenkephalin in white blood cells. In order to pursue this investigation, we needed to develop methods for isolating white blood cells and for measuring proenkephalin products within the isolated white cells. Using methods modified from, A. Boyum (*Scand. J. Immunol.* 5, Suppl 5: 2-8,1976), we are validating procedures for reliably isolating white blood cells from small samples of canine blood. Five ml of blood were collected in EDTA and mixed with one part 6% dextran in 0.9% saline. Red blood cells were allowed to settle for thirty-five minutes at room temperature. The supernatant was removed, diluted with 10 ml of saline, centrifuged and the cells were washed twice with saline. After washing, the white cells were resuspended in 5 ml of saline and counted. The cell counts were compared to whole blood to determine percent recovery. The white cell suspension was centrifuged again, the supernatant was discarded, and the cellular constituents were extracted with 5 ml of hot water to stop enzymatic reactions. The average number of white cells recovered was $8.5 \pm 2.1 \times 10^3$ cells/μl for 10 trials. This compares with $12.2 \pm 2.9 \times 10^3$ cells/μl prior to isolation and represents a 71.5% ± 2.0% recovery. In order to measure proenkephalin products, rabbits were immunized with a synthetic peptide corresponding to the C-terminal 15 amino acid sequence of proenkephalin conjugated to keyhole limpet hemocyanin. The resulting antiserum binds the C-terminal heptapeptide, met-enkephalin-arg-phe (MEAP) with high affinity. MEAP was radioiodinated and a radioimmunoassay was developed. The assay appears to identify significant concentrations of immunoreactive proenkephalin in isolated white cells and suggests we can measure proenkephalin in the white cells from 10 microliters of whole blood. Once fully validated we plan to study the role of white cell enkephalins during cardiovascular and drug related stresses. Supported by NIDA & local funds.

Abstract #26

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Miao-Xiang He, M.D.

Department: Integrative Physiology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

DOWNREGULATION OF CARDIAC CONTRACTION BY INORGANIC PHOSPHATE (Pi). Miao-Xiang He, and H. Fred Downey. Dept. Integ. Physiol. Univ. N. Tex. Hlth. Sci. Ctr., Ft. Worth, TX

Myocardial contraction declines rapidly at the onset of ischemia to decrease energy demand and thus reduce injury. The mechanism has not been identified. We examined energetics at the onset of ischemia with 0.5 s time resolution in isolated rat hearts. We found: 1) Pi increased prior to and faster than the decrease in left ventricular pressure (LVP) during the first 10 s after the onset of ischemia induced by decreasing coronary flow 70% (Isch-1); 2) LVP was linearly correlated to Pi; 3) Phosphorylation potential (PP, log[ATP]/[ADP][Pi]) and ADP changed faster than LVP at the onset of Isch-1. To further identify the mediator which downregulates contraction, less severe ischemia was induced by reducing flow 50% (Isch-2). A brief repetitive ischemia protocol was used to achieve 0.5 s time resolution of ^{31}P-NMR. LVP decreased less during Isch-2 (by 41$\pm$2% vs. 50$\pm$1% in Isch-1) and Pi was lower (6.2$\pm$0.3 vs. 8.5$\pm$1.0 mM), but there were no differences in PP and ADP between Isch-1 and Isch-2. The linear regressions of LVP-Pi during Isch-2 and Isch-1 were superimposed, but LVP-PP and LVP-ADP regressions during Isch-2 diverged from that of Isch-1. At PP < 4.75 M^{-1}, LVP-PP regressions during Isch-2 and Isch-1 were superimposed but at PP > 4.75, LVP was higher during Isch-2. LVP-ADP regression during Isch-2 was elevated above that of Isch-1. The results indicate that Pi may be the energetic mediator modulating myocardial contractile downregulation at the onset of ischemia. (Supported by NIH Grant HL35027 and a Faculty Research Grant)

Abstract #27

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Edward L. Orr, Ph.D.

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty_X_ Staff___

Read instructions and fit abstract inside the space given below:

CORONARY OCCLUSION INCREASES HISTAMINE LEVELS IN CORONARY VENOUS AND COLLATERAL CIRCULATIONS OF THE DOG. E.L. Orr, A.A. Khan and K.W. Scheel. University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas. 76107

Based primarily on histological evidence, acute coronary occlusion results in activation (degranulation) of cardiac mast cells. Since histamine is always released upon degranulation of mast cells, then histamine should be released and subsequently appear in the coronary blood in response to coronary occlusion. To test this hypothesis, hearts of anesthetized adult mongrel dogs were exposed via a thoracotomy at the 4th intercostal space and subjected to acute occlusion of the left anterior descending coronary artery (LAD). Blood samples were obtained from the femoral artery, coronary sinus, venous drainage from the LAD perfusion territory and retrograde collateral flow before and at various times after LAD occlusion. Histamine levels were measured in the blood plasma by a sensitive and specific radioenzymatic method. Acute coronary occlusion of the LAD resulted in the following time-dependent peak increases in plasma histamine levels: coronary sinus = 108-190\%, cardiac vein = 56-81\%, and collateral retrograde flow = 1.3-10 fold. No increase in plasma histamine level of blood samples from the femoral artery were observed. These results confirm the release of histamine from mast cells after coronary occlusion, and suggest that mast cell products may be involved in the regulation of coronary and collateral blood flow. Supported by grants from the NIH and American Heart Association, Texas Affiliate

Abstract #28

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: G. M. Rocha, M.D., Ph.D.

Department: Integrative Physiology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

HYPEROSMOTIC STRESS INDUCES AN ATTENUATED VASOCONSTRICTIVE EFFECT ON AGED MICE ARTERY.

G.M. Rocha, M. J. Foster, P. B. Raven. Dept. of Integrative Physiology, University of North Texas Health Science Center, USA 76107.

These experiments were designed to observe if Na^+/H^+ exchange is involved in arterial response of the aged mice. Tail arteries of aged (24-30 months old) and control young (3 months old) B6D2F1 mice were submitted to a hyperosmotic stress or to α_1-adrenergic receptor mediated arterial reactivity. Isolated arteries about 300 μm in diameter were cannulated and pressurized to 40 mmHg with the intraluminal flow arrested. Measurements of the arterial internal diameter were performed *in vitro* by a video dimension analyzer system. Phenylephrine (10^{-10} to 10^{-5} M) was used to induce α_1-adrenergic receptor mediated constriction; mannitol (10 to 200 mM) was used to increase the osmolar range of saline solution in the vessel bath. Phenylephrine dose-dependently induced comparable vasoconstriction in aged (n=5) and young (n=3) mice arteries. Hyperosmotic stress, induced vasoconstriction in both aged (n=3) and young (n=6) mice arteries. However, the hyperosmotic-induced vasoconstrictive effect was significantly attenuated in aged when compared to young mice arteries ($t=3.74$; $p<0.05$). These results suggest that the attenuated vasoconstrictive response of the aged mice tail artery due to hyperosmotic stress are independent of Na^+/H^+ ion exchange at the smooth muscle that occurs with α_1-receptor coupled vasoconstriction.

Abstract #29

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Jami R. Kern

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

HUMAN VASCULAR TISSUE EQUIVALENT

Jami R. Kern*, P.B. Raven†¥, S.D. Dimitrijevich*¥; Departments of *Biochemstry and Molecular Biology and Surgery; †Physiology; ¥Cardiovascular Research Institute; UNTHSC, FT. WORTH, TX 76107

Failure of the vascular tissue, and primarily the cardiovasculature, remains a major cause of death in this country. Surgical intervention using donor tissue, while successful, is hindered by a limited supply of quality tissue and the need for long term immunosuppression. Tissue replacement utilizing *in vitro* generated grafts constructed using human cellular components is becoming a viable alternative. We propose to establish a Human Vascular Tissue Equivalent (HVTE) using human vascular endothelial cells, vascular smooth muscle cells, fibroblasts and a matrix of type I collagen. It is hypothesized that the radial alignment of smooth muscle cells and fibroblasts constituting the tubular blood vessel structure will result from the use of the Rotating Wall Vessel (RWV). This vessel, developed at NASA's Johnson Space Center, creates an environment of microgravity ideal for cell growth. The adventia will first be constructed with human fibroblasts within a collagen matrix, and then the media consisting of densely packed smooth muscle cells will be formed. Finally the vessel will be endothelialized. In difference to other proposed models, this HVTE will not only have the structure and cell alignment of vasculature, but will also be based on a non-contracting matrix. To date we have developed methods for culturing the blood vessel component cells (endothelial and smooth muscle cells and fibroblasts) and have established a preliminary three dimensional flat model lacking the radial cell alignment. We hope to translate these accomplishments into a successful HVTE by combining the use of the RWV and our experience in forming non-contracting equivalents of other human tissues.

Abstract #30

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Juan Wang

Department: Pharmacology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

REGULATION OF CARDIAC PHOSPHOINOSITIDE-SPECIFIC PHOSPHOLIPASE C ISOENZYMES. Juan Wang and E.E. Quist, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

Cardiac tissue contains several phosphoinositide-specific phospholipase C (PLC) isoenzymes but their regulatory mechanisms are poorly understood. Our objectives have been to identify and characterize the membrane and cytosolic PLC isoenzymes in rat and canine myocardium in assays using [3H]-phosphatidylinositol (PI) and phosphatidylinositol 4,5 bisphosphate (PIP_2) as substrates. Rat and dog heart microsomal membranes possess Ca^{2+} activated PLC activity which hydrolyze PI and PIP_2. PLC activity is higher in rat than dog membranes but both are maximally activated by 200 nM Ca^{2+}. Cytosol PLC is inactive under physiologic conditions unless assayed in the presence of high (mM) Ca^{2+} and 1 mM deoxycholate. To determine if specific cytosolic PLC activators of membrane PLC were present, cytosol was fractionated either on a S-200 sephacryl HR or anion exchange DE-52 columns A DE-52 cytosolic fraction eluting with 100 mM NaCL activated membrane PLC activity by 3-4 fold in dog microsomes and 40-60 % in rat microsomes. This 100 mM fraction itself did not contain PLC and eluted with an apparent molecular weight of 38 kDa on a sephacryl 200 column. The activator protein increased Vmax of PLC activity without changing the Ca2+ affinity. ATP and GTP were also not required for activation. In addition, combining 200 mM NaCL DE-52 fractions with membranes also resulted in significant increases in PLC activity. This fraction alone possessed inactive PLC as determined from western blotting indicating that activation resulted from combination of inactive cytosolic PLC isoforms with the membranes. Together the results of this investigation indicate that membrane PLC is activated by an unidentified cytosolic 38 kDa protein. Furthermore cytosolic PLC isoforms may be activated by binding or translocating to membranes. Further studies will be required to resolve the physiological significance of these modes of cardiac PLC activation.

Abstract #31

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Lara Keyser

Department: Pharmacology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CHARACTERIZATION OF A NOVEL INOSITOL TETRAPHOSPHATE STIMULATED Ca^{2+} TRANSPORTER IN CARDIAC MEMBRANES. Lara Keyser and E. E. Quist, Pharmacology, University of North Texas. Health Science Center at Fort Worth, Fort Worth, Texas, 76107

Cardiac junctional sarcoplasmic reticulum (J-SR) contain a novel Ca^{2+} transporter which is stimulated by inositol tetraphosphate (IP_4). This transporter is postulated to facilitate Ca^{2+} loading of J-SR in conjunction with the SR-Ca^{2+}-ATPase. Because this transporter operates by facilitated diffusion and therefore does not require ATP, we postulate that it acquires energy for Ca^{2+} transport by coupling to transport of other ion(s) or via a membrane potential. To test this possibility, the effects of Na^+, K^+, H^+ and Cl^- on IP_4 stimulated $^{45}Ca^{2+}$ uptake into J-SR vesicles were examined. IP_4 stimulation was below baseline at a pH of 6.5 whereas maximal IP_4 stimulation occurs at pH 7.0. IP_4 stimulation was less at pH of 7.4 and 8.0. IP_4-stimulated Ca^{2+} transport was not dependent or modulated by Na^+, K^+ or choline. In the absence of Cl^-, IP_4 stimulated Ca^{2+} transport was blocked. This observation suggests that IP_4-stimulated Ca^{2+} transport is dependent on Cl^-. In conclusion, these studies indicate that both H^+ and Cl^- may serve as allosteric regulators or may be co-transported with Ca^{2+}. Further studies will be required to study the mechanism by which H^+ and Cl^- regulate the Ca^{2+} transporter in J-SR vesicles or in liposomes reconstituted with the purified Ca^{2+} transporter.

Abstract #32

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: **Victoria L. Rudick**

Department: **Anatomy and Cell Biology**

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty__X__ Staff___

Read instructions and fit abstract inside the space given below:

THE HUMAN APOA-I GENE IN MDCK CELLS: CONSEQUENCES OF ITS EXPRESSION. **V.L. Rudick+, F. Wei*, and M.J. Rudick*, +Dept. of Anatomy & Cell Biology, Univ. of N. TX Health Science Ctr. at Ft. Worth; Ft. Worth, TX 76107; *Biology Dept., Texas Woman's Univ., Denton, TX 76204.**

MDCK cells expressing human apoA-I (N2A cells) not only target apoA-I, but also exhibit several unexpected alterations in cell morphology due to changes in glycogenesis and lipid biosynthesis. Exogenous gene expression depends on gene copy number and integrative state. Synthesis of encoded protein is then directly proportional to mRNA content, but for secretory proteins, as apoA-I, intracellular events influence the amount of protein finally exported. Southern hybridization analysis of low molecular weight and genomic DNA indicated that apoA-I genes had recombined into the MDCK genome. Also N2A cells had 10 fold more apoA-I gene copies than endogenous apoA-I secreting Caco-2 cells, but the levels of $mRNA_{apoA\text{-}I}$ are similar. Northern hybridization revealed that N2A cells had a mRNA corresponding in size to that of the Caco-2 cells and a larger one representing a fusion transcript. The rate of apoA-I secretion in N2A was half that of Caco-2. Thus, the final rate of apoA-I export is affected by one or more prior steps. Immunoelectron microscopy located apoA-I within the Golgi complex and specifically with spirally arranged Golgi-derived membranes, and with whorls of smooth membranes. Both have been implicated in lipid synthesis, and incorporation of [2-^{3}H]glycerol into a chloroform:methanol fraction showed that N2A cells produce 3-4-fold more lipid than MDCK cells. Both N2A and Caco-2 cells have large, relatively electron translucent areas containing flocculent material and lacking boundary membranes. These also develop in response to apoA-I gene expression, although apoA-I does not appear to associate with them. N2A cells had 3 times more glycogen than MDCK cells, suggesting that the inclusions might be glycogen. Two different methods were then used to detect cellular glycogen ultrastructurally in both cell types, osmium-iron complexation and periodic acid oxidation followed by reduction of chelated bismuth. Both demonstrated that the pools consisted of glycogen. (+Supported by grant #95G-153 American Heart Association, Texas Affiliate).

Abstract #33

CLINICAL STUDIES
AND
COMMUNITY PROJECTS

34	David Gibson, Ph.D.	OFFICE OF CLINICAL RESEARCH
35	Paul Brittain*	ADMINISTRATION OF A HEALTH STATUS QUESTIONNAIRE IN SEVERAL OUTPATIENT SETTINGS--THE CLINICAL OUTCOMES RESEARCH STUDY
36	Harold W. Keller, Ph.D.	INTRODUCTION TO INDUSTRY PRACTICE: A MODEL FOR COLLABORATION
37	Patricia Cappelletti*	DEFINING EFFECTIVE APPROACHES OF BIOTECH COMPANIES TO COMMERCIALIZE TECHNOLOGY
38a	Henri Migala	INTEGRATING POPULATION-BASED PRINCIPLES AND PUBLIC HEALTH COMPETENCIES INTO FAMILY MEDICINE EDUCATION
38b	Henri Migala	DEVELOPMENT OF A DEDICATED COMMUNITY-BASED RURAL AND MINORITY HEALTH EDUCATION PROGRAM IN FAMILY MEDICINE

*Student Presenter

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: David Gibson, Ph.D.

Department: Office of Clinical Research

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

OFFICE OF CLINICAL RESEARCH
David Gibson, Ph.D
University of North Texas Health Science Center
at Fort Worth
3500 Camp Bowie Boulevard
Fort Worth, Texas 76107-2699

The University of North Texas Health Science Center has taken the innovative action of forming an Institutional Office to manage the clinical research process at the medical school. Since it's inception in 1996, the Office of Clinical Research (OCR) has developed or managed clinical studies for five first time Principal Investigators and in it's first full year of operation the OCR will see a 300% to 500% increase in activity for Sponsored Clinical Research. As an Institutional office the OCR has been in position to facilitate multiple interdepartmental clinical studies including studies on Bronchitis, pain management and hypertension and anticipates numerous additional institutionally wide clinical programs being implemented during 1997. During the coming year the Institution, through the Office of Clinical Research, is focusing on several clinical indications including wound healing, Alzheimer's disease, pain management, cardiology, several indications in OB/GYN and allergies.

Abstract #34

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Paul Brittain

Department: Office of Clinical Outcomes Research, Epidemiology, and Statistics

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ADMINISTRATION OF A HEALTH STATUS QUESTIONNAIRE IN SEVERAL OUTPATIENT SETTINGS--THE CLINICAL OUTCOMES RESEARCH STUDY. Paul Brittain, John Licciardone, and Cheryle Yarbrough. Office of Clinical Outcomes Research, Epidemiology, and Statistics, University of North Texas Health Science Center at Ft. Worth, Ft. Worth, Texas 76107.

The Office of Clinical Outcomes Research, Epidemiology, and Statistics (OCORES) of the University of North Texas Health Science Center at Ft. Worth (UNTHSC) is responsible for the development and implementation of clinical outcomes research projects throughout the numerous outpatient clinics at UNTHSC. Beginning in January 1996, OCORES research personnel began designing the first phase of what is to become an institutional-based clinical outcomes research study. Phase 1 of this study involves collection of baseline health status information through the administration of the "Short Form with 36 Questions Health Status Questionnaire" (SF-36) to all patients seen at each of the outpatient clinics at UNTHSC. The SF-36 is a well-established survey instrument designed for patients' self-reporting of their current health status. Survey administration began in the Family Medicine Department's Central Clinic in April 1996 and has since expanded to include all six Family Medicine Clinics and the Osteopathic Manipulative Medicine Clinic. Each patient's survey responses are linked to key demographic and diagnostic variables, such as age and current diagnoses, to create a complete profile for each patient. Phase 2 of this project will involve testing hypotheses generated from trends and patterns seen in the data collected from phase 1. This poster describes the methodology employed in phase 1 of the Clinical Outcomes Study. In addition, the challenges faced by the OCORES research team in the planning and implementation of this study are discussed to highlight the practical difficulties of clinical outcomes research. Finally, implications of this study to UNTHSC from the clinical to the administrative levels are considered.

