National Heart, Lung, and Blood Institute

Annual Report of Intramural Research

October 1, 1989
to
September 30, 1990

CLINICAL BRANCHES

NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04175-04 -CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Revascularization of the heart via the process of angiogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ellis F. Unger, M.D.	Senior Staff Fellow	CB	NHLBI
Matie Shou, M.D.	Visiting Fellow	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

Veterinary Resources Branch, NIH

LAB/BRANCH: Cardiology Branch

SECTION: Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	2.8	PROFESSIONAL:	2.0	OTHER	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An unsolved problem in cardiology is how to provide relief for patients with severe atherosclerotic coronary disease, in whom conventional revascularization techniques (i.e., angioplasty, bypass surgery) are not feasible. This situation arises (not uncommonly) when all three major coronary arteries are diseased along the majority of their length. Our Laboratory is interested in the application of angiogenic therapy to this problem, that is, enhancement of coronary collateral blood flow via the pharmacologic induction of blood vessel growth.

We have developed a canine model whereby angiogenesis can be directed to ameliorate myocardial ischemia. The left anterior descending coronary artery (LAD) was occluded gradually over a 2 week period by placing an ameroid constrictor around the artery. At the same setting, the internal mammary artery (IMA) was implanted into the region supplied by the LAD. We have previously demonstrated that collaterals develop between the IMA and the territory normally perfused by the LAD in this model, supplying nutritive collateral blood flow. By positioning a tube in the distal IMA, we were able to provide a continuous retrograde infusion directly into the vessel from an implanted pump, at the point where collaterals would develop between the IMA and the coronary circulation. Heparin, 15 or 150 units/hr, or saline vehicle alone were infused into the IMA. After 8 weeks, the IMA provided a greater proportion of maximal collateral flow in heparin treated dogs ($23\pm5\%$, $N=16$) than in saline treated dogs ($10\pm3\%$, $N=12$, $p<0.05$). Thus, in a preparation where collaterals are established between an extracardiac artery and the coronary circulation, angiogenic agents can be targeted locally by means of a continuous infusion at the anastomotic site, and administration of heparin in this preparation promotes the formation of collaterals between the extracardiac artery and the coronary circulation. In an ongoing experiment, we are attempting to determine whether heparin exerts a similar collateral-promoting effect when given systemically (by repeated subcutaneous injections) rather than locally.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04808-03 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth factors in myocardial angiogenesis, atherogenesis & embryogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Ward Casscells, M.D.	Senior Staff Fellow	CB NHLBI
Edith Speir, B.S.	Biochemist	CB NHLBI
Shashi Shrivastav, Ph.D.	Special Volunteer	CB NHLBI
Zu-Xi Yu, M.D.	Special Volunteer	CB NHLBI
Ya-Min Fu, M.D.	Special Volunteer	CB NHLBI
Paolo Spirito, M.D.	Special Volunteer	CB NHLBI
Victor Ferrans, M.D.	Chief, Section on Ultrastructural Pathology	PB NHLBI
Stephen E. Epstein, M.D.	Chief	CB NHLBI

COOPERATING UNITS *(if any)*

Section on Ultrastructure, Pathology Branch, NHLBI

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

For the past year we continued to focus on the cell physiology of basic and acidic fibroblast growth factors, using both in vitro and in vivo models.

The FGFs and their receptors were also found to be expressed in embryonic tissues but not in the adult, where the peptides are stored. In vascular injury, as in embryonic vessels and cultured vascular cells, FGFs and their receptors were re-expressed. Smooth muscle cell proliferation was inhibited by exposure of the cells to bFGF conjugated to the ribosome inhibitor saporin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04823-02 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Myocardial Ischemia and Hypertrophic Cardiomyopathy (Title change)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sect.	NHLBI CB
Vasken Dilsizian, M.D.	Clinical Investigator	NHLBI CB
Arshed Quyyumi, M.D.	Senior Investigator	NHLBI CB
Lameh Fananapazir, M.D.	Senior Investigator	NHLBI CB
Robert O. Bonow, M.D.	Deputy Chief	NHLBI CB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis and Nuclear Cardiology Sections

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Exercise induced defects during thallium scintigraphy that normalize at rest occur commonly in patients with hypertrophic cardiomyopathy. However, it is not know whether or not these defects are indicative of myocardial ischemia. To assess the pathophysiologic relevance of reversible thallium defects, 50 patients with hypertrophic cardiomyopathy underwent exercise thallium scintigraphy and measurement of myocardial metabolism and hemodynamics during pacing stress. Thirty-seven patients (74%) had thallium abnormalities that normalized after 3 hours of rest. Of these 37 patients, 27 (73%) had metabolic evidence of myocardial ischemia during rapid atrial pacing. Four of 13 patients (31%) with normal thallium scans also had evidence of pacing-induced myocardial ischemia, a significantly lower prevalence ($p<0.01$). Seven of 10 asymptomatic patients had reversible thallium defects, with 4 of these having evidence of myocardial ischemia during pacing stress. Thus, exercise thallium scintigraphy identifies hypertrophic cardiomyopathy patients with evidence of inducible myocardia ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04825-02 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocardial sensitivity in patients with chest pain and normal coronary arteries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diagnostic Sec.	NHLBI	CB
Arshed Quyyumi, M.D.	Senior Investigator	NHLBI	CB
Lameh Fananapazir, M.D.	Senior Investigator	NHLBI	CB
Stephen E. Epstein, M.D.	Chief	NHLBI	CB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis Section, NHLBI