Abstract #35

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Harold W. Keller

Department: Office of Research and Biotechnology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff X

Read instructions and fit abstract inside the space given below:

INTRODUCTION TO INDUSTRY PRACTICE: A MODEL FOR COLLABORATION. H. W. Keller, R. W. GRACY, T. YORIO, University of North Texas Health Science Center, Fort Worth, TX 76107-2699

This graduate course serves as a model of collaboration between area industry, small businesses, the City of Fort Worth and the University of North Texas Health Science Center. The idea for this course developed at the Health Science Center and was made possible through the cooperation and networking activities with the City of Fort Worth's Strategy 2000. This group was charged with developing a strategic plan for the city that focused on the health care and biotechnology industries. Networking breakfasts were held monthly where leaders from area businesses, industry and educational organizations met to plan and discuss the economic future of Fort Worth. As an outcome of these meetings, a number of industry representative were invited to discuss the creation of a graduate course that introduced students to the organization and operation of industry practice. This course was offered for the first time in the fall of 1996. It was designed to help graduate students prepare for industry positions and broaden their career path horizons. Sixteen evening class sessions were held with graduate students, postdoctorates, faculty, course instructors, and interested industry leaders in attendance. Twenty-five course instructors came from Abbott Laboratories, Alcon Laboratories, Electronic Monitors, MicroChem Laboratory, TALEM, INC, and the UNT Health Science Center. Instructors volunteered their time to present practical topics to students, including industry organization, regulatory and environmental affairs, research and development (pharmaceuticals and medical devices - instrumentation), manufacturing, quality assurance and quality control, marketing and sales, budgeting, new product development process, clinical studies, intellectual property, entrepreneurial - small business environment and career planning. Students received two credit hours and either a pass or fail grade. Overall course evaluations from the students gave highly favorable ratings for instructional presentation and content, including the importance of the biomedical sciences to industry. Plans call for offering this course again in the fall of 1997. This successful community/health science center collaboration will serve as a model for other courses and joint programs in the future.

Abstract #36

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Patricia Cappelletti

Department: Biomedical Sciences

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DEFINING EFFECTIVE APPROACHES OF BIOTECH COMPANIES TO COMMERCIALIZE TECHNOLOGY, P. CAPPELLETTI AND H. LAL, DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER, FORT WORTH, TEXAS 76107-2699

The objective of the study is to define approaches to commercialize technology that are used by biotech companies originating from academic institutions and to determine the most effective ones. The hypothesis being tested is that approaches used by biotech companies for commercialization of technology significantly influence the success of these companies and that a combination of approaches is found most profitable. Availability of products on the market is a measure of success. Five categories of approaches were identified: (1) rights to proprietary technology, (2) origins of technology, (3) protection of technology, (4) sources of funding, and (5) new product focus. A survey of biotech companies is being conducted through a questionnaire in order to determine effective approaches used. Data returned from the survey will be statistically analyzed using multiple logistic regression analysis to test the relationships between multiple variables and to determine the best model for further analysis. The results are expected to show that successful companies (having products on the market) (1) acquire their technologies from academic institutions, (2) patent their technologies prior to out-licensing arrangements, (3) grant rights to their proprietary technologies to other companies to develop into products and/or market, (4) obtain the largest percentage of funding from sources other than private placements such as public offerings, and (5) focus on innovative new rather than "me-too" products.

Abstract #37

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Henri Migala, MA

Department: Family Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

INTEGRATING POPULATION-BASED PRINCIPLES AND PUBLIC HEALTH COMPETENCIES INTO FAMILY MEDICINE EDUCATION

Henri Migala, MA; Larry Johnson, MSW

The principal objective of this project is the development of a professional academic medical scholarship environment in the department's community-based ambulatory teaching clinics. The project begins with introducing basic public health principles to the faculty, who in turn, educate the students to practice family medicine with a greater community healthcare awareness. This new perspective will then be applied into actual community-based clinical services. The success of this new approach will then be examined to determine project efficacy. Family medicine faculty will collaborate with public health and other institutional faculty to develop community-based applied research projects to evaluate project development and success. The outcomes of these research projects will be used to further develop faculty training and student educational programs. Each component of this project allows for professional academic scholarship development and dissemination of information through appropriate academic forums. Faculty development, medical education, population-based health care services and community-based outcomes research projects will all be used to develop scholarship activities.

Abstract #38a

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Henri Migala, MA

Department: Family Medicine

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

DEVELOPMENT OF A DEDICATED COMMUNITY-BASED RURAL AND MINORITY HEALTH EDUCATION PROGRAM IN FAMILY MEDICINE

Henri Migala, MA; Barbara Adams,

Given the significant minority and rural underserved populations and areas of Texas, the increased recognition for a greater degree of cultural-sensitivity training for primary care clinicians and the need for a more community-oriented approach to clinical practice, the Department of Family Medicine has developed an innovative program specifically designed to integrate primary care training, cultural-sensitivity and public health into the professional development and formation of our primary care physicians. This program will provide our medical students with a dedicated longitudinal series of community-based clinical experiences that focus on rural health, minority health, community health, cultural-sensitivity and public health training in the primary care environment.

Abstract #38b

EYE RESEARCH

39	James E. Turner, Ph.D.	NORTH TEXAS EYE RESEARCH INSTITUTE
40	Terry K. Wiernas*	HUMAN CORNEAL EPITHALIAL CELLS EXPRESS FUNCTIONALLY-COUPLED B_2-BRADYKININ RECEPTORS: PHARMACOLOGICAL CHARACTERIZATION USING AGONISTS AND ANTAGONISTS
41	Rajnee Agarwal	EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA ISOFORMS (TGF-ß 1-3) AND RECEPTORS ((TGF-ß RI-RIII) IN CULTURED HUMAN TRABECULAR MESHWORK CELLS
42	Ganesh Prasanna, Ph.D.	REGULATION OF ENDOTHELIN-1 AND BIG ENDOTHELIN-1 LEVELS BY TUMOR NECROSIS FACTOR - α IN HUMAN NON-PIGMENTED CILIARY EPITHELIAL CELLS IS PROTEIN KINASE-C DEPENDENT
43	Wenhong Tao, Ph.D.	ENDOTHELIN RECEPTOR A IS EXPRESSED AND MEDIATES THE $[Ca^{2+}]_i$, AND TRABECULAR MESHWORK CELLS
44	Rouel S. Roque, M.D.	MICROGLIA-DERIVED CYTOTOXIC FACTOR(S) INDUCE APOPTOSIS OF 661W PHOTORECEPTOR CELLS IN VITRO
45	Harold J. Sheedlo	RETINAL PROGENITOR CELLS: IMMUNOCYTOCHEMICAL AND REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION ANALYSES
46	James E. Turner, Ph.D.	RPE CONDITIONED MEDIUM INFLUENCES PHOTORECEPTOR CELL SURVIVAL AND MATURATION IN THE RAT RETINA
47	Tammy Hancock Nelson	AN ANTIBODY TO RPE SECRETED PROTEIN SINHIBITS RETINAL DEVELOPMENT AND PHOTORECEPTOR CELL MATURATION

48	Scott Krueger*	PHOTO-OXIDATIVE STRESS LEADS TO APOPTOSIS OF 661W MOUSE PHOTORECEPTOR CELLS
49	Wendi Lambert*	EXPRESSION OF TRANSFORMING GROWTH FACTOR-β ISOFORMS AND THEIR RECEPTOR mRNAs IN CULTURED HUMAN LAMINA CRIBROSA CELLS
50	Martha E. Stokely*	RETINA TROPHICALLY SUPPORTS OLIGODENDROCYTES FROM THE OPTIC NERVE *IN VITRO*
51	Michael H. Chaitin, Ph.D.	CD44 IN THE RETINA

*Student Presenter

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: James E. Turner, Ph.D.

Department: North Texas Eye Research Institute

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

NORTH TEXAS EYE RESEARCH INSTITUTE
James E. Turner, Ph.D., Director. University of North Texas Health Science Center.

Background The North Texas Eye Research Institute (NTERI) was established in 1992 as one of the Centers of Excellence at the University of North Texas Health Science Center at Fort Worth. The purpose of the NTERI is to serve as an academic and research focus for basic and clinical science activities within the vision community of Fort Worth and the surrounding areas. Consequently, over 20 faculty are involved in visual science research and have appointments to the NTERI. These faculty are located in three basic science departments (Anatomy and Cell Biology, Pharmacology, and Biochemistry and Molecular Biology) at the UNT Health Science Center, at the Alcon Research Laboratories and within the ophthalmology community of Fort Worth.

Within NTERI there are a number of groups of faculty interested in retina research, ocular diabetes, autoimmune diseases of the eye, optic nerve regeneration, glaucoma, corneal wound healing and aging. Specific areas of research interests within the various groups include: neovascularization, trophic factors, wound healing, aging, cell death, retina transplantation, glaucoma and diabetic complications. Broad technical areas of expertise are found though faculty interests within the NTERI to include: morphology, cell biology, biochemistry, molecular biology, pharmacology, electrophysiology and clinical expertise. All of the areas of expertise are supported by modern sophisticated equipment and a skilled technical staff.

Abstract #39

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Terry K. Wiernas

Department: Pharmacology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

HUMAN CORNEAL EPITHELIAL CELLS EXPRESS FUNCTIONALLY-COUPLED B_2-BRADYKININ RECEPTORS: PHARMACOLOGICAL CHARACTERIZATION USING AGONISTS AND ANTAGONISTS. T. K. Wiernas, B. W. Griffin and N. A. Sharif. Molecular Pharmacology Unit, Alcon Laboratories, Fort Worth, TX 76134 and Department of Pharmacology, University of North Texas Health Sciences Center at Fort Worth, TX 76107. Bradykinin (BK) and Lysyl-bradykinin (Lys-BK) are peptides which are released at high nanomolar concentrations into the tear-film of ocular allergic patients. We hypothesized that these peptides may activate specific receptors on the ocular surface, especially the corneal epithelium (CE), and thus the CE cells may represent a potential target for these kinins. The aim of the present studies was to determine the presence of and the pharmacological characteristics of BK receptors on normal cultured primary (CEPI) and SV40 virus-transformed human corneal epithelial (CEPI-17-CL4) cells using the accumulation of [^{3}H]inositol phosphates ([^{3}H]IPs) as a bioassay. BK induced a maximal 1.95 - 2.51 fold ($n = 17$ - 26) stimulation of [^{3}H]IPs accumulation in these cells. The molar potencies of BK and some of its analogs were very similar in both cell types (e.g. EC_{50} for BK = 3 nM, Lys-BK = 0.9 nM, Tyr^{8}BK = 19 nM and Des-Arg^{9}BK = 14 μM). The BK-induced responses were competitively antagonized by the B_2-selective BK antagonist HOE-140; Icatibant; ($pA_2 = 8.5$; $K_i = 2.9$ nM). The rank order of potency of agonist BK-related peptides, coupled with the antagonism of the BK-induced [^{3}H]IPs by the specific B_2-antagonist, strongly suggests that a B_2-receptor subtype is involved in mediating functional PI responses in the CEPI-17-CL4 and CEPI cells. The CEPI-17-CL4 cells represent a good *in vitro* model of the human corneal epithelium to further study the role of BK receptors in its physiology and pathology such as in allergic/inflammatory conditions, potential wound healing and other functions of the cornea. Supported by Alcon Laboratories.

Abstract #40

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Rajnee Agarwal

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA ISOFORMS (TGF-ß 1-3) AND RECEPTORS (TGF-ß RI-RIII) IN CULTURED HUMAN TRABECULAR MESHWORK CELLS

((R.Agarwal[1], W.Lambert[1], A.F. Clark[2], S.E. Wilson[3] and R.J. Wordinger[1])) North Texas Eye Research Institute and Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, TX.[1]; Glaucoma Research, Alcon Laboratories, Fort Worth, TX.[2]: Eye Institute, the Cleveland Clinic Foundation, Cleveland, OH.[3].

Purpose: Growth factors, acting through high affinity receptors, are known to control critical cell functions via paracrine and autocrine mechanisms. The microenvironment within the human trabecular meshwork (TM) may be established and maintained by growth factors within the aqueous humor and those produced locally by human trabecular meshwork cells (HTM). However, we lack basic information as to which growth factors and growth factor receptors are expressed by normal HTM cells. The purpose of this study was to determine which isoforms of TGFß (TGFß 1-3) and which TGFß receptors (TGFß RI-RIII) are expressed by normal cultured HTM cells. **Methods**: Total cellular RNA isolation, DNA synthesis, RT-PCR and agarose gel electrophoresis were performed using well characterized HTM cell lines from 6 day, 48 day, 6 month, 2 year, 18 year, 54 year and 80 year old donors. The PCR primers for TGFß isoforms and TGFß receptors were designed using Entrez (NCBI, Bethesda, MD.) and Oligo 4.0 (National Biosciences Inc., Plymouth, MN.) To verify PCR product specificity, nucleic acid sequencing was performed by cloning PCR products in the TA Cloning Vector (Invitrogen, San Diego, CA.) and sequencing with Sequenase 2.0 (United States Biochemical, Cleveland, OH.). **Results**: We detected mRNA's for TGFß-2 and TGFß-3 in all cell lines. Messenger RNA for TGFß-1 was variably expressed. We detected mRNA's for TGFßR-1, TGFßR-II, and TGFßR-III in all cell lines. Message for an alternatively spliced form of TGFßR-II was detected in all cell lines and with the exception of the 54 year old cell line and alternatively spliced form of TGFßR-I was detected in all cell lines. **Conclusion**: These results indicate that cultured HTM cells express all TGFß isoforms and receptor types. The interaction of members of the TGFß family within the human TM may be important for normal function. Support: Glaucoma Research Foundation (RJW); NIH EY-10056 (SEW). "None"

Abstract #41

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Ganesh Prasanna Ph.D.

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

REGULATION OF ENDOTHELIN-1 AND BIG ENDOTHELIN-1 LEVELS BY TUMOR NECROSIS FACTOR - α IN HUMAN NON-PIGMENTED CILIARY EPITHELIAL CELLS IS PROTEIN KINASE-C DEPENDENT. G. Prasanna, A. I. Dibas, K. White, W. Tao, and T. Yorio. Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76116.

Purpose. To determine the role of protein kinase C following the treatment with tumor necrosis factor-α (TNF-α) on the levels of endothelin-1 (ET-1) and Big endothelin-1 (Big ET-1) in SV-40 transformed human non-pigmented epithelial (HNPE) cells. **Methods.** Intracellular ET-1 and Big ET-1 levels were monitored using the indirect immunofluorescence technique in HNPE cells following treatments with TNF-α, phorbol esters and staurosporine. Experiments included individual and combination treatments of agonists and antagonists. Incubation times ranged from 15 - 60 minutes. Control cells were untreated throughout the incubation period. Quantitation of fluorescence intensities were made by measuring changes in the gray-scale levels of photomicrographs between treatments and controls. **Results.** In HNPE cells, TNF-α increased the immunofluorescence for ET-1 and Big ET-1 in a time dependent manner. Phorbol esters (PMA) incubated for 30 minutes exhibited the same fluorescence intensity as that observed from TNF-α incubation for 60 minutes. The combination of TNF-α and PMA increased the fluorescence for both peptides compared to untreated controls but remained similar to that observed for either treatments alone. Staurosporine pre-treatment followed by TNF-α treatment resulted in a loss of fluorescence for both peptides. **Conclusions.** Stimulation of Big ET-1 and ET-1 synthesis by TNF-α and PMA may involve a PKC-dependent step(s) that can be inhibited by staurosporine. Supported in part by an Advanced Research Program Grant from The Texas Higher Education Coordinating Board.

Abstract #42

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Wenhong Tao

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ENDOTHELIN RECEPTOR A IS EXPRESSED AND MEDIATES THE $[Ca^{2+}]_i$ MOBILIZATION IN HUMAN CILIARY SMOOTH MUSCLE, CILIARY NONPIGMENTED EPITHELIAL, AND TRABECULAR MESHWORK CELLS

W. Tao, and T. Yorio Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Forth Worth, TX 76107

Purpose: To investigate if endothelin is an active cytokine in the regulation of aqueous humor by human ciliary body and trabecular meshwork. **Methods:** Endothelin receptor gene expression was probed with reverse transcription of polymerase chain reaction (RT-PCR). $[Ca^{2+}]_i$ mobilization was characterized with vedio image microscopy and Fura-2AM fluorescent probe. **Results:** Endothelin receptor A (ET_A) is detected with RT-PCR in cultured human ciliary smooth muscle (HCM) cells, ciliary nonpigmented epithelial cells (HCE), and trabecular meshwork cells (HTM). The mRNA phenotype was verified with restriction enzyme digestion. No ET_B was detected with RT-PCR. The $[Ca^{2+}]_i$ of HCM cells was increased from 57.3 ± 7.1 nM to 328 ± 108 nM (n=23) (P=0.018) by 1 nM endothelin-1 (ET-1). In HCE cells, $[Ca^{2+}]_i$ rose from 40.1 ± 2.9 nM to 90.0 ± 9.9 nM (n=55) (P<0.001) with same concentration of ET-1. Similarly, ET-1 (1 nM) increased the $[Ca^{2+}]_i$ from 51.3 ± 5.7 nM to 184.8 ± 46.7 nM (n=19) (P<0.001) in the HTM cells. The agonist for ET_B, S-6-c, did not show any effects on $[Ca^{2+}]_i$ transient in all three types of cells. **Conclusion:** ET_A, not ET_B, is expressed and responsible for mediating the signal for $[Ca^{2+}]_i$ mobilization by ET in the human ciliary smooth muscle, ciliary nonpigmented epithelial, and trabecular meshwork cell.

Abstract #43

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: ROUEL S. ROQUE, M.D.

Department: DEPARTMENT OF ANATOMY AND CELL BIOLOGY

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

MICROGLIA-DERIVED CYTOTOXIC FACTOR(S) INDUCE APOPTOSIS OF 661W PHOTORECEPTOR CELLS IN VITRO. R.S. Roque[1], A.A. Rosales[1], A.M. Brun[1], N. Agarwal[1], and M.R. Al-Ubaidi[2]. [1]Department of Anatomy and Cell Biology and North Texas Eye Research Institute, University of North Texas Health Science Center at Fort Worth, TX 76107; [2]Department of Ophthalmology and Visual Sciences, The University of Illinois at Chicago, IL 60612.

Studies in our laboratory suggest that retina-derived microglial cells release molecule(s) that may promote photoreceptor cell death in the dystrophic rat retina. We have begun to characterize these molecules and determine the mechanisms involved in the microglia-mediated photoreceptor cell death. The difficulty of obtaining pure populations of photoreceptor cells has necessitated the use photoreceptor cells (661w) generated from transgenic mice retinas expressing the SV40T antigen directed by human interstitial retinoid-binding protein promoter. Cultures of 661w cells were treated for 24-48 hours with basal medium (DMEM with 0.1% bovine serum albumin) or basal medium conditioned by activated microglial cells (MGCM) in the presence or absence of heat or serum treatment. The cultures were then assayed for cell death using colorimetric techniques to measure lactate dehydrogenase released by dead cells. The mechanism of cell death was investigated using terminal deoxynucleotidyl transferase-mediated fluorescein-12-dUTP nick end labeling and electron microscopic analysis of MGCM-treated cells. LDH measurements suggest that about 50% of 661w cells grown in MGCM were dead after 48 hours of treatment as compared with 20% of those in basal medium. Heat treatment at 100°C X 10 min. or supplementation of the media with 1% serum resulted in parallel findings. A large number of MGCM-treated cells labeled positively for apoptosis (as shown by the incorporation of fluorescein-labeled dUTP at 3'-OH ends of fragmented DNA) unlike those treated with basal medium. Electron microscopy of MGCM-treated cultures revealed 661w cells in various stages of apoptotic cell death characterized by compaction of nuclear chromatin, fragmentation of nuclei, and/or cellular budding. Our study shows that activated microglial cells induce degeneration of cultured photoreceptor cells via release of soluble heat-stable molecule(s). Moreover, our study suggests that the MGCM-induced photoreceptor cell death involves apoptosis similar to that described in animal models of retinal degeneration such as the dystrophic rat retina.
(Supported by NIH EY10766)

Abstract #44

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Harold J. Sheedlo

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty✓ Staff___

Read instructions and fit abstract inside the space given below:

RETINAL PROGENITOR CELLS: IMMUNOCYTOCHEMICAL AND REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION ANALYSES H.J. Sheedlo, R.J. Wordinger, R. Agarwal and J.E. Turner University of North Texas Health Science Center/North Texas Eye Research Institute, Fort Worth, TX 76107.

Expression of stem-, photoreceptor- and glial-cell proteins and the mRNA for nestin, an intermediate filament protein and early neuroepithelial cell marker, c-fos, an immediate early gene (IEG), basic fibroblast growth factor (FGF-2) and epidermal growth factor (EGF) in retinal progenitor cells and explants were determined by immunocytochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR). For this study, pure populations of progenitor cells were isolated from retinal explants of 2-day-old normal rats that were cultured in conditioned media (CM) of transformed neonatal rat (tnr) retinal pigment epithelial (RPE) cells for ~2 weeks. Progenitor cells were processed for immunocytochemical localization of nestin, opsin, arrestin, neuron-specific enolase (NSE) and cellular retinaldehyde-binding protein (CRALBP). cDNA was generated from total RNA of progenitor cells and retinal explants and probed with primers for nestin, c-fos, FGF-2 and EGF. The PCR products were separated in agarose gels. As shown by immunocytochemistry, over 80% of progenitor cells expressed opsin, arrestin and NSE, while about 30% and 1% were immunoreactive for CRALBP and nestin, respectively. As determined by RT-PCR, retinal progenitor cells expressed the message for nestin and FGF-2, but not EGF. However, the retinal explants showed the presence of message for these proteins. The message for the IEG c-fos was not detected in either the retinal progenitor cells or explants. Progenitor cells grown in tnrRPE-CM formed a unique lamination pattern without cellular contact suggesting a trophic relationship. In conclusion, FGF-2 expression by progenitor cells may provide a mechanism to autoregulate cell division and/or for cell fate determination. Similar cells from human retinal explants may serve as a therapy in ocular diseases such as some forms of retinitis pigmentosa and macular degeneration. Support provided by NIH grant EY 04337.

Abstract #45

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: James E. Turner, Ph.D.

Department: North Texas Eye Research Institute/Dept. of Anatomy & Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

RPE CONDITIONED MEDIUM INFLUENCES PHOTORECEPTOR CELL SURVIVAL AND MATURATION IN THE RAT RETINA J.E. Turner, T.A. Rogers, T.H. Nelson, H.J. Sheedlo Department of Anatomy and Cell Biology/North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX.

Purpose. To assess the effects of RPE conditioned medium (RPE-CM) on rat retinal development *in vitro* and *in vivo*, with particular emphasis on photoreceptor cell maturation. **Methods.** RPE-CM, diluted 50% with a chemically-defined media from transformed neonatal rat (tnr)RPE cultures, was added to explants of retinas from E18 or PN2 Long Evans rats. Control explants were treated with defined media or 10% fetal bovine serum. Explants were examined by phase contrast microscopy for neurite outgrowth and at day 7 were dissociated and prepared for immunocytochemical analysis for opsin, arrestin and cellular retinaldehyde binding protein (CRALBP) expression. Furthermore, CM from tnrRPE cells was injected into the vitreous of 7-day-old Long Evans rats and retinas were analyzed by light and electron microscopy, and opsin immunocytochemistry 7 days post surgery. **Results.** In retinal explants supplemented with tnrRPE-CM, long, ganglion cell-like neurites were detected after 3 days, while controls showed no such activity. Analysis of dissociated E18 explants showed that approximately 40% of the cells were opsin$^+$, while only 14% and 23% of the cells from explants grown in serum or defined medium respectively, expressed this photoreceptor marker. Additionally, immunocytochemical analysis of dissociated explant cells, in PN2 explants, revealed that at 7 days after RPE-CM treatment, 80% of the retinal progenitor cells expressed opsin, compared to 20% for cells grown in defined medium or serum. In addition, vitreal injections of RPE-CM into PN7 eyes initiated an increase in inner/outer segment lengths and increased survival of ectopic photoreceptor cells in the inner nuclear layer, 1 week post-injection, when compared to retinas of sham-injected eyes. **Conclusions.** The *in vitro* and *in vivo* results suggest that a protein(s) in the RPE-CM plays a key role in normal retinal development, particularly in photoreceptor cell survival and outer segment maturation. We call this putative protein the RPE cell line-derived retina trophic factor or RPE-RTF.
(Supported by NIH grant EY04377.)

Abstract #46

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Tammy Hancock Nelson

Department: North Texas Eye Research Institute/Dept. of Anatomy & Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

AN ANTIBODY TO RPE SECRETED PROTEINS INHIBITS RETINAL DEVELOPMENT AND PHOTORECEPTOR CELL MATURATION T.H. Nelson, N. Ling, H.J. Sheedlo 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.

Purpose. To assess the effects of a polyclonal antibody to proteins secreted (RPE-SP) by a spontaneously transformed (tnr)RPE cell line on *in vitro* and *in vivo* rat retinal development with particular emphasis on photoreceptor cell maturation. **Methods.** A polyclonal antiserum was produced in sheep against proteins in a conditioned media (CM) from tnrRPE-CM and futher purified to an antibody. The RPE-SP antibody was added to explants of postnatal day 2 (PN2) Long Evans rats at dilutions of 0.25 to 50% with RPE-CM and observed for 7 days by phase contrast microscopy. Control explants were treated with either RPE-CM, diluted 50% with serum-free defined medium, defined medium or 10% fetal bovine serum only. At 7 days, explants were dissociated and prepared for immunocytochemical analysis for the photoreceptor cell marker opsin. In addition, vitreal and subretinal space injections of the RPE-SP antiserum or antibody were made into 4 and 7 day neonatal Long Evans rat eyes and prepared for light and electron microscopy at 7, 14, 21 and 28 days post surgery. Control eyes received injections of either preimmune serum or normal sheep IgGs. **Results.** Retinal explant cultures supplemented with RPE-SP antiserum or antibody adsorbed with tnrRPE-CM nullified the long neurite ganglion cell-like outgrowth normally seen by 3 days in RPE-CM treated cultures. By 5 days the antiserum/antibody treatment caused explant degeneration. Extensive retinal degeneration, eventually leading to microphthalmia, was seen in neonatal eyes receiving subretinal space injections of the antiserum. Those retinas receiving antiserum/antibody vitreal injections were significantly thinner, demonstrated by photoreceptor cell degeneration and compromised outer segment maturation and survival when compared to controls. Some regions of the retina were almost completely devoid of photoreceptor cells. **Conclusions.** These *in vitro* and *in vivo* studies suggest that RPE-SP antiserum/antibody nullifies the actions of RPE secreted protein(s) crucial to the survival of the developing retina and in particular that of photoreceptor cells. We call this putative protein the RPE cell line-derived retina trophic factor or RPE-RTF. (Supported by NIH grant EY04377).

Abstract #47

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Scott Krueger

Department: Anatomy and Cell Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

PHOTO-OXIDATIVE STRESS LEADS TO APOPTOSIS OF 661W MOUSE PHOTORECEPTOR CELLS. (S Krueger[ab], R Krishnamoorthy[a], I-H Pang[a,b], E Martin[b], P Magnino[b], M J. Crawford[a], M Al-Ubaidi[c], S K. Jain[d], and N Agarwal[a], and R J Collier[ab])) UNT Health Science Center[a], and Alcon Laboratories[b], Fort Worth, TX. Uni. of Illinois[c], Chicago, IL; LSU Medical Center[d], Shreveport, LA.

Purpose. To determine effects of photic insults on lipid peroxidation, glutathione (GSH), cytoplasmic calcium ($[Ca^{2+}]_i$), apoptosis and viability in transformed mouse photoreceptor (661W) cells. Methods. Cultured 661W photoreceptor cells were exposed to fluorescent visible light (3-4 mW/cm^2) for varying durations up to 7 hrs. Control cells were shielded from light for similar time intervals. After light exposure, cell cultures were evaluated for: cell viability (Formazan assay, Promega); apoptosis, TUNEL and DNA laddering; lipid peroxidation by measuring malonyldialdehyde (MDA) levels by thiobarbituric acid (TBA) reaction and GSH depletion. Changes in intracellular calcium were monitored by Fura-2 dye in cells exposed to 10mW/cm^2 from a light microscope. Results. Photo-oxidative stress to 661W cells resulted in significant loss of cell viability after a four hour exposure (≈40%), linearly decreasing out to 7 hrs when no viable cells were detected. A significant number of cells died by apoptosis as determined by TUNEL and DNA laddering. Light exposure resulted in increased MDA formation (≈80%) with a significant decrease in GSH levels (≈1.6X). The MDA accumulation and GSH depletion were prevented by the inclusion of N-acetyl cysteine (2mM) and thiourea (7 mM) respectively. The cytoplasmic $[Ca^{2+}]_i$ levels significantly increased with time prior to cell morphology changes. Conclusions. Exposure of 661W cells to photo-oxidative stress leads to apoptotic cell death. Cell death was preceded by lipid peroxidation and increased intracellular calcium. Further studies are underway to elucidate the mechanism(s) of apoptosis by measuring changes in gene expression (i.e., Bcl-2, Bax). Supported by Alcon Laboratories, Inc., E and C5.

Abstract #48

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Wendi Lambert

Department: Anatomy and Cell Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EXPRESSION OF TRANSFORMING GROWTH FACTOR-ß ISOFORMS AND THEIR RECEPTOR mRNAs IN CULTURED HUMAN LAMINA CRIBROSA CELLS ((W. Lambert[1], R. Agarwal[1], A.F. Clark[2], and R.J. Wordinger[1])) North Texas Eye Research Insitute and Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, TX[1]; Glaucoma Research, Alcon Laboratories, Fort Worth, TX[2].

Purpose: The lamina cribrosa (LC) is an area within the optic disk that serves to protect and guide the axons of the retinal ganglion cells through the sclera to form the optic nerve. Changes in the extracellular matrix (ECM) within the LC have been observed in primary open angle glaucoma and may involve one of the resident cell populations, the LC cells. Growth factors are known to regulate ECM production. However, the role of growth factors and growth factor receptors in the normal structure and function of the LC has not been extensively studied. The purpose of this study was to begin to determine which growth factors and growth factor receptors are expressed in cultured human LC cells. **Methods:** Total cellular RNA isolation, cDNA synthesis, RT-PCR, and agarose gel electrophoresis were performed using well characterized LC lines from a neonatal, 64 year, and 65 year old donors. A normal human astrocyte cell line (Clonetics Corp., San Diego, CA.) and human astrocytoma cell line were also studied. The PCR primers were designed using Entrez (NCBI, Bethesda, MD.) and Oligo 4.0 (National Biosciences, Plymouth, MN.). **Results:** Message for transforming growth factor beta-2 (TGFß-2), TGFß-3, TGF-ßRI, TGFß-RII and alternatively spliced forms of TGFß-RI and TGFß-RII were detected in the all samples. Message for TGFß-1 and TGFß-RIII were not detected in any of the cell lines studied. **Conclusions:** The results from this study demonstrate that cultured human LC cells express message for growth factors and growth factor receptors which may be important to the normal structure and function of the lamina cribrosa. ("None")

Abstract #49

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Martha E. Stokely

Department: Anatomy and Cell Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

RETINA TROPHICALLY SUPPORTS OLIGODENDROCYTES FROM THE OPTIC NERVE *IN VITRO*, M. E. Stokely*, and S. J. Moorman. Dept. Anatomy & Cell Biology, UNT Health Science Center at Fort Worth, Ft. Worth, TX 76107.

It has been proposed that the mortality rate (approx. 50%) of optic nerve oligodendrocytes during development is regulated by competition for a combination of available trophic factors. The retina is one possible source for those trophic factors. To test this, oligodendrocytes from P5 rat optic nerve were grown in poly-lysine coated dishes under three conditions: 1) unsupplemented DMEM/F12 media, n=15; 2) unsupplemented DMEM/F12 media containing retinal explants (plated 5 days earlier from P0 litter-mates), n=45; 3) F12 media supplemented with heat inactivated horse serum, conalbumin, vitamin C, glucose, and insulin, n=60. Dishes were observed at 1, 3, and 5 days.

Oligodendrocytes appeared unhealthy and survived less than 5 days in unsupplemented DMEM/F12 media. In either supplemented F12 media or DMEM/F12 with retinal explants, oligodendrocytes appeared healthy, survived, differentiated and grew. These results suggest the retina is a possible source of trophic factors for oligodendrocytes in the optic nerve.

Supported by the UNT Health Science Center at Fort Worth.

Abstract #50

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: MICHAEL H. CHAITIN, PH.D.
Department: ANATOMY & CELL BIOLOGY

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

CD44 IN THE RETINA. Michael H. Chaitin, Raghu Krishnamoorthy and Neeraj Agarwal. Department of Anatomy and Cell Biology and North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX.

The transmembrane glycoprotein CD44 is a cell adhesion molecule that is distributed in a wide variety of cells and tissues. It's main ligand is hyaluronic acid. Other ligands include chondroitin sulfates, fibronectin, laminin and collagens I and VI. Certain forms of CD44 can also bind trophic factors such as bFGF. Recently, the involvement of standard and variant forms of CD44 in tumor cell metastasis, tissue differentiation and apoptosis has been studied. We have been studying CD44 in the mouse retina to determine its role in retinal adhesion, retinal degeneration and the response of the retina to extracellular signals. For these studies we have used immunoperoxidase and immunogold labeling, Western blotting and Northern blotting. Immunocytochemistry has shown CD44 to be localized to the Muller cell apical microvilli which project into the interphotoreceptor matrix (IPM). On Western blots, retinal CD44 is found to be 90 kD which represents the standard form of the molecule. On Northern blots, the predominant mRNA transcript is about 4.5 kb which is identical to that for mouse brain astrocytes. Muller cells are retinal glial cells related to astrocytes. Thus, the data shows the presence of CD44 in glial cells within the neural retina and indicates that CD44 may play a role in the adhesion of the retina to the IPM. Additionally, CD44 is in a prime location to respond to trophic factors sequestered within the IPM.

Abstract #51

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*Student Presenter

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Walter J. McConathy, Ph.D.

Department: Institute of Nutrition and Chronic Disease Prevention

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

INSTITUTE OF NUTRITION AND CHRONIC DISEASE PREVENTION

Michael Clearfield, D.O, Director, and Walter J. McConathy, Ph.D., Deputy-Director University of North Texas Health Sciences Center, Fort Worth, Texas.

Purpose: The long-term mission of this Institute is to promote good health by emphasis on sound nutritional practices. The Institute will have three broad areas of focus: **Basic and Applied Research; Higher Education; and Public Education; and Community Service.**

Activities: The Institute will address the role of nutrition in preventing cardiovascular disease, diabetes, and the improvement of the quality of life during aging. The initial focus will involve cardiovascular risk factors, lipoprotein metabolism, and lipid lowering drugs. In the long term, research activities will focus on the nutritional components and molecular mechanisms of disease processes at the cell, organ, and whole organism level while clinical activities will include the development of programs in the area of diabetes, preventive cardiology, rehabilitation for those with vascular disease, osteoporosis, and dietary intervention in the aging process and cancer.

Summary: Based on available experts at UNTHSC, this Institute will enhance collaborative research between physicians and basic scientists of a number of Departments. More importantly, it will promote interactions with the local and national professional community in the area of sound nutritional practices leading to the prevention of chronic disease.

Abstract #52

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Karen R. Murray

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

THE MC ARDLE 7777 CELL LINE AS A TOOL FOR STUDYING THE STRUCTURE AND FUNCTION OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE (LCAT).

Karen R. Murray, Amir Ayyobi, Maya Nair, P. Haydn Pritchard and Andras G. Lacko

Department of Biochemistry and Molecular Biology, University of North Texas Health Science Center and Atherosclerosis Specialty Laboratory, St. Paul's Hospital, University of British Columbia, Vancouver, B.C., CANADA.

Recombinant LCAT is now available from several sources. However, the characteristics of these recombinant enzyme forms is of concern, especially regarding the the affinity of the respective enzymes toward HDL. Therefore, the production of LCAT by a liver cell line that is likely to resemble the plasma enzyme appeared desirable. The recombinant LCAT secreting cell line was prepared by a double transfection procedure using McArdle 7777 cells. The transgene includes a methallothienin promoter that is expected to respond to Zn^{++} ions. The McArdle 7777-LCAT cells were grown to 80% confluency in Dulbecco's modified Eagle's medium with 20% serum. Subsequently, the cells were incubated with serum-free Opti-MEM with increasing concentrations of zinc (0 μM--50 μM). The culture medium was harvested at 24, 48 and 72 hrs. from the control and zinc supplemented cutures and used for the determination of LCAT activity.