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to investigate the causes of chest pain in patients found to have angiographically normal coronary arteries during cardiac catheterization, cardiac sensitivity to catheter manipulation, pacing at various stimulus intensities, and intracoronary injection of contrast media were examined in several groups of patients undergoing cardiac catheterization. Right heart (especially right ventricular) catheter manipulation and pacing, and intracoronary contrast media injection, provoked chest pain typical of that previously experienced in 29/36 (81%) of patients with chest pain and angiographically normal coronary arteries and 15/33 (46%) symptomatic patients with hypertrophic cardiomyopathy. In contrast, only 2/33 (6%) symptomatic patients with coronary artery disease experienced their typical chest pain with these sensitivity tests ($P<0.001$). None of the 10 patients with valvular heart disease but without the chest pain syndrome, experienced any sensation whatsoever with these tests. Thus, patients who have chest pain despite angiographically normal arteries have abnormal cardiac sensitivity to a variety of stimuli, which maybe of causal importance to the chest pain syndrome or may contribute to their perception of ischemia-induced pain. The same phenomenon was also commonly seen in symptomatic patients with hypertrophic cardiomyopathy. Whether this phenomenon represents abnormal activation of pain receptors within the heart, or abnormal processing of visceral afferent neural impulses in the peripheral or central nervous system is unknown.

Endocardial sensitivity in patients with chest pain and normal coronary arteries

The causes of chest pain in patients found to have angiographically normal coronary arteries during cardiac catheterization remain controversial. Cardiac sensitivity to catheter manipulation, pacing at various stimulus intensities, and intracoronay injection of contrast media were examined in several groups of patients undergoing cardiac catheterization. Right heart (especially right ventricular) catheter manipulation and pacing, and intracoronary contrast media injection, provoked chest pain typical of that previously experienced in 29 of 36 (81%) patients with chest pain and angiographically normal coronary arteries and 15 of 33 (46%) symptomatic patients with hypertrophic cardiomyopathy. In contrast, only 2 of 33 (6%) symptomatic patients with coronary artery disease, experienced their typical chest pain with these sensitivity tests ($P<0.001$). None of 10 patients with valvular heart disease, but without a chest pain syndrome, experienced any sensation whatsoever with these tests. Cutaneous pain threshold testing demonstrated that patients with chest pain and normal coronary arteries actually had a higher pain threshold to thermal stimulation compared to patients with coronary artery disease or hypertrophic cardiomyopathy. Further, no relationship existed between cardiac sensitivity and cutaneous sensitivity testing.

Thus, patients who have chest pain despite angiographically normal coronary arteries may have abnormal cardiac sensitivity to a variety of stimuli. This increased sensitivity may be of causal importance to the chest pain syndrome, or may contribute to their perception of ischemia-induced pain. The same phenomenon was also commonly seen in symptomatic patients with hypertrophic cardiomyopathy. Whether this represents abnormal activation of pain receptors within the heart or abnormal processing of visceral afferent neural impulses in the peripheral or central nervous system is unknown.

Project Number: Z01 HL 04825-02 CB

6. NHLBI INTRAMURAL PROJECT CODING SHEET

Instructions: Place a checkmark next to each category that applies to this project. The degree or percentage of relevance to each category may be requested from you at a later date. Please enter additional information where requested.

___ AIDS or related

___ Biotechnology

✓ Clinical study

Ethnicity: all

Gender: both

Age range: 18 - 71 years

___ Diabetes

___ Diagnostic imaging/radiology

___ Digestive Diseases

___ Endocrinology

___ Fish oil

___ Genetic disorder

(Specify:_____)

___ Genome mapping and sequencing

___ Human fetal tissue

___ Laser technology

___ Neurotoxicology

___ Nutrition

___ Organ transplantation

___ Prevention

___ Toxicology

********* ORPHAN DISEASES *********

✓ Cardiomyopathies

___ Cooley's anemia

___ Cystic fibrosis

___ Epstein-Barr Virus

___ Familial emphysema

___ Hemophilia

___ Kawasaki Disease

___ Lipid disorders

___ Lupus - Systemic Erythematosis

___ Sarcoidosis

___ Sickle cell disease

___ Sjogren's

___ Von Willebrand Factor & related

___ Other

(Specify: _____)

** SEXUALLY-TRANSMITTED DISEASES **

___ Cytomegalovirus (CMV)

___ Hepatitis (Type: _____)

___ Herpes

B/A/D: Basic: ____ + Applied: 100% + Developmental: ____ = 100 Percent

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04826-02 CB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Investigation & Management of Arrhythmias in Hypertrophic Cardiomyopathy Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Lameh Fananapazir, M.D.,	Head, Clinical Electrophysiology Laboratory	NHLBI CB
Anthony C. Chang M.D.	Staff Fellow	NHLBI CB
Marc Ovadia M.D.	Staff Fellow	NHLBI CB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Clinical Electrophysiology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.6	0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
- ☐ (b) Human tissues
- ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sudden cardiac death (SCD) and syncope are frequent complications in patients with hypertrophic cardiomyopathy (HCM). We have previously shown that in HCM patients, a strong association exists between inducibility of ventricular tachycardia (VT) at electrophysiologic (EP) studies and a history of sudden cardiac arrest (SCA) and syncope. To obtain a better understanding of the mechanisms responsible for of SCA and syncope in HCM patients, we performed detailed hemodynamic and EP assessment of sudden cardiac arrest survivors (N=30) and HCM patients with syncope (N=55). We also assessed