Abstract #53

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

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Research Appreciation Day 1997

ABSTRACT FORM

First Author: Samuel Chuang

Department: Pharmacology

Graduate Student_X_ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

A Single *in vitro* Point Mutation Within the Positive *Cis*-acting Element in the First Non-translated Exon Silences Transcription of the Human Apolipoprotein B Gene in HepG2 Cells

By Samuel S. Chuang and Hriday K. Das
Department of Pharmacology,
University of North Texas- Health Science Center at Fort Worth,
Fort Worth, Texas 76107

Hepatic cell-specific expression of the human apolipoprotein B (apoB) gene is controlled by at least four *cis*-acting elements located within the (-128 to +122) promoter region (S. S. Chuang and H. K. Das, *Biochem. Biophys. Res. Commun.* **220:** 553-562, 1996). Two *cis*-acting positive elements (-104 to -85; -84 to -60) are located upstream of the start of transcription. A negative element (+20 to +40) and a strong positive element (+43 to +53) are located in the first non-translated exon of the human apolipoprotein B gene. *Trans*-acting factors BRF-2, BRF-1, BRF-3, and BRF-4 interact with the above four cis-acting elements respectively. In this study, we examine the roles, the upstream positive elements (-104 to -85) and (-84 to -60) may play in modulating transcriptional regulation of the apoB gene by downstream elements (+20 to +40) and (+43 to +53). Using *in vitro* mutagenesis and transient transfection experiments in HepG2 cells, the *cis*-acting element (-84 to -60) has been found to be absolutely necessary for the function of the upstream positive element (-104 to -85) as well as the downstream negative (+20 to +40) and positive (+43 to +53) elements. *In vitro* mutagenesis of the downstream positive element (+43 to +53) and transfection of the of the mutant promoter constructs in HepG2 cells reveal that nucleotide G at position (+51) is essential for the strong positive activity of the element (+43 to +53). A single substitution point mutation of nucleotide G at position (+51) to either A or T reduces apoB gene transcription in HepG2 cells by 90%. These results suggest that a single substitution point mutation *in vivo*, of nucleotide G to either A or T at position (+51) in the downstream positive promoter element may have potential to cause the autosomal-dominant disorder heterozygous hypobetalipoproteinemia.

Abstract #54

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Yun Bai

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

RAT CARDIAC CAM KINASE II AND CALCINEURIN REGULATE EMBRYONIC GENE EXPRESSION BY A MECHANISM OF REPRESSION/DEREPRESSION IN VIVO IN TRANSGENIC MICE Y. Bai and S. R. Grant, Dept. of Biochem.UNTHSC @ Ft. Worth,TX 76107
Recent studies have demonstrated that cardiomyocytes receptors which signal to the nucleus through a mechanism of protein kinase C (PKC) activation transcriptionally up-regulate a variety of genes of embryonic origin. This list includes contractile proteins such as β-myosin heavy chain, the actins, the troponins, or other embryonic genes like atrial nateuretic factor (ANF). Evidence has begun to accumulate that embryonic gene expression can also be regulated by beta-adrenergic receptor nuclear signaling. Signaling through beta Adr R does not appear to be coupled through PKC activation. Preliminary studies from our laboratory suggest that a calcium/calmodulin dependent nuclear signaling cascade(s) plays a central role in the cellular expression of sarcomeric contractile protein. A calcium/calmodulin dependent nuclear signaling pathway appears to be regulated through beta adrenoreceptor subtype signaling. The calcium-dependent enzyme, cardiac CaM Kinase II negatively regulates contractile protein genes and ANF. This research project has proposed to explore a functional role in vivo studies of exogenous CaM Kinase II and calcineurin (CaN) regulating transcription of cardiac embryonic gene. We will study this signaling cascade *in vivo*. βeta 1-Adr R signaling through CaM Kinase II activation transcriptionally silences the cardiac ANF, skeletal Actin, cardiac actin in the adult mouse myocardium. Second, β2-Adr R signaling through the CaM-dependent protein phosphatase, CaN, derepresses (activates) these genes in vivo. The study has proposed to access a functional role for exogenous CaM kinase II or CaN activity in regulating the repression of the cardiac embryonic genes using a transgenic mouse model for inducible. Transgenic mice expressing the tetracycline transactivator protein (tTA) in heart tissue serve as the basic system. Inducible CaN or CaM Kinase II expression plasmids have been constructed. These constructs will be directly injected into heart tissue of TG mice harboring the tet transactivator protein tTA. Tetracycline (tet) induction of CaN or CaM Kinase II in TG mice will be regulated *in vivo* and repression/derepression of the embryonic genes in TG mice will be detected by Northern analysis, protein abundance by Western analysis. The physiological alterations of mice hearts are studied. At present, I have constructed the tet inducible CaN gene expression plasmid. Next step is to inject tet inducible CaN plasmids system into transgenic mice heart. Further, the levels of the cardiac embryonic genes are going to be analyzed.

Abstract #55

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Richard Easom

Department: Biochemistry and Molecular Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

DEPHOSPHORYLATION AND DEACTIVATION OF CaM KINASE II IN PERMEABILIZED βTC3 CELLS IS MEDIATED BY PROTEIN PHOSPHATASES 1 and 2C. Richard A. Easom, Natalie R. Filler, Jimmy L. Tarpley and Harshika Bhatt, Biochemistry and Molecular Biology, University of North Texas Health Science Center, Fort Worth, TX.

The activation and induction of autonomous CaM kinase II by glucose in pancreatic β–cells has implicated this enzyme in the regulation of insulin secretion. This autonomy is mediated by the autophosphorylation of a threonine 286/287 in its regulatory domain and persists as long as this residue remains phosphorylated. In order to delineate the regulatory mechanism responsible for kinase deactivation, we studied the involvement of protein phosphatases in α-toxin-permeabilized βTC3 cells. In these cells, Ca^{2+} induced the dose-dependent activation of CaM kinase II such that the proportion of the enzyme in autonomous form was 0.67 ± 0.25 % and 39.39 ± 1.90 % in the presence of 50 nM and 10 μM Ca^{2+}, respectively. Following activation, the return of Ca^{2+} to pre-stimulatory levels (50 nM) resulted in the rapid decline in autonomous kinase activity such that basal activation levels were achieved by 2.0 min. The rate of this reverse was not affected by cyclosporin A or FK-506 eliminating the involvement of PP-2B. By contrast, CaM Kinase II deactivation was slowed but not prevented by okadaic acid and calyculin A (1 μM), inhibitors of PP-1 and -2A. The preferential inhibition by calyculin A over okadaic identified this phosphatase activity as PP-1. EDTA also prolonged autonomy levels in the absence of stimulatory concentrations of Ca^{2+} further suggesting the involvement of a Mg^{2+}-dependent protein phosphatase such as protein phosphatase 2C. Near total prevention of CaM Kinase II deactivation was only achieved in the combined presence of EDTA and okadaic acid. These data suggest the dephosphorylation and deactivation of CaM Kinase II in the β-cell is mediated through a complex interaction of PP-1 and PP-2C. (Supported by NIH DK-47925 to R.A.E.).

Abstract #56

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Harshika Bhatt

Department: Biochemistry and Molecular Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EFFECT OF GLUCAGON LIKE PEPTIDE-1 (GLP-1) ON GLUCOSE STIMULATED INSULIN SECRETION IN PANCREATIC β-CELLS IS MEDIATED BY CaM KINASE II.

H. Bhatt, *A.Dibas, *T.Yorio and R.A.Easom. Dept. of Biochemistry & Mol. Biology and *Dept of Pharmacology. University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

While it has been established that glucose is the major insulin secretaogogue, with increased intracellular Ca^{2+} a critical signal, the biochemical mechanisms of insulin secretion are not completely known. Additionally, factors other than glucose, secreted in response to oral nutrients are required for the physiological response of the β-cells to glucose. Glucagon-Like Peptide-1 (7-37) [GLP-1(7-37)] is a gluco-incretin hormone which plays an important role in glucose homeostasis. Although, GLP-1 (7-37) - stimulated insulin secretion from the β–cell is associated with an increase in cAMP accumulation, little is known about the signal transduction pathways used by this peptide. In the current study, the glucose responsive pancreatic β-cell lines (βTC3 and INS-1) used as a model to study insulin secretion and an involvement of the Ca^{2+}-Calmodulin dependent protein Kinase - CaM Kinase II. we recently demonstrated that GLP-1 (7-37) increased the free cytosolic calcium level ([Ca]i), and stimulated insulin secretion in a glucose-dependent manner. Similarly, GLP-1 induced the significant activation of CaM Kinase II which accompanied an increase in [(Ca)i] and insulin secretion in the presence of glucose (0.1 - 20.0 mM) and GLP-1(0 - 10 nM). These findings documented a novel GLP-1-sensitive Ca^{2+} signalling pathway via CaM Kinase II activation, that might play an important role in the regulation of insulin secretion from β-cells.

(Supported by NIH DK47925)

Abstract #57

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Michael C. Lawrence

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

GENE TRANSFER INTO PANCREATIC ISLETS BY RECOMBINANT ADENOVIRUS. Michael C. Lawrence and Richard A. Easom, UNTHSC at Fort Worth, Fort Worth, TX 76107.

The transfer of DNA into mammalian cells in order to examine metabolic processes has been limited by the relative inefficiency of available techniques for gene transfer. Previous gene transfer studies on transformed clonal β-cells have employed physical techniques such as lipofection or electroporation. Although these techniques have a high incidence of co-transfection of two plasmids into a single cell, which have given insight to the regulation and activation of chimeric-reporter gene constructs, their overall transfection efficiency is only 10-20%. Meaningful insights into the impact of overexpression of genes on acute regulation of insulin release will require much higher efficiencies. Moreover, primary β-cells, which may provide more accurate information, are more difficult to transfect, as they are isolated in the form of multicellular aggregates. One method to achieving increased gene transfer into primary β-cells is by recombinant adenovirus. Recombinant virions can be produced by the co-transfection of the circular viral genome with a plasmid containing a fragment of the viral genome and the gene of interest. Infection of isolated pancreatic islets by recombinant adenovirus has shown to have gene transfer efficiencies of 60-100%. The recombinant protein may be expressed at 10-20% of the total cellular proteins. Here we describe the process of constructing a recombinant adenovirus. (Supported by the Advanced Research Program of the Texas Higher Education Co-ordinating Board).

Abstract #58

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Sarah Boyle

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

FK-506 INHIBITS INSULIN GENE EXPRESSION LEADING TO DECREASED INSULIN SYNTHESIS AND SECRETION.

Sarah Boyle and Richard Easom. Department of Biochemistry and Molecular Biology, University of North Texas health Science Center, Fort Worth, Texas, 76107-2699

It has been postulated that glucose regulation of insulin gene expression may be in part mediated by changes in intracellular Ca^{2+}. Based on the established role of the Ca^{2+}-calmodulin dependent phosphatase (pp-2B), calcineurin, to regulate IL-2 expression in T-lymphocytes, it was of interest to evaluate the potential of this enzyme to similarly regulate insulin gene expression. Calcineurin is inhibited by FK-506, a drug that is widely used as an immunosuppresant in tissue transplantation. Because of its potential use in the prevention of the onset of the autoimmune, Type I diabetes, this study has addressed the effects of FK-506 on insulin secretion in primary islet cells, by static secretion experiments, and insulin gene expression in the INS-1 β cell line by Northern blot analysis. FK-506 (0-10 nM) had no acute effect (i.e. at 24 hours) on glucose stimulated insulin secretion in primary islet cells, but caused a slight dose dependent decrease in insulin secretion at 48 hours. FK-506 had a significant effect (0-10nM) on glucose-stimulated insulin gene transcription at 24 hours as indicated by the decrease in the amount of mRNA detected by Northern blot analysis. This data is consistent with the hypothesis that sustained insulin secretion in response to stimulus such as glucose requires new synthesis of insulin mediated by gene transcription. The acute release of insulin could be the result of the fusion of stored secretory granules with the plasma membrane. Western blot analysis of INS-1 cell proteins confirmed the presence of calcineurin in the INS-1 cells. These findings by the above experiments suggest that FK-506 may have direct effects to inhibit insulin gene transcription, leading to decline in insulin mRNA levels, insulin synthesis and subsequent insulin secretion. (Supported by Advance Research Program of the Texas Higher Education Coordinating Board to R.E.A.)

Abstract #59

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Gary Frank Scott

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

REGULATION OF INSULIN GENE EXPRESSION BY CALCIUM/CALMODULIN KINASE Gary Frank Scott and Richard A. Easom Department of Biochemistry and Molecular Biology UNT-HSC, Fort Worth, TX 76107.

Our laboratory is interested in the potential role of Ca^{2+}-dependent kinases in the regulation of insulin biosynthesis and secretion in the β-cells of the mammalian pancreas. Insulin biosynthesis by transcription of the insulin gene in the pancreatic β-cell is dependent on the interaction between trans-acting transcription factor proteins and cis-acting DNA sequence elements located in the untranscribed 5' promoter region within 350 bp upstream from transcription start site. Previous studies have established that human IUF-1 (or IPF-1/STF-1/**IDX-1**, a β-cell specific high consensus mammalian homeodomain transcription factor) plays an important role in glucose-sensitive insulin gene transcription. Since the transcriptional potency of this factor is dependent or its phosphorylation state, we have studied the ability of CaM kinases to modulate **IDX-1.** We have identified using EMSA (electrophoretic mobility shift assay of DNA-protein factor complexes) the presence of **IDX-1** in βTC3 (mouse insulin-secreting tumor) cell nuclear extracts. IUF-1/**IDX-1** binding has been characterized to two different length DNA probes containing the human insulin gene IUF-1 specific binding site (CT2 and CT2/IEB) using EMSA. The specific band produced by **IDX-1** association with human insulin promoter DNA elements was validated by successful competition by unlabeled DNA and its modulation (supershift) by the introduction of the anti-**IDX-1** antibody. Since β-cell glucose uptake and metabolism produces a rise in intracellular Ca^{2+}, nuclei were isolated from α-toxin permeabilized βTC3 cells treated with $10\,\mu M Ca^{2+}$ (or control, $0.05\,\mu M Ca^{2+}$) in the presence of [α–^{32}P]-ATP. After incubation of these radiolabelled nuclear extracts with unlabeled transcription factor recognition-site oligonucleotide promoter sequences, this novel modification of the EMSA assay revealed Ca^{2+}-dependent DNA-phospho-protein complexes, likely containing **IDX-1**. These data are consistent with a role of CaM Kinase in the regulation of insulin gene expression.

Abstract #60

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Kimberly Ann Krueger

Department: Biochemistry

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CALCIUM-STIMULATED PHOSPHORYLATION OF MAP-2 IN PANCREATIC β-CELLS IS MEDIATED BY CaM KINASE II. K.A. Krueger, H. Bhatt, M. Landt and R.A. Easom, UNTHSC at Fort Worth, Fort Worth, TX 76107 and Washington University School of Medicine, St. Louis, MO 63110.

An elevation of intracellular Ca^{2+} is a critical signal in the initiation of insulin secretion from the pancreatic β-cell but the mechanism involved is not understood. Previously, we have demonstrated that the multifunctional Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) is activated by glucose in isolated rat islets implicating this enzyme in the secretory process, but its cellular targets are unidentified. One of the best substrates of CaM kinase II *in vitro* that could function in secretory events, is the microtubule-associated protein, MAP-2. The current study represents an evaluation, *in situ*, of MAP-2 as a substrate of CaM kinase II using a permeabilized β-cell model. By immunoblot analysis, the presence of MAP-2 in the βTC3 cell was established. The > 250 kDa protein was efficiently immunoprecipitated by antibodies raised against rat brain MAP-2, and was significantly phosphorylated *in situ* in α-toxin-permeabilized βTC3 cells. Increases of Ca^{2+} induced a concentration-dependent phosphorylation of MAP-2 which temporally coincided with the Ca^{2+}-dependent activation of CaM kinase II. Ca^{2+}-induced phosphorylation of MAP-2 was not inhibited by an inhibitor of protein kinase A (H-89) at concentrations that prevented phosphorylation induced by forskolin, but was abrogated by KN-93 and K252a, inhibitors of CaM kinase II. Two-dimensional tryptic phosphopeptide mapping revealed that the sites phosphorylated by CaM kinase II *in vitro*, while distinct from sites phosphorylated by PKA *in vitro*, were homologous to those sites phosphorylated *in situ* upon incubation of the βTC3 cells with increased free Ca^{2+}. These data provide evidence that MAP-2 is phosphorylated by CaM kinase II in the pancreatic β-cell *in situ*, and that this event may provide an important link in the mediation of Ca^{2+}-dependent insulin secretion. (Supported by NIH grant DK-47925).

Abstract #61

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORT

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Emma M. Ings

Department: Biochemistry and Molecular Biology

Graduate Student ✓ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

PHOSPHORYLATION OF SYNAPSIN I BY CAM KINASE II IN PERMEABILIZED PANCREATIC β-CELLS Emma M. Ings, Kim A. Krueger and Richard A. Easom Department of Biochemistry and Molecular Biology University of North Texas Health Science Center, Fort Worth, Texas, 76107-2699

We have previously demonstrated that glucose activates the multifunctional Ca^{2+}/calmodulin-dependent protein kinase (CaM Kinase II) in isolated rat pancreatic islets in a manner that correlates with insulin secretion. This observation implies a fundamental role of CaM Kinase II to regulate Ca^{2+}-sensitive insulin exocytosis but a full understanding of this potential relationship requires the identification of its intracellular substrates. In order to address this question the Ca^{2+}-induced phosphorylation of candidate proteins has been evaluated by selective immunoprecipitation from β-cells made permeable to Ca^{2+} and high specific activity [γ-^{32}P]ATP. Initially, Ca^{2+} was shown to stimulate the rapid (30 sec-2 min) phosphorylation of synapsin I (M_r~ 85KDa) in α-toxin-permeabilized βTC3 cells with a Ca^{2+} dependency similar to that required to activate CaM Kinase II (i.e. >500nM Ca^{2+}). Protease (endoproteinase Glu-C) digestion of synapsin I revealed that the larger proportion of phosphate incorporated in response to Ca^{2+} was at a site selective for CaM Kinase II. This data is consistent with the ability of CaM Kinase II to phosphorylate synapsin I in β cells. Immunofluoresence analysis identified the presence of synapsin I in βTC3 cells at plasma membrane regions. Immunoblot analysis however, demonstrated that synapsin I was not associated with insulin secretory granule membranes isolated from β–cell insulinoma tissue. Furthermore, islets were shown to express synapsin I to a much lesser extent than βTC3 cells. This study confirms synapsin I as a substrate for CaM Kinase II in transformed clonal pancreatic β-cells but its role in normal physiological insulin secretion is questioned by the lack of association with secretory granules and its low level expression in primary islets. (Supported by NIH grant DK-47925 to R.A.E. E.M.I. is a recipient of an International Work Placement Student Scholarship from the Graduate School of Biomedical Sciences.).

Abstract #62

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: PARAMJIT BHOGAL

Department: MICROBIOLOGY AND IMMUNOLOGY

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DETERMINATION OF CYCLIC ADP-RIBOSE LEVELS IN CULTURED HUMAN CELLS. P. BHOGAL, R. EASOM and R. ALVAREZ-GONZALEZ. Depts. of Biochem. and Mol. Biol. and Microbiol. and Immunol., University of North Texas Health Science Center at Fort Worth, TX 76107-2699.

Mobilization of intracellular Ca^{++} is a signaling mechanism that is of fundamental importance to cellular physiology. Cyclic adenosine diphosphoribose (cADPR) is a recently discovered metabolite of nicotinamide adenine dinucleotide (NAD) that appears to play a role in the Ca^{++} - induced Ca^{++} - release from intracellular stores. Previous research has allowed the determination of intracellular cADPR levels indirectly, through the quantification of calcium concentrations. However, cADPR in human cells has never been directly measured.

Cells were harvested using a 20% (w/v) trichloroacetic acid treatment. The cell extract was taken through dihydroxyboronyl-Bio-Rex for affinity chromatography purification of nucleotides containing two or more riboses. Purified material was then treated with phosphodiesterase to hydrolyze all phosphoanhydride bonds. However under these conditions, cADPR remains intact due to its ring structure. The enzyme degradation products were isolated using dihydroxyboronyl-Bio-Rex again. Next, we converted cADPR to ADPR, using NAD glycohydrolase isolated from *Bungarus fasciatus* and the products generated were isolated using a third step of affinity chromatography. Following derivitization of ADPR to ε-ADPR via chloroacetaldehyde at 60° C and boronate purification on a PBA - 60 column, HPLC - fluorescence detection allowed for the quantification of this compound. Prelimary results using this method have shown a positve peak corresponding to ε -AMP deriving from cADPR in pancreatic ß cells under 0.1mM glucose conditions. We propose to use this method to accurately quantify the amount of endogenous levels of cADPR in both normal and transformed human cells. (This research project was funded in part by the Texas Advanced Research Program.)

Abstract #63

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Gustavo Pacheco-Rodriguez, Ph.D.

Department: MICROBIOLOGY AND IMMUNOLOGY

Graduate Student___ Medical Student___ Postdoctoral Fellow ✓ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DISTRIBUTION OF POLY(ADP-RIBOSE) GLYCOHYDROLASE IN DIFFERENT SUBNUCLEAR FRACTIONS

G. Pacheco-Rodriguez, and R. Alvarez-Gonzalez.

Depts. of Biochem. & Mol. Biol. and Microbiol. and Immunol., University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699.

The poly(ADP--ribosyl)ation of DNA-binding proteins is a reversible post-translational pathway that is elicited in the cell nucleus by genotoxic agents that lead to the formation of breaks on DNA. Protein-bound ADP-ribose polymers are synthesized by poly(ADP-ribose) polymerase (PARP) [E.C. 2.4.2.30] and degraded by poly(ADP-ribose) glycohydrolase (PARG) *in vivo*. Here, we have determined the distribution of PARG activity in distinct functional domains of the cell nucleus. Nuclei were isolated from rat liver tissue and fractionated into separate chromatin, nuclear envelope, and nuclear matrix structures by: i) endogenous endonuclease digestion (to eliminate most of the chromosomal DNA); ii) low salt and high salt treatments (to extract histone and non-histone proteins); and iii) a Triton X-100 detergent extraction (to separate the nuclear envelope from the nuclear matrix fraction). The identity of these structures was confirmed by the biochemical analysis of their protein components following SDS-polyacrylamide gel electrophoresis (PAGE) and Coomassie blue staining. The total amount of PARG activity associated with each fraction was determined by high resolution PAGE, autoradiography and densitometric analysis of the [^{32}P]radiolabeled ADP-ribose formed utilizing protein-free ADP-ribose polymers of 2-70 residues in size as a substrate. Our assay was highly specific and sensitive to the femtomole level. Excellent reproducibility was observed with both, crude extracts of eucaryotic nuclei and purified PARG. Our results show that roughly equivalent amounts (35-45%) of the total PARG activity were associated with chromatin and the nuclear envelope. By contrast, a smaller portion of the enzyme (10-20%) was obtained with the nuclear matrix fraction. We also observed that pure histones and nuclear matrix proteins inhibited the activity of purified PARG. Therefore, this phenomenon has to be taken into consideration for the accurate measurement of PARG activity in both chromatin and the nuclear matrix.

This project was supported by grants GM45451 from NIH and 9678-014 from the Texas Advanced Research Program.

Abstract #64

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Jean Rawling, Ph.D.

Department: MICROBIOLOGY AND IMMUNOLOGY

Graduate Student___ Medical Student___ Postdoctoral Fellow ✓ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

POLY(ADP-RIBOSYL)TION OF TRANSCRIPTION FACTOR IIF (TFIIF)

J.M. Rawling and R. Alvarez-Gonzalez.

Department of Microbiology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699.

We have investigated the susceptibility of eukaryotic transcription factors as targets for covalent modification by poly(ADP-ribose) polymerase (PARP) [E.C. 2.4.2.30]. Human recombinant TFIIF (RAP30/74), TFIIB, and TBP of TFIID were incubated with calf thymus PARP and [^{32}P]-labeled NAD$^+$ at 37°C. Upon lithium dodecyl sulfate-PAGE and autoradiography, a band of radioactivity co-migrated with each TFIIF subunit. No radioactivity was associated with either TFIIB or TBP. TFIIF modification depended on the presence of nicked DNA, which is essential for PARP activity. Association of [^{32}P] with TFIIF increased in a time-dependent manner between 30 sec and 5 min. The degree of modification also increased with TFIIF concentration and with NAD$^+$ concentration. RAP74 was modified preferentially to RAP30 in both the time course and dose response studies. Using tris-borate/EDTA PAGE, we confirmed that the radioactive species associated with the TFIIF subunits were covalently-bound ADP-ribose polymers. Chemical stability studies indicated that carboxylate residues are the principal amino acid acceptors of poly(ADP-ribose) on both TFIIF subunits. We conclude that TFIIF is a specific substrate for covalent poly(ADP-ribosyl)ation in a pure enzyme system.

Funded by grants 009768-14 from the Texas Advanced Research program and GM45451 from the National Institutes of Health.

Abstract #65

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 199

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORT

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Anita I. Zvaigzne

Department: Department of Biochemistry and Molecular Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow XXX Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EFFECT OF ACTIVE SITE MODIFICATION ON THE TERMINAL MARKING DEAMIDATION OF TRIOSEPHOSPHATE ISOMERASE. Anita I. Zvaigzne; John M. Talent; Neil Agarwal and Robert W. Gracy; Department of Biochemistry and Molecular Biology; University of North Texas Health Science Center at Fort Worth; 3500 Camp Bowie Boulevard; Fort Worth, TX 76107

The conformational change which results from the opening and closing of the hinged lid over the catalytic center of triosephosphate isomerase is transmitted to the subunit interface of the dimer and eventually leads to the spontaneous, specific deamidation of Asn-71. This is followed by the deamidation of Asn-15 approximately 5Å away on the neighboring subunit and leads to destabilization of the protein and degradation. However, it has not been established whether this molecular wear and tear occurs via an intra-subunit or inter-subunit transmission of the conformational change. We have studied the first step in the terminal marking by reacting the active site Glu-165 with the substrate analogue, 3-chloroacetol phosphate (CAP) which immobilizes the hinged lid. Under mild deamidation conditions the native homodimer readily deamidated. In contrast, the CAP-modified homodimers were resistant to deamidation. Heterodimers composed of one native subunit and one CAP-subunit showed intermediate deamidation. When the native and CAP-labeled subunits were resolved by electrophoresis in urea gels, it was found that the unlabeled subunit had preferentially deamidated. These data coupled with molecular modeling considerations show that restricting movement of the hinged lid prevents deamidation of Asn-71 on that subunit, but that the other subunit with the free hinged lid functions independently and still deamidates. Thus, the conformationally induced wear and tear appears to be largely intra-subunit. These observations clearly link catalysis with terminal marking of the protein and suggests a general mechanism for how other enzymes may wear out.
(This work was supported by a National Institute of Aging MERIT Award (AG01274) to R.W. Gracy and by the Robert A. Welch Foundation (B0502). A.I. Zvaigzne is supported by a Welch Postdoctoral Fellowship.)

Abstract #66

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Kent S. Boles

Department: Microbiology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CLONING AND CHARACTERIZATION OF THE HUMAN HOMOLOGUE OF A MURINE NATURAL KILLER CELL RECEPTOR

Kent S. Boles and Porunelloor A. Mathew, Department of Microbiology and Immunology, UNT Health Science Center, Fort Worth, TX 76107

Natural killer (NK) cells play an important role in defense against tumor cells and viral infections. 2B4 is a novel signaling molecule expressed on NK and some T cells and functions in target cell killing. 2B4 is a 66 kDa integral membrane protein with one transmembrane region in its monomeric structure. It has been classified as a member of the immunoglobulin gene superfamily. Anti-2B4 monoclonal antibodies directed against cultured NK cells and non-MHC restricted T cells greatly enhances their destruction of tumor cells. Therefore, further characterization of the 2B4 molecule and identification of the human homologue could be major contributions to medicine. In pursuit of the latter goal, a human genomic library in phage λ was screened with the full length, murine, 2B4 cDNA. Six plaques displayed positive signals initially. Further screening will demonstrate if these clones are truly positive. Isolated clones will be characterized by sequencing. cDNA clones would also be made from human NK cell mRNA. Future studies should include the function of the 2B4 molecule expressed in human NK cell lines.

Abstract #67

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Yukfung Lee

Department: Microbiology and Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow_x_ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

TRANSCRIPTIONAL REGULATION OF EXPRESSION OF A MURINE NATURAL KILLER CELL RECEPTOR:

Yukfung Lee, Susan Stepp and Porunelloor Mathew, Department of Microbiology and Immunology, UNT Health Science Center, Fort Worth, TX 76107

Natural killer (NK) cells are large granular lymphocytes that mediate the killing of various tumor cells and virally infected cells. The molecular basis of NK cell recognition and activation by target cells is poorly understood. We have previously identified, cloned and characterized a receptor,2B4, expressed on murine NK cells. It is found that 2B4 is not only expressed on all NK cells, but also expressed on macrophages and those T cells which display NK-like killing. Comparison of 2B4 with sequences in the GenBank database showed that 2B4 is a novel member of immunoglobulin supergene family. In order to further understand the transcriptional regulation of the 2B4 gene, we obtained a genomic 2B4 clone in the P1 system including the sequence of the 2B4 gene promoter region and determined the sequence 5' to the first exon by cycle sequencing. The promoter region was PCR amplified and subcloned into pCR2.1 vector. Using nuclear protein extract from a T cell line, CTLL-2, the interaction of the promoter with nuclear proteins was studied by electrophoretic mobility shift assay (EMSA).The result showed that a 100bp 5' fragment (-89 to +11) of the promoter region interacted with nuclear protein(s) expressed only in CTLL-2 cells, which express 2B4, whereas, such specific binding was not seen in Jurkat, Peer (both T cell line) or Sp2/o (B cell line) cells which do not express 2B4. Competition EMSA showed that the binding was sequence specific. Experiments are being done to determine the exact DNA binding region within this 100 bp promoter region and to clone the nuclear factors.

Abstract #68

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Pisate John Kamthong

Department: Biochemistry & Molecular Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EFFECT OF CYCLIC ADENOSINE MONOPHOSPHATE ON INDUCED MACROPHAGE COLONY-STIMULATING FACTOR TRANSCRIPTION P. John Kamthong, Fu-Mei Wu, Ming-chi Wu Department of Biochemistry & Molecular Biology, UNT-HSC, Fort Worth, Texas 76107

Monocyte/macrophage colony-stimulating factor (M-CSF), a glycoprotein hematopoietic growth factor, is required for growth, differentiation, and functioning of monocytes and macrophages. TPA and Interleukin-1 (IL-1), had been shown previously in our lab to induce M-CSF production in human pancreatic cancer cell line -- MIA-PaCa2. And cyclic adenosine monophosphate (cAMP) decreased such induced M-CSF production by unknown mechanism. Several published data identified transcriptional factors, which are activated and bind to the cis-acting sites in 5'-flanking region and induce expression of M-CSF gene, such as NF-kB, AP-1, SP-1 etc. This triggered our interest to investigate the roles of those transcriptional factors in the expression of human M-CSF. We found by gel mobility shift assay that treatment of MIA-PaCa2 cells with TPA and IL-1 activated NF-kB binding to the cis-acting consensus sequences. TPA and IL-1 also increased AP-1 binding to its consensus sequence. While cAMP treatment decreased both NF-kB and AP-1 binding induced by either IL-1 or TPA. We speculated that cAMP exerts its inhibitory effect on M-CSF production at the transcriptional level. This exhibition attempt to answer the question at what level the cAMP involvement is. The demonstration of the effects of cAMP on interaction between transcriptional factors (NF-kB & AP-1) and consensus cis-acting sequence in 5'-flanking region of M-csf gene from IL-1 or TPA induced MIA-PaCa2 cells, as well as quantitation of M-CSF mRNA by northern blot analysis in respective conditions would implicate that cAMP inhibitory effect is at the transcriptional level.

Abstract #69

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: ADNAN DIBAS

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow_X_ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

THE ATP-DEPLETING REAGENT "IODACETAMIDE" INDUCES THE DEGRADATION OF PROTEIN KINASE C ALPHA (PKCα) IN LLC-PK$_1$ PIG KIDNEY CELLS.

Adnan Dibas[1], Julie Wood[1], Abdul J. Mia[2] and Thomas Yorio[1].
[1]Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX. 76107 and [2]Division of Science and Mathematics, Jarvis Christian College, Hawkins, TX. 75765.

The alkylating reagent iodoacetamide, a potent inhibitor of sulfhydryl proteases, was found to stimulate the selective degradation of protein kinase C alpha (PKCα) isoform. Treatment of LLC-PK$_1$ cells with iodoacetamide (0.5-15 mM) for 30-90 minutes at room temperature followed by western blotting on total cell homogenate, revealed the absence of an 80 KDa protein and the appearance of an 50 KDa band that was still recognized with the antibody. Serine protease inhibitor, metalloprotease inhibitors and leupeptin failed to prevent the degradation of PKCα. The degradation persisted at 4 ^{o}C and in the absence of Ca^{2+}. Iodoacetamide had no direct effect on purified PKCα. In conclusion, the degradation of PKCα is a novel phenomenon. The degradation process could not be inhibited by known protease inhibitors or in the absence of Ca^{2+} or at 4 ^{o}C and appears to involve interactions with an unknown intermediates.

This research is supported in part by a grant from the U.S. Department of the Army # DAMD17-95-C-5086

Abstract #70

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: **Sandra Rodriguez**

Department: **Anatomy and Cell Biology**

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

GOLGI COMPLEX REASSEMBLY AFTER BREFELDIN A TREATMENT IN MDCK CELLS TRANSFECTED WITH THE GENE FOR HUMAN GROWTH HORMONE. **S. Rodriguez and V.L. Rudick, Dept. of Anatomy & Cell Biology, UNT Health Science Ctr., Ft. Worth, TX 76107.**

The unique architecture of the Golgi apparatus allows it to perform a myriad of functions related to cell secretion, but while much is known about Golgi structure and function, questions remain concerning the assembly and maintenance of its stacked cisternal morphology. To this end several laboratories have investigated events involving Golgi fragmentation such as occurs during mitosis or microtubule depolymerization, but little work has been done to explain Golgi reassembly. Previously in our laboratory Madin Darby canine kidney (MDCK) cells were transfected with pXGH5 containing the human growth hormone (hGH) gene to obtain permanently expressing clones. It was shown that one of these contained twice as many Golgi stacks as untransfected cells and the assembly of the stacks could be modulated by altering growth conditions. Thus, this system provided an ideal means of examining Golgi assembly by treating the hGH-secreting clones with the pharmacological agent brefeldin A (BFA), known for its ability to disrupt the Golgi, and then following hGH in the absence of the drug as the Golgi reforms. Our hypothesis was that hGH secretion would be perturbed by BFA and would necessitate new protein synthesis to allow Golgi reassembly before secretion proceeds. In this preliminary presentation the aims were three fold. First, we correlated the number of Golgi stacks in three clones (designated 3C, 4F, and 3A) with the amount of hGH secreted using two methods. Immunofluorescent labeling with anti-hGH antibody revealed perinuclear staining with 3C being the brightest followed by 4F and 3A, respectively. These data were corroborated by a radioimmune assay that measured the amounts of hGH secreted. Initial rates of secretion (ng secreted/hour/ 10^4 cells) were 760 (3C), 290 (4F), and 125 (3A). Second, in order to follow Golgi during disruption and reassembly, we used immunocytochemistry to assess the feasability of employing various Golgi and endoplasmic reticulum (ER) markers to track these organelles. The TRITC-conjugated lectins concanavalin A (Con A; specific for ER, cis and medial Golgi) and wheat germ agglutinin (WGA; specific for trans cisternae and trans Golgi network) were then used along with anti-hGH to localize the hormone within the various compartments. Third, following BFA treatment, Golgi reassembly and hGH activity were monitored using both immunocytochemistry and radioimmunoassay. These data will be used to investigate protein inhibition on Golgi reformation and hGH secretion.

Abstract #71

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Bangdong Wei

Department: Microbiology and Immunology

Graduate Student X Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

THE GLOBAL REGULATOR CsrA CONTROLS BOTH CARBON METABOLISM AND MOTILITY IN *Escherichia coli.* B. Wei, *A.-M. Brun-Zinkernagel and T. Romeo. Department of Microbiology and Immunology, *Department of Anatomy and Cell Biology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107-2699

The carbon storage regulator gene, *csrA*, has been previously shown to direct central carbon flux in *E. coli.* It positively regulates glycolysis, while it negatively controls gluconeogenesis and the glycogen biosynthetic and catabolic pathways. This study was designed to explore the effects of *csrA* on the Krebs cycle, the glyoxylate shunt, which is required for growth on acetate as a sole carbon source, and cell motility. A *csrA::kanR* mutation caused *E. coli* to exhibit a variable lag in growth when acetate was the major carbon source. The specific activities of two Krebs cycle enzymes, isocitrate dehydrogenase and citrate synthase, and two unique glyoxylate shunt enzymes, isocitrate lyase and malate synthase, in MOPS media supplemented with acetate plus succinate, or glucose, and in Kornberg media with glucose, were examined in wild type and *csrA::kan* strains. Although the levels of the Krebs cycle enzymes were not affected, the two glyoxylate shunt enzymes were significantly lower in this *csrA* mutant, suggesting that CsrA positively controls the expression of the *aceBAK* operon. The study of an *aceB::lacZ* operon fusion indicated that CsrA does not regulate the expression of this operon at the transcriptional level. Comparison of these *csrA* wild type and mutant strains on semisolid tryptone agar plates showed that *csrA* is required for cell motility. Negative staining electron microscopy revealed that the *csrA::kan* cells could not form flagella. Motility is an important property of bacteria which allows them to move toward an attractant, such as a nutrient, or away from a repellent. Based on our study that CsrA regulates the glyoxylate operon and flagellar formation, it appears that CsrA plays a significant role in the adaptation of bacteria to unfavorable nutritional conditions.

Abstract #72

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: CHANG, WOO-JIN

Department: MICROBIOLOGY & IMMUNOLOGY

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

A Study to Elucidate the Biological Role of Glycogen in *Escherichia coli*. **Woo-Jin Chang and Tony Romeo. Department of Microbiology & Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107**

Glycogen is synthesized by numerous species of bacteria, including *Escherichia coli*. Studies of the biochemistry, enzymology and genetic regulation of this polysaccharide macromolecule have been intensively pursued over the past 40 years, and a wealth of knowledge has consequently accumulated. However, the ultimate scientific question, "Why do bacteria make glycogen?", still remains unanswered. To elucidate the biological role of bacterial glycogen, a collection of glycogen-deficient mutants were obtained by random insertional mutagenesis using a *Tn10kan* transposon. Strains were characterized by P1*vir* transduction, complementation, and Southern hybridization analysis to determine whether the *kanR* insertions had occurred within the *glg* operon, which contains structural genes for glycogen biosynthesis. Resulting glycogen-deficient mutants, along with their isogenic parent strain, were exposed to stress conditions such as long-term starvation, heat-shock and hyperosmolarity. Preliminary data indicate that glycogen-deficient mutants are more sensitive to heat-shock than the wild-type strain but showed little change in sensitivity to hyperosmolarity. In addition, heat shock sensitivity varied with the growth phase, with stationary phase cells exhibiting higher resistance than exponentially growing cells. We hypothesize that intracellular glycogen is synthesized to confer resistance to heat shock, and possibly to other physiological stresses.

Abstract #73

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Mark E. Hart, Ph.D.

Department: Department of Microbiology & Immunology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

EXPRESSION OF ALPHA TOXIN (*HLA*) IN AN *AGR* DELETION MUTANT OF *STAPHYLOCOCCUS AUREUS*. Mark E. Hart, and Wei Si, Department of Microbiology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107-2699.

The gram-positive bacterium, *Staphylococcus aureus* causes a number of diseases in humans ranging from endocarditis and osteomyelitis to toxemias such as toxic shock syndrome and toxic food poisoning. The bacterium's disease diversity has been attributed to its ability to produce greater than thirty extracellular and cell wall-associated proteins most of which have been shown to be involved with some aspect of disease. Some of these, most notably alpha-toxin, staphylococcal enterotoxin B, and toxic shock syndrome toxin, are coordinatedly expressed in a temporal fashion. They accumulate, in vitro, during the postexponential phase of growth, while cell wall-associated proteins such as coagulase and protein A are preferentially made during exponential growth. This coordinate expression of exoproteins is regulate by several distinct chromosomal loci, and of these, the accessory gene regulator (*agr*) is by far the best characterized. Recent studies with *S. aureus* suggest that the *agr* regulatory system is central for the expression of exoproteins. To further examine the role of *agr* in exoprotein production, we determined the levels of alpha-toxin (*hla*) message in an *agr* deletion mutant. Spent media from *S. aureus* RN6911 (*Δagr::tetA[M]*) grown in the presence or absence of tetracycline to late exponential phase was added to early exponential phase cultures of *S. aureus* RN6911. RNA isolated from samples taken at 0, 30, and 60 minutes after treatment with spent media was probed for *hla* mRNA by Northern analysis. Alpha-toxin message levels were substantially increased in cultures receiving spent media from RN6911 grown in the presence of tetracycline when compared to cultures receiving spent media from RN6911 grown in the absence of the antibiotic. These data show that expression of *hla* can be modulated independently of *agr*. We are currently exploring the possibility that a secreted protein factor(s) represses *hla* expression in post-exponential growth. (NIH grant AI36934 and Faculty Research Grant awarded to M.E.H)

Abstract #74

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

POSTER PRESENTATION

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Lisa M. Hodge

Department: Department of Microbiology & Immunology

Graduate Student ✓ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

Cloning of Hemagglutinin and Nucleoprotein Genes from Influenza Virus Using Reverse Transcriptase-Polymerase Chain Reaction

Lisa M. Hodge, Haifa Al-Khatib, and Jerry W. Simecka

Department of Microbiology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, 76107

Influenza infection has a major health and economic impact on the United States, causing an estimated 10,000-40,000 deaths annually. However, the current vaccine is not 100% effective; therefore, it is necessary to continue to develop improved vaccines. Construction of a recombinant vaccine using molecular biology techniques involving adenovirus and *Salmonella* vectors, chimeric proteins or naked DNA would provide a solution. Because influenza has highly variable proteins, such as hemagglutinin (HA) and nucleoprotein (NP), between different strains, it is necessary to devise a rapid technique to clone these genes for vaccine development. In our current studies, we propose an approach using the reverse transcriptase polymerase chain reaction (RT-PCR) for cloning of these genes. RT-PCR was chosen because it is relatively easy and sensitive, allowing amplification from small quantities of viral RNA. From isolated A/PR/8/34 viral RNA, cDNA was generated by reverse transcription of the viral template (RT). Using the polymerase chain reaction (PCR), we were able to amplify the cDNA. Primers specific for HA or NP were designed with NcoI and XhoI restriction endonuclease sites for easy directional cloning into various vectors. These primers produce PCR products with the expected sizes for the HA (890 base pairs) and NP (1518 base pairs) genes. We are currently cloning the PCR products for partial sequencing to confirm the identity of PCR generated products. Restriction digestion analysis of the putative NP PCR products, generated using NP primers, produced fragments consistent with the sequence of NP. Our goal is to use our technique to generate PCR products and clone vaccine antigens from a variety of influenza virus strains for universal use. This may prove to be a valuable tool for the development of new recombinant vaccines. This work is support by a UNTHSC Faculty Research Grant to J.W.S.

Abstract #75

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Harold W. Keller

Department: Office of Research and Biotechnology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff X

Read instructions and fit abstract inside the space given below:

THE DEVIL'S CIGAR. Harold W. Keller,[1] K. C. Rudy,[2] James Murray,[2] University of North Texas Health Science Center, Fort Worth, TX 76107-2699[1] and Botanical Research Institute of Texas, Fort Worth, Texas 76102-4060 and Living Science Center, 703 NW Green Oaks Blvd, Arlington, TX 76015.[2]

The Devil's Cigar is an unusual and rare ascomycete fungus that was first described as new after being discovered in the Austin, Texas area by Charles Peck in 1893. It was first given the name *Urnula geaster* and later recognized as the sole species of a separate genus, *Chorioactis geaster* (Pk) Eckblad. Since then it has been collected from several localities in Texas and two sites in Japan. This operculate, cup fungus belongs in the family Sarcosomataceae and order Pezizales but has earned the common name "Devil's Cigar" for two good reasons: first, the three-inch apothecium is a dark brown, velvety, tough, spindle-shaped tube that somewhat resembles a short cigar; second, as it matures, it splits open from its apex to form a sizable earth star that audibly hisses releasing a smokelike cloud of spores. In 1991 it was discovered growing in River Legacy Parks, Arlington (Tarrant County), Texas. In the park it grows exclusively in association with decaying cedar elm stumps and is now known from over 60 different sites, making this area the "devil's ashtry", mycologically speaking. Its growth habit, development, spore release mechanism, seasonality patterns and geographical distribution are being recorded by park staff, visitors and volunteers. It has only been found in the fall, first appearing about September 1st and thereafter can be found in the park up until December. It has recently been found in Crystal Canyon in Arlington as well as near Joe Pool Lake and Possum Kingdom Lake. In the latter case, it was growing in association with a red cedar tree. This bizarre, cigarlike, exploding fungus has been adopted as a totem for River Legacy Parks and the new Living Science Center. It has been featured in *The Mycologist* and *The Mycophile,* used as a fund raiser for the new Living Science building through the sale of illustrated t-shirts bearing colored, fungal fruiting bodies, and has created public awareness and interest in the ecology of the park. The t-shirts are used as part of an incentive program for volunteers: white t-shirts are sold to the general public, green t-shirts are given to volunteers with 50 hours of service and navy blue t-shirts for 200 hours of service. The photographs presented here were taken by James Murray from Arlington, Texas. There is a proposal in the Texas state legislature to make this fungus the official state fungus for Texas.

Abstract #76

NEUROSCIENCE AND BEHAVIOR

77	Harbans Lal, Ph.D.	SUBSTANCE ABUSE INSTITUTE OF NORTH TEXAS
78	Yaprak Egilmez Harrison	EFFECT OF BUSPIRONE ON ETHANOL SELF-ADMINISTRATION DURING ACUTE ETHANOL WITHDRAWAL
79	Marianna Jung*	SEX DIFFERENCES IN PTZ- AND MCPP-LIKE STIMULI PRODUCED BY ETHANOL WITHDRAWAL
80	Meghan Selvig	ENVIRONMENTAL STRESS: IMPACT ON THE STUDY OF ANXIETY
81	Cathy L. Bell Horner	THE α SUBUNIT OF RECOMBINANT $GABA_A$ RECEPTORS INFLUENCES INTERACTION OF PRICROTOXIN-SITE LIGANDS
82	Ren-Qi Huang, Ph.D.	CHARACTERIZATION OF RECOMBINANT $GABA_A$ RECEPTOR RUNDOWN AND THE POTENTIAL MECHANISM(S)

*Student Presenter

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Harbans Lal

Department: Pharmacocology and SAINT

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

SUBSTANCE ABUSE INSTITUTE OF NORTH TEXAS (SAINT)

Harbans Lal, Executive Director
University of North Texas Health Science Center, Fort Worth, Texas

The Substance Abuse Institute of North Texas (SAINT) was established in 1993 by the Department of Pharmacology and the Department of Psychiatry and Human Behavior. This consortium of professionals includes researchers active in the study of the physiological basis of substance addiction and is actively involved in research to develop new drug therapies which will aid in the withdrawal and abstinence from substances of abuse. Several research grants from NIAAA are focused on treatment of alcohol withdrawal, while two large contracts from NIDA concentrate on developing an antagonist to block the reinforcing effects of cocaine. Other current projects include investigations on the contribution of genetic factors on the consumption of cocaine and use of gene-modified animals to determine the underlying neurochemical processes involved in cocaine self-administration.

Educational Activities of members of the institute include programs to study the effectiveness of substance abuse treatment policies in our institution, graduate and post-graduate training of research professionals, and strong interactions between members to develop and extend research programs. The institute hosts research conferences and cosponsors seminars with area groups. International speakers and visiting scientists are attracted to the University of North Texas Health Science Center campus to interact and perform research with members of the institute.

Abstract #77

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Yaprak Egilmez Harrison

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow_X_ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

EFFECT OF BUSPIRONE ON ETHANOL SELF-ADMINISTRATION DURING ACUTE ETHANOL WITHDRAWAL Y. Egilmez Harrison, C.J. Wallis, J. Grewal, H. Lal. Department of Pharmacology and SAINT, University of North Texas Health Science Center, Fort Worth, TX 76107.

Buspirone, a nonbenzodiazepine anxiolytic drug, exerts its actions primarily through 5-HT1A receptors. Given the pharmacological evidence that 5-HT1A receptors are involved in the control of ethanol preference and voluntary intake in rats, the present study examined the effect of chronic buspirone on ethanol self-administration during acute ethanol withdrawal. Male Long Evans rats were trained to self-administer ethanol (10% w/v) in a sweetened-solution fading procedure under an FR2 schedule of reinforcement. Once a stable intake of EtOH was established rats were given a nutritionally balanced liquid diet with or without (control) ethanol (4.5%) for 10 days. During the last 3 days of this chronic regimen buspirone (10 mg/kg/day) or saline (% 0.9) was administered i.p. Twelve hours after removal of the ethanol/control diet, rats were tested for ethanol intake in the same self-administration paradigm. Chronic treatment with buspirone significantly decreased ethanol intake in rats going through withdrawal. There was no significant effect of buspirone on ethanol intake of rats from the control group. Similarly, the same regimen of chronic buspirone had no significant effect on saccharin intake in a separate group of animals. These results suggest that chronic administration of buspirone may be useful in suppressing ethanol withdrawal symptoms and thus decrease relapse of ethanol intake. (Supported by NIAAA #AA09567).

Abstract #78

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Research Appreciation Day 1997

ABSTRACT FORM

First Author: Marianna Jung

Department: Pharmacology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

SEX DIFFERENCES IN PTZ- AND MCPP-LIKE STIMULI PRODUCED BY ETHANOL WITHDRAWAL. M.E. Jung, H. Lal, and C.J. Wallis. Department of Pharmacology and SAINT, University of North Texas Health Science Center, Fort Worth, TX 76107.

This experiment determined if male, female and ovariectomized (OVX) female rats differ in the type or intensity of anxiogenic stimuli produced by ethanol withdrawal. Intact male rats, OVX, and sham female rats were trained on a two-lever choice task with either: 1) the GABA-A indirect antagonist, pentylenetetrazole (PTZ, 16 mg/kg, IP) versus saline; or 2) a 5-HT1b/2a/2c partial agonist, m-chlorphenylpiperazine (m-CPP, 1.2 mg/kg, IP) versus saline. The ED50 for PTZ or m-CPP was measured using a cumulative dosing regime. Prior to treatment with ethanol, the ED50 for either PTZ or m-CPP did not differ for the three groups (sex, surgery). During acute ethanol withdrawal (AEW, 12 h, 6.5%, 10 d), significantly fewer sham-operated females selected the drug lever after a saline injection as compared to male or OVX animals. The ED50 for all three groups was reduced during protracted ethanol withdrawal (PEW, 36 h) as compared to pre-ethanol treatment values (2-3 fold reduction). The ED50 of m-CPP for sham female rats during PEW was not significantly different than those of OVX or male rats, but did show a trend toward a higher ED50. Thus, this study demonstrates that while fewer intact female rats exhibited a spontaneous EW-induced PTZ- and m-CPP-like anxiogenic stimulus than OVX or male rats, all of the animals showed increased sensitivity to the anxiogenic stimuli produced by PTZ and m-CPP durng PEW. Thus, ovarian factors appear to reduce the spontaneous development of a PTZ- or m-CPP-like stimulus during AEW despite an overall increase in sensitivity of these systems. (Supported by NIAAA #AA06890 and #AA09567)

Abstract #79

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Meghan Selvig

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff XX

Read instructions and fit abstract inside the space given below:

ENVIRONMENTAL STRESS: IMPACT ON THE STUDY OF ANXIETY. Selvig M., Lal H., and Wallis CJ. Department of Pharmacology and SAINT, University of North Texas Health Science Center

Two operant models of anxiety are currently in use in our laboratory. These models require male Long Evan Hooded rats to detect an anxiety-like stimulus produced by injection of the anxiogenic drug penteylenetetrazol (PTZ) or 1-(3-Chloro-phenyl) piperzine HCL (mCPP) and discriminate these stimuli from that produced by saline. A correct choice is expressed as ten presses on the appropriate lever (drug vs saline; fixed ratio 10 ; fr10), and the behavior is reinforced by delivering a 45 mg food pellet to the rat for each correct choice. Data are expressed as the percent of animals in a test squad that select the drug lever (% Drug lever selection). If 80% or more of the animals press the drug lever, a treatment is considered to substitute for the anxiogenic drug. Under normal test conditions less than 20% of animals injected with saline press the drug lever.

During the past several months the trained animals were exposed to unpredictable noise, vibration, and smells resulting from heavy construction in the animal care facility.

During the pre-construction period, less than 20% of 180 animals (mCPP or PTZ trained) responded on the drug lever after a saline injection. During the construction period 80 % to 90% of the same animals were responding on the drug lever after a saline injection. These data indicate that the stressful conditions produced by construction substituted of either drug. Once construction ended, the percent of animals responding on the drug lever after a saline injection slowly declined, finally returning to the 20% baseline. These data support the hypothesis that while the primary actions of these drugs differ (PTZ is a GABA antagonist and mCPP is a serotonin agonist), their discriminative stimuli depend on a common factor found in stress. Supported by NIAAA grants AA09567 and AA10545

Abstract #80

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Cathy L. Bell Horner

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

THE α SUBUNIT OF RECOMBINANT $GABA_A$ RECEPTORS INFLUENCES INTERACTION OF PICROTOXIN-SITE LIGANDS. Cathy L. Bell-Horner, Ren-Qi Huang and Glenn H. Dillon. Dept. of Pharmacology, University of North Texas Health Science Center at Fort Worth, TX 76107

We have defined previously the interactions of picrotoxin-site ligands with the α1β2γ2 configuration of recombinant $GABA_A$ receptors, and the influence of GABA on these interactions. The current experiments tested the hypothesis that the α subunit influences the interactions of picrotoxin (PX) and U-93631 [4-dimethyl-3-t-butylcarboxyl-4,5-dihydro(1,5-a) quinoxaline], a novel ligand to the picrotoxin site, with recombinant $GABA_A$ receptors. Whole-cell patch clamp recordings were obtained from HEK293 cells stably expressing α6β2γ2 or α3β2γ2 receptors. Roughly one-half EC_{50} concentrations of GABA were applied to α6β2γ2 or α3β2γ2 configurations in the presence of varying [PX] or [U-93631]. PX and U-93631 inhibited GABA-activated Cl^- current (reduction in initial peak current and enhanced rate of current decay) in the α6β2γ2 configuration in a concentration-dependent manner. The IC_{50} for U-93631 shifted approximately 10-fold (from 42 to 3.5 μM) at the end of a 10 s application of GABA to α6β2γ2 receptors, indicating enhancement of U-93631 effect with GABA-bound receptors. Inhibition of Cl^- current by PX was similarly enhanced by GABA in the α6β2γ2 receptor. GABA enhanced the drug effect by increasing the association rate (k_{+1}) for PX in the GABA-bound receptor. These results are comparable to those obtained in α1β2γ2 receptors. In α3β2γ2 receptors, PX and U-93631 also inhibited GABA-activated Cl^- current in a concentration-dependent manner. However, the shifts in IC_{50} values due to the presence of GABA were significantly smaller (2-3 fold) in α3β2γ2 receptors, for both U-93631 and PX. Subsequent experiments demonstrated that the distinct effect of PX and U-93631 on α3β2γ2 receptors is at largely due to slower channel activation kinetics in this receptor. Our results indicate the α subunit of recombinant $GABA_A$ receptors influences the interaction of picrotoxin and the novel picrotoxin-site ligand U-93631 with these receptors. The distinct effect of U-93631 and PX in α3-containing receptors appears not to be due to structural differences in the picrotoxin-site of the α3 subunit, but instead is due to slower channel gating kinetics in the α3β2γ2 configuration of the $GABA_A$ receptor. (Support: NIH R29 ES07904 and TX Advanced Research Program # 009768-027).

Abstract #81

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Ren-Qi Huang, Ph.D.

Department: Pharmacology

Graduate Student___ Medical Student___ Postdoctoral Fellow X Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

CHARACTERIZATION OF RECOMBINANT $GABA_A$ RECEPTOR RUNDOWN AND THE POTENTIAL MECHANISM(S) Ren-Qi Huang and Glenn H. Dillon. Department of Pharmacology, University of North Texas, Health Science Center at Forth Worth, TX 76107

Progressive time-dependent decline in ion channel function (rundown) has been reported in several systems, and may be due to disruption of the intracellular environment. Rundown of $GABA_A$ receptor-mediated Cl^- current, as well as the potential underlying mechanism(s), has not been well-characterized. We used the whole-cell patch clamp technique to determine: 1) whether rundown occurs in stably expressed (HEK293 cells) rat $\alpha3\beta2\gamma2$ $GABA_A$ receptors; and 2) if rundown does occur in this receptor, what factors contribute to it. When resting $[Ca^{2+}]_i$ was buffered to relative high level by 4 mM EGTA, response to low [GABA] (20 μM) did not show rundown, with or without ATP in the intracellular solution, in the whole-cell configuration. However, with or without the presence of ATP, high [GABA] (2 mM) induced significant rundown, which was observed by decreases in both the maximum GABA-induced current and GABA EC_{50} (from 129 to 38 μM). Moreover, repetitive application of high [GABA] demonstrated that receptor desensitization does not account for the rundown. Subsequently, we conducted perforated patch recordings using the pore-forming antibiotic amphotericin B (240 μg/ml), to prevent intracellular washout. The absence of rundown in perforated patch recordings indicates the intracellular substrate(s) required to maintain the function of $GABA_A$ receptors is dialyzed by the whole-cell pipette. The rundown was completely prevented by 4 mM Mg^{2+}-ATP and low resting $[Ca^{2+}]_i$ buffered with the internal EGTA (10 mM). Rundown of GABA response was induced by high $[Ca^{2+}]_i$ or ATP-free internal medium. Intracellular introduction of W-7 (100 μM), a inhibitor of Ca^{2+}/calmodulin, retarded the rate of rundown of GABA responses. However, cypermethrin, a potent inhibitor of Ca2+/calmodulin-dependent phosphatase, had no apparant effect on the rundown. Our results demonstrate that the rundown of $GABA_A$ receptor function is concentration-dependent, due to dialysis of an intracellular substrate(s). Furthermore, both ATP and low $[Ca^{2+}]_i$ are necessary to stabilize the function of the $GABA_A$ receptor. A Ca^{2+}/calmodulin-dependent protein phosphatase may be involved in modulation of rat $\alpha3\beta2\gamma2$ $GABA_A$ receptors.
(Support by NIH R29 ES07904 and TX ARP# 009768-027)

Abstract #82

WOUND HEALING AND MICROGRAVITY

83	S. D. Dimitrijevich, Ph.D.	WOUND HEALING RESEARCH INSTITUTE
84	Tamara J. Reese	THE EFFECT OF MICROGRAVITY ON HUMAN TISSUE
85	Joshua Slater	DEVELOPMENT OF THE ZEBRAFISH VESTIBULAR SYSTEM DEPENDS ON NORMAL GRAVITY
86	Kristi D. Henderson*	STUDIES OF OCULAR WOUND HEALING IN SIMULATED ZERO GRAVITY USING THE CORNEAL IMPLANT MODEL
87	Jill Moore*	DEVELOPMENT OF A DERMAL EQUIVALENT MODEL TO INVESTIGATE FACTORS THAT REGULATE THE DIFFERENTIATION, FUNCTIONAL ACTIVITIES, AND DISAPPEARANCE OF MYOFIBROBLASTS
88	S. D. Dimitrijevich, Ph.D.	THE ROLE OF KERATOCYTES IN CORNEAL WOUND HEALING

*Student Presenter

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

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ABSTRACT FORM

First Author: Wound Healing Research Institute

Department: _____

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

A key role of the Wound Healing Research Institute, established in 1992, is to translate research results into viable treatments that minimize the pain and suffering caused by debilitating consequences of problem wounds. Its five-fold mission includes: expanding knowledge of the process of injury and wound healing using novel *in vitro* models and molecular biology techniques; application and innovative approaches such as the use of hyperbaric medicine, growth factors, tissue replacement therapies to problem wounds to prevent amputation and permanent disability; 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. Funding from federal, state and private agencies and organizations supports various projects conducted within the institute. Faculty from basic science departments and the departments of general and family practice, internal medicine, pathology, surgery and hyperbaric medicine make up the research staff of the institute.

Abstract #83

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Tamara J. Reese

Department: Biochemistry & Molecular Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff X

Read instructions and fit abstract inside the space given below:

THE EFFECT OF MICROGRAVITY ON HUMAN TISSUE

T.J. Reese*, J.G. Mills*†, and S.D. Dimitrijevich*†,
***Department of Biochemistry and Molecular Biology and**
†Surgery; †Wound Healing Research Institute; UNTHSC, Ft.Worth, TX 76107.

Rotating Wall Vessel cell culture systems (RWVs) have been developed by NASA to simulate microgravitational (μg) environment on Earth. This environment greatly reduces the shear stress exerted on the cells and improves mass transfer, thus optimizing nutrient supply and waste removal from the cells' immediate vicinity. RWVs have been shown to greatly improve the proliferation of difficult to grow cells. Normal and neoplastic cells are also induced to form aggregates ("organoids").

We have developed several *in vitro* models of human tissue such as the skin and cornea based on the non-contracted connective tissue matrix composed of type I collagen and appropriate fibroblasts. In the present study we are interested in the effect of microgravity on skin genesis and implications in different phases of tissue repair.

Preliminary studies have resulted in three patented RWV modifications, which specifically fit the needs of our experimental protocols. In new RWVs, assembly of the dermal equivalent (DE) proceeds without contraction and cell proliferation. Normal human fibroblasts randomly populate DE and appear to synthesize collagen and GAGs. Normal human keratinocytes attach, proliferate and differentiate on the DE under simulated μg expressing skin specific cytokeratins. While fibroblasts do not express αSMA, both FGF1 and 2 are detected in the DE and epidermal part of SE. DE and a new model of dermal wound healing are being considered for flight experiments.

We are grateful for the support by NASA Biotechnology/Microgravity Program.

Abstract #84

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Joshua Slater

Department: Anatomy and Cell Biology

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___ Other X

Read instructions and fit abstract inside the space given below:

DEVELOPMENT OF THE ZEBRAFISH VESTIBULAR SYSTEM DEPENDS ON NORMAL GRAVITY. J. Slater[1], R. Cordova[1], C. Burress[1], and S.J. Moorman. Department of Anatomy and Cell Biology, UNT Health Science Center at Fort Worth, Ft. Worth, TX 76107 and [1]North Side High School of Medical Professions, Ft. Worth, TX 76106

It has been suggested that stimulus dependence is a general feature of all developing sensory systems. To date, it has only been possible to test this idea for the developing vertebrate vestibular system in the microgravity environment of a Space Shuttle mission. For technical reasons, all attempts to perform these experiments have yielded inconclusive results. NASA designed the **R**otating **W**all **P**erfused **V**essel (RWPV) to simulate microgravity for cells in culture on earth. We replaced the culture medium with aquarium water allowing the RWPV to be used to simulate a microgravity environment for zebrafish eggs. Zebrafish eggs were collected once a week for 16 weeks within 3 hours after they were laid and fertilized. About 50% of those eggs were transferred into a beaker with aquarium water and about 50% were placed in the RWPV. The zebrafish eggs/hatchlings were maintained in the RWPV for either 72 or 96 hours. (hatching occurs between 48 and 72 hours after fertilization). The hatching rate in the RWPV was >80% and identical to that of the controls. RWPV animals (n > 250) displayed a swimming behavior that was indistinguishable from the control animals when illuminated from above. However, when illuminated from below, RWPV animals swam either dorsal surface up or laying on their side, they corkscrewed, swam vertical loops, and occasionally even swam upside down. At 72 hours, 20 out of 57 RWPV animals were missing one or more otoliths. In contrast, 100% of the control animals (n > 50) had 2 otoliths on each side. At 96 hours, no RWPV animals were missing otoliths and there was no significant difference between the size of the utricular otolith between the RWPV and the control animals. However, at 96 hours, the saccular otolith was significantly smaller in the RWPV animals. Immediately upon removal from the RWPV, experimental animals showed some signs of compensatory eye rotation, but with a much less clear relationship between the orientation of the eye and the direction of gravity than the age-matched control animals. This difference is still very obvious one day later. These results support the idea that the development of the equilibrium receptor system in zebrafish is dependent on the presence of normal gravity. (The first 3 authors contributed equally to this work and are listed in the order that they did rotations in the lab.)

Abstract #85

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Kristi D. Henderson

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

STUDIES OF OCULAR WOUND HEALING IN SIMULATED ZERO GRAVITY USING THE CORNEAL IMPLANT MODEL

Kristi D. Henderson* and S.D. Dimitrijevich*†, *Department of Biochemistry and Molecular Biology and †Surgery; North Texas Eye Research Institute; Wound Healing Research Institute; UNTHSC, Ft Worth, TX 76107.

Fibroblasts are the major cellular components of the connective tissue and play a crucial role in the wound healing process. When activated fibroblasts divide, migrate, synthesize the extracellular matrix, participate in tissue contraction and may be phagocytic. Most of these functions are based on precise cell-matrix interactions mediated by integrins. These cell surface receptors are dimeric glycoproteins which interact with specific sequences on the ECM macromolecules (e.g. collagen). Thus, integrin expression is a sensitive indicator of cellular responses to the changes in the extracellular microenvironment. The effects of zero gravity on wound healing are not known. In our attempts to study this microenvironment *in vitro,* we hypothesize that the repair process will be enhanced due to reduction in tissue stress and abundance of nutrients. In order to prove or disprove this hypothesis we will use the corneal implant model, which we have begun to study in 1g environment. This model is initiated by implanting punch biopsies of human corneal stroma into collagen type I gel matrix. Initially, only the responses of the fibroblasts will be studied. These will include cell proliferation, matrix biosynthesis and expression of specific integrins. The results obtained at 1g will be compared with the results obtained from identical experiments conducted at simulated 0g. We expect that these studies will lead to a better understanding of the tissue repair process at zero gravity. (Support from NASA Microgravity program is anticipated.)

Abstract #86

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Jill Moore

Department: Biochemistry and Molecular Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

DEVELOPMENT OF A DERMAL EQUIVALENT MODEL TO INVESTIGATE FACTORS THAT REGULATE THE DIFFERENTIATION, FUNCTIONAL ACTIVITIES, AND DISAPPEARANCE OF MYOFIBROBLASTS

Jill Moore and S.D. Dimitrijevich
Department of Biochemistry and Molecular Biology and Surgery; The Wound Healing Research Institute, UNTHSC, Fort Worth, TX 76107

Wound healing is a sequential process involving synchronized functions of cellular and matrix components. In the final stages of the repair process, wound closure is effected by tissue contraction. The main cell involved in contraction is the myofibroblast which has been studied extensively due to its role in normal as well as abnormal healing situations. In particular, investigations into excessive contraction conditions that lead to significant scarring have shown myofibroblasts to be present. Thus far, however, experiments to study myofibroblasts and the factors that regulate their functions have used dermal equivalent models that do not provide a realistic account of the wound healing process. Our objective was to develop a dermal equivalent model that can mimic the "in vivo" wound healing situation more accurately by remaining quiescent until stimulated to contract. Dermal equivalents, consisting of collagen type I and infant fibroblasts obtained from human foreskin, were set up in 24 well plates that were either coated (with 2% BSA in PBS or agarose) or non-coated. In addition, different test factors including serum, TGFB-1 and calcium were studied as inducers of contraction. Results indicate that 2% BSA in PBS coated plates provided the adequate amount of tension necessary to promote contraction of the dermal equivalents upon stimulation, while keeping the controls quiescent. This model will be used in future experiments to test the effects of other factors on myofibroblasts and their role in contraction, as well as a model to identify inhibitors to the contraction process. Information gained from these investigations will be helpful in treating individuals who suffer from painful scarring that results from excessive contraction.

Abstract #87

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: S. D. Dimitrijevich

Department: Biochemistry & Molecular Biology and Surgery

Graduate Student___ Medical Student___ Postdoctoral Fellow___ Faculty X Staff___

Read instructions and fit abstract inside the space given below:

THE ROLE OF KERATOCYTES IN CORNEAL WOUND HEALING

***¥S.D. Dimitrijevich, *T.J. Reese, †L. Taliana, *Department of Biochemistry and Molecular Biology and Surgery; ¥North Texas Eye Research Institute; UNT HSC, Ft. Worth, Texas, †Div. of Biomol. Eng., CSIRO/ UNSW, Sydney, Australia.**

Contraction of cutaneous wounds has been attributed to myofibroblasts, a differentiated phenotype of fibroblasts. It has also been suggested that migration of these cells generates tractional forces which may be one of the mechanical factors involved. The goal of this project is to provide a better understanding of wound contraction which may take place during the repair of the corneal stroma. Initially we probed the non-confluent monolayers of cultured stromal fibroblasts with fluorescently labeled antibodies against cytoskeletal proteins (e.g. α-smooth muscle actin, desmin, vimentin). In order to address the question in a more relevant manner a new *in vitro* model of the stroma was developed and characterized. This model is based on the three-dimensional outgrowth of keratocytes, and is generated by implanting a stromal punch biopsy into a collagen type I gel matrix. The behavior of keratocytes in these human and bovine Stromal Equivalents, were compared by histochemical and transmission electron microscopic methods. The non-contractile phenotype of both the bovine and human keratocytes showed positive expression of desmin and vimentin. The α-smooth muscle actin was expressed only under the culture conditions which lead to generation of myofibroblast phenotype. The migration and proliferation of keratocytes from the implanted stromal biopsies did not cause matrix contraction over a culture period of several weeks.
The new method of generating stromal equivalent provides a valuable model for studying the role of keratocytes in stromal wound healing.

Supported by NASA Biotechnology and Microgravity Program and APA(I) scholarship to LT; Ocular tissue supplied by Alcon Laboratories, Inc.

Abstract #88

STUDENT ORAL PRESENTATION COMPETITION

(1:30) Shelley R. McDonald	APOLIPOPROTEIN E DEFICIENCY ACCELERATES SPATIAL LEARNING AND MEMORY DECLINE IN AGING MICE
(1:45) Robert Carter, III	ROLE OF THE SKELETAL MUSCLE PUMP DURING RECOVERY FROM EXERCISE
(2:00) Johnathan D. Tune	INSULIN IMPROVES OXYGEN UTILIZATION EFFICIENCY BY ENHANCING GLUCOSE UPTAKE DURING MODERATE HYPOPERFUSION IN CANINE LEFT VENTRICLE
(2:15) Matthew J. Crawford	PHOTO-OXIDATIVE STRESS DOWN-REGULATES THE ACTIVITY OF TRANSCRIPTION FACTOR NF-kB IN 661W PHOTORECEPTOR CELLS
(2:30) Rustin E. Reeves	$pI_{Cln\ m}$ mRNA EXPRESSION IS RESPONSIVE TO CHANGES IN CELL VOLUME AND INTRACELLULAR OSMOLALITY
(2:45) ***BREAK***	
(3:00) Lori Johnson	GROWTH OF *STAPHYLOCOCCUS AUREUS* INSIDE TITANIUM DIFFUSION CHAMBERS IMPLANTED IN THE PERITONEAL CAVITIES OF RATS
(3:15) Debra White	MOLECULAR CLONING OF THE GLOBAL REGULATOR *csr*A FROM *Salmonella typhimurium*
(3:30) Harlan Jones	IgA AND Th2 CYTOKINE RESPONSES DEVELOP AFTER INTRANASAL IMMUNIZATION WITH INFLUENZA VACCINE PLUS CHOLERA TOXIN
(3:45) Rusty Crum	ANALYSIS OF THE TRANSPOSON INSERTION SITE IN A HEMOLYSIN AND LIPASE DEFICIENT STRAIN OF *STAPHYLOCOCCUS AUREUS*

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Shelley R. McDonald

Department: Pharmacology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

APOLIPOPROTEIN E DEFICIENCY ACCELERATES SPATIAL LEARNING AND MEMORY DECLINE IN AGING MICE. McDonald, S.R., Smith, J., and Forster, M.J. Department of Pharmacology, Geriatrics Education and Research Institute, UNTHSC, Fort Worth, TX 76107 and Department of Biochemical Genetics and Metabolism, The Rockefeller University, New York, NY 11021.

Apolipoprotein E (apoE) is found in three allelic variants: E2, E3 and E4. Epidemiological studies associate E4 with an increased risk for developing Alzheimer's disease (AD), while E2 is associated with decreased risk. Studies show that the degree of risk for AD is paralleled by the ability of the allelic variants to protect neuronal cell cultures against cytotoxcity of H_2O_2 and β-amyloid, an abnormal protein implicated in AD pathogenesis (E2>E3>E4> control). If apoE protects against oxidative stress *in vivo*, then animals that are missing the apoE gene would be expected to have greater age-associated neuronal damage and loss of learning/memory capacities. This hypothesis was tested by comparing the age-associated decline in learning and memory capacities in apoE deficient mice (EO) and wild type background controls (WT). A place learning task was used to measure spatial learning and memory; this task required mice to swim to a platform hidden in a tank of opacified water. All mice were obtained from the Rockefeller University and divided into groups to be tested cross-sectionally at 5 months and 18 months of age. When 5 months of age, the EO and WT mice did not significantly differ in their abilities to acquire or retain the position of the hidden platform or to relearn a new position when the platform was moved. Both WT and EO mice at 18 months showed age-associated increases in the latencies to reach the platform, but path length was significantly increased only in the EOs. After a 60-h retention interval of no testing, the ability to locate the platform was comparable to that of the acquisition phase for all groups except the 18 month EOs, which had a decreased ability to remember the location they had previously learned. When the platform was reversed, young control mice and young apoE deficient readily learned the new location. The older apoE deficient mice, however, were much slower in learning the new position. The current findings suggest that apoE is important for maintaining CNS function in aging mice and protecting against age-associated functional loss.
(Supported by NIH-NIA Grant A607695)

Submit to Laura Barber, Graduate School of Biomedical Sciences DEADLINE: March 3, 1997

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Robert Carter, III

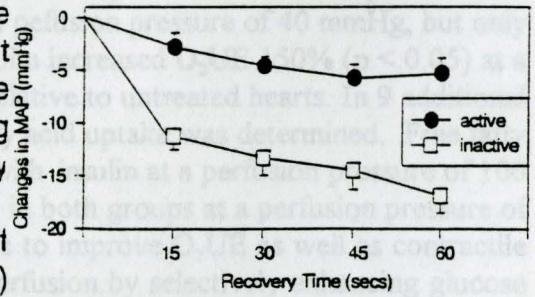
Department: Department of Integrative Physiology

Graduate Student X Medical Student ___ Postdoctoral Fellow ___ Faculty ___ Staff ___

Read instructions and fit abstract inside the space given below:

ROLE OF THE SKELETAL MUSCLE PUMP DURING RECOVERY FROM EXERCISE. R Carter, III, DE Watenpaugh, WL Wasmund, SL Wasmund, ML Smith, FACSM. Department of Integrative Physiology, UNTHSC, Ft. Worth, TX 76107-2699.

This study tested the hypothesis that the initial rapid decrease in blood pressure during inactive recovery from exercise is due,in part to lack of the skeletal muscle pump, which is an important mechanism for venous return during exercise. Twelve healthy volunteers underwent 2 exercise sessions each consisting of a warm-up followed by 3 min of cycling at 60% of maximal heart rate (HR), followed by 5 min of recovery: 1) seated (inactive); or 2) loadless pedaling (active), in random order. Thirty min separated the 2 protocols. Mean arterial pressure (MAP, photoplethysmography), cardiac output (CO, Doppler echocardiography), thoracic impedance (TI) and HR were measured continuously during each bout of exercise. Total peripheral resistance (TPR) was calculated from MAP and CO. Results: The MAP changes from peak exercise to min 1 of recovery are shown in the figure.Therewere significant differences between inactive and active recovery during the first minute of recovery from submaximal exercise (* $p<0.05$). Maintenance of central blood volume (TI) and CO paralleled the maintenance of MAP. Conclusion: These data suggest that engaging the skeletal muscle pump by loadless pedaling markedly improves the support of MAP during recovery from submaximal exercise. (Supported, in part, by NIH grant HL-49266)



UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Johnathan D. Tune

Department: Integrative Physiology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

INSULIN IMPROVES OXYGEN UTILIZATION EFFICIENCY BY ENHANCING GLUCOSE UPTAKE DURING MODERATE HYPOPERFUSION IN CANINE LEFT VENTRICLE

Johnathan D. Tune, Wayne W. Loney, Robert T. Mallet, and H. Fred Downey. Department of Integrative Physiology, University of North Texas Health Science Center, Fort Worth, Texas 76107

When myocardial oxygen supply is reduced, increased utilization of glucose may be advantageous, since oxidation of glucose requires less oxygen per ATP produced than does oxidation of fatty acids. Insulin stimulates glucose uptake and oxidation in mammaliam myocardium. This study tested the hypothesis that insulin improves myocardial contractile function and oxygen utilization efficiency (O_2UE) under low flow conditions by enhancing glucose metabolism and diminishing fatty acid metabolism. Perfusion pressure was lowered from 100 (control) to 40 mmHg in the left anterior descending coronary artery of 21 anesthetized, open chest dogs. Regional glucose uptake, lactate uptake, power index (heart rate · systolic pressure · segment shortening), and O_2UE (power index / myocardial oxygen consumption) were determined without ($n = 6$) and with intravenous insulin (4 U / min, $n = 6$). Glucose uptake increased with insulin ($p < 0.05$) while lactate uptake decreased ($p < 0.05$). Without insulin, power index decreased 97% ($p < 0.0001$) at a pefusion pressure of 40 mmHg, but only 47% ($p > 0.05$) with insulin. Insulin increased O_2UE 150% ($p < 0.05$) at a perfusion pressure of 40 mmHg relative to untreated hearts. In 9 additional experiments, myocardial free fatty acid uptake was determined. Free fatty acid uptake decreased ($p < 0.05$) with insulin at a perfusion pressure of 100 mmHg and decreased ($p < 0.05$) in both groups at a perfusion pressure of 40 mmHg. Thus, insulin appears to improve O_2UE as well as contractile function during coronary hypoperfusion by selectively enhancing glucose utilization. (Supported by NIH HL 35027, HL 50441 and Tex. Ad. Res. Prog. 9768).

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Matthew J. Crawford

Department: Anatomy and Cell Biology

Graduate Student X Medical Student X Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

PHOTO-OXIDATIVE STRESS DOWN-REGULATES THE ACTIVITY OF TRANSCRIPTION FACTOR NF-kB IN 661W PHOTORECEPTOR CELLS. ((M. J. Crawford[1], R. Krishnamoorthy[1], M. Al-Ubaidi [2], and N. Agarwal [1])) Department of Anatomy and Cell Biology, UNT Health Science Center [1]; U. of Illinois, Chicago, Illinois [2].

Purpose. Photo-oxidative stress has been shown to lead to 661W photoreceptor cell death via an apoptotic pathway, but the mediators of this process have yet to be elucidated. We hypothesize that down-regulation of the transcription factor NF-kB is a mechanism by which injured photoreceptor cells commit suicide. Methods. Cultured 661W photoreceptor cells were exposed to fluorescent visible light (3-4 mW/cm^2) for periods up to 4 hours. Control cells remained in a dark environment for the same time period. Following experimental conditioning, nuclear extracts were prepared and analyzed by electrophoretic mobility shift assays (EMSA) using end-labeled consensus sequence of double strand NF-kB and its mutant form oligos. Results. Photo-oxidative stress to 661W cells results in significant lowering of NF-kB activity as shown by EMSA in light exposed 661W cells as compared to dark exposed controls. The specificity of NF-kB binding was shown by competition with cold NF-kB oligos and mutant NF-kB oligos. Conclusions. Exposure of 661W cells to photo-oxidative stress leads to down regulation of NF-kB activity. These findings are significant in supporting NF-kB's role in the pathogenesis of ocular disease. Further studies are underway to determine if down regulation of NF-kB is involved in the apoptotic pathways of in vivo models of retinal dystrophies and photo-oxidative stress.

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UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Rustin E. Reeves

Department: Anatomy and Cell Biology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

pI_{Cln} mRNA EXPRESSION IS RESPONSIVE TO CHANGES IN CELL VOLUME AND INTRACELLULAR OSMOLALITY. R.E. Reeves[1], M. Coca-Prados[2], and P.R. Cammarata[1].Department of Anatomy and Cell Biology[1], University of North Texas Health Science Center/North Texas Eye Research Institute, Fort Worth, TX 76107 and Department of Ophthalmology and Visual Science[2], Yale University School of Medicine, New Haven, CT 06510.

Cell volume fluctuations actuate specific membrane transport/channel pathways which result in the net accumulation or loss of osmotically active solutes. The relationship between cell volume and mRNA expression of pI_{Cln}, a chloride channel regulatory protein associated with the myo-inositol efflux pathway in cultured bovine lens epithelial cells (BLECs), was evaluated in this study. Two distinct experimental approaches were designed to define the circumstances that alter mRNA expression of pI_{Cln}. For rapid cellular shrinkage, BLECs maintained to confluency in physiologic medium (MEM, 257 mOsm) were switched to sodium hypertonic medium (MEM +116mM additional NaCl, 480 mOsm), hereafter referred to as SHM. For rapid cellular swelling, BLECs were acclimated in SHM for 12 hours then switched to MEM for up to 24 hours. Gradual cellular swelling in response to intracellular polyol accumulation was achieved in BLECs exposed to 40 mM galactose medium (Gal) and the expression of pI_{Cln} mRNA was monitored. BLECs exposed to hypertonic medium conditions (raising medium osmolality) upregulate the expression of pI_{Cln} mRNA. When exposed to hypotonic medium conditions (lowering medium osmolality), rapid cell swelling ensues, and BLECs respond by moderately downregulating the expression of pI_{Cln} mRNA. Gal exposure (gradual cell swelling) showed a slight upregulation of pI_{Cln} mRNA expression only under certain experimental conditions. These data suggest a converse relationship exists between pI_{Cln} mRNA expression and changes in cell volume and/or intracellular osmolality. Hypertonicity induces BLECs to rapidly shrink, concentrating internal solutes and causing active accumulation of osmolytes. Ensuing upregulation of pI_{Cln} mRNA suggests a preparatory response via an unknown signaling mechanism that "senses" the loss of cell volume and/or an increase in intracellular osmolality; therefore, preparing the cell for the impending need to efflux osmolytes from cell to medium. Hypotonicity prompts rapid cell swelling, normally eliciting osmolyte efflux. Succeeding downregulation of pI_{Cln} mRNA suggests an inhibitory response via a mechanism that "senses" the gain in cell volume and/or a decrease in intracellular osmolality, alleviating the need for further efflux of osmolytes. Supported by Public Health Service Award EY05570 (PRC).

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ABSTRACT FORM

First Author: Lori Johnson

Department: Microbiology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

GROWTH OF *STAPHYLOCOCCUS AUREUS* INSIDE TITANIUM DIFFUSION CHAMBERS IMPLANTED IN THE PERITONEAL CAVITIES OF RATS. Lori A. Johnson, and Mark E. Hart, Department of Microbiology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107-2699.

Staphylococcus aureus is a gram positive bacterium causing a wide variety of diseases, among them being toxic shock syndrome, toxic food poisoning, osteomyelitis and endocarditis. The pathogenesis of *S. aureus* is due in part, to its capacity to produce over thirty cell wall-associated and extracellular proteins; most of which have been shown to be virulence factors as the result of extensive *in vitro* studies. However, little is known about the expression of these proteins in an *in vivo* environment. Recent studies have shown that whole cell protein profiles of *S. aureus* grown *in vivo* differ from those of *in vitro* grown cells. These data have lead us to hypothesize that as *S. aureus* enters and adapts to the host environment, genes expressed specifically in the host environment may include additional virulence factors not expressed when the bacterium is grown *in vitro*. Therefore, the overall goal of this study is to generate a cDNA library from total RNA isolated from *in vivo* grown *S. aureus* cells. This cDNA will undergo subtractive hybridization with total RNA isolated from *in vitro* grown *S. aureus* to remove genes expressed in both environments. The remaining genes represent those specifically expressed in the *in vivo* environment. In order to accomplish this goal, we first determined the growth capabilities of *S. aureus* in titanium diffusion chambers surgically implanted in the peritoneal cavities of rats. Rats implanted with chambers were allowed to recover for three days before the chambers were inoculated with various concentrations of *S. aureus* cells. Three days later, the contents of the implanted chambers were recovered and total cell numbers were determined by growth on agar plates. Chambers inoculated with 10^5 cells resulted in an increase in cell number to 10^7-10^8 per chamber. Higher cell inoculums resulted in no appreciable increase in cell numbers. To determine if sufficient RNA can be obtained from recovered cell numbers, total RNA was isolated from 10^5-10^8 cells. Ribosomal RNA bands were easily detected on ethidium bromide stained agarose gels for RNA isolated from 10^6-10^8 cells while bands for 10^5 cells were barely visible. These data indicate that *S. aureus* can be grown in the described *in vivo* environment and that sufficient total RNA can be isolated from recovered cell numbers to generate the desired cDNA library. (NIH grant AI36934 and Faculty Research Grant awarded to M.E.H)

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: Debra White

Department: Microbiology and Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

MOLECULAR CLONING OF THE GLOBAL REGULATOR *csrA* FROM *Salmonella typhimurium*

D. White, M. E. Hart, Roy Curtiss, III and T. Romeo
University of North Texas Health Science Center, Fort Worth, TX. 76107-2699

Several studies have indicated that the physiological status of a bacterial pathogen, as evidenced by the growth phase of the infecting inoculum, can significantly affect the resulting pathogenic process. Stationary phase cultures are notably more resistant to environmental stresses than exponential phase cultures and they exhibit enhanced virulence. Studies in *Escherichia coli* have identified a gene known as *csr*A, carbon storage regulator, which encodes a global regulator of carbon metabolism and of various genes expressed in early stationary phase of growth. A homolog of *csr*A in the plant pathogen *Erwinia carotovora* has been shown to regulate its virulence properties. The current study was initiated to determine whether *csr*A affects virulence of mammalian pathogens as well. The *csr*A region of the mammalian pathogen *Salmonella typhimurium* was amplified by PCR using primers based on the *E. coli* sequence. The PCR products were treated with Bal 31 exonuclease to remove an unclonable region downstream from *csr*A, which was previously found in *E. coli*. The resulting products were treated with Klenow fragment, ligated into pUC19 plasmid and used to transform DH5-alpha. Several clones with phenotypic properties expected of *csr*A were isolated. These clones will be used to characterize the molecular and regulatory properties of the *csr*A *Salmonella* gene, including its role in mammalian infections. (National Science Foundation)

ORAL PRESENTATION

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

Research Appreciation Day 1997

ABSTRACT FORM

First Author: HARLAN JONES

Department: MICROBIOLOGY AND IMMUNOLOGY

Graduate Student_X_ Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

IgA AND Th2 CYTOKINE RESPONSES DEVELOP AFTER INTRANASAL IMMUNIZATION WITH INFLUENZA VACCINE PLUS CHOLERA TOXIN Harlan Jones and Jerry W. Simecka, Dept. Microbiology and Immunology, University of North Texas Health Science Center, Forth Worth, Texas 76107 and Dept. Biology, Southern University, Batan Rouge, LA 70813

Although current vaccine strategies for influenza protect the lower respiratory tract, induction of mucosal immunity along the upper respiratory tract is limited. In this study, we investigate the immune response after intranasal immunization (i.n.) of influenza vaccine plus the mucosal adjuvant, cholera toxin (CT). We examined the B cell and T helper cell subset (Th1 and Th2) responses as they serve as mediators of immunity. BALB/c mice were i.n. and intradermally (i.d.) immunized with influenza vaccine plus CT (0 and 7 days). Total RNA was isolated from lung tissue and mononuclear cells from the lung, nasal passages and spleen (10 and 14 days) post immunization (p.i.). IgA and IgG responses were determined in these tissues by ELISPOT assay. Influenza specific IgA responses predominated in both nasal passages (3, 000 IgA AFC/10^6 cells) and lungs (2,400 IgA AFC/10^6 cells) after i.n. immunization. Little or no IgA responses were present within the spleen by either route of immunization. IgG responses were present in all tissues after i.n. immunization with the responses were highest in the lung (1,600 IgG AFC/10^6 cells). However, IgG, but not IgA, responses developed after i.d. immunization.

Cytokine mRNA expression of the T helper cytokines (IL-2, IFN-γ, IL-4, and IL-5) were examined within the lung at day 10 p.i. by relative RT-PCR. ß2-microglobulin, a housekeeping gene, was used to normalize mRNA responses between samples. The levels of IL-4 and IL-5 mRNA, which are both produced by Th2 cells, were increased after i.n. immunization with antigen alone or in combination with CT. The inclusion of CT enhanced IL-5 mRNA expression. Increased mRNA expression of Th1 cytokines, IL-2 and IFN-γ, were apparent only after inclusion of CT as an adjuvant. These results indicate that Th2 cells are activated after i.n. immunization, in addition to developing IgA responses. This is consistent with Th2 cells role in mucosal immunity. However, the mucosal adjuvant, CT may also facilitate activation of Th1 cells, in addition to enhancing IgA and Th2 cell responses. (This work is supported by a Faculty Research Grant to JWS and NIH Bridge Grant to R. Kaman.)

Submit to Laura Barber, Graduate School of Biomedical Sciences **DEADLINE: March 3, 1997**

UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH

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ABSTRACT FORM

First Author: Rusty Crum

Department: Department of Microbiology & Immunology

Graduate Student X Medical Student___ Postdoctoral Fellow___ Faculty___ Staff___

Read instructions and fit abstract inside the space given below:

ANALYSIS OF THE TRANSPOSON INSERTION SITE IN A HEMOLYSIN AND LIPASE DEFICIENT STRAIN OF *STAPHYLOCOCCUS AUREUS*. Rusty M. Crum, and Mark E. Hart, Department of Microbiology and Immunology, University of North Texas Health Science Center, Fort Worth, TX. 76107-2699.

Staphylococcus aureus is a human pathogen that is capable of producing over thirty extracellular and cell wall-associated proteins; many of which, are virulence factors. To date, expression of many of these factors is regulated by three distinct genetic loci found on the staphylococcal chromosome but other regulators may exist. Recently, we generated a transposon mutant library of *S. aureus* in an effort to isolate additional regulatory loci. Transposon mutants were screened and several were found to be deficient in hemolysin and lipase activities. Because the structural genes for lipase and hemolysin are not linked, we considered the possibility that the insertion occurred in a regulatory element responsible for the expression of these genes. To test this hypothesis, we used Southern analysis to demonstrate that the transposon inserted once in the staphylococcal chromosome and the insertion did not occur in any of the three known regulatory systems. A portion of the insertion site was cloned and sequenced. Analysis of the sequence with staphylococcal DNA databases revealed the sequence to be homologous to regions within the staphylococcal rRNA operons. Currently, we are assessing the relationship between the transposon insertion event and the observed mutant phenotype by attempting to transduce the mutation from the mutant strain back into the parental strain. Unexpectedly, during the course of this study it was observed that a small portion of the parental strain colonies appeared to be deficient in catalase production; a key characteristic in the identification of the genus, *Staphylococcus*. Diagnostic analysis indicated that this variant is indeed, *S. aureus*. Because the variation in catalase activity is unrelated to the transposon mutant phenotype, we decided to further examine the cause of variation in catalase activity. In order to do this, we are currently attempting to clone the catalase gene from *S. aureus*. The catalase genes from *Escherichia coli* and *Bacillus subtilis* were compared and primers were generated from highly conserved regions within the genes. Chromosomal DNA from *S. aureus* was isolated and is currently being used to isolate the catalase gene by polymerase chain reaction (PCR). (NIH grant AI36934 and Faculty Reserch Grant awarded to M.E.H.)

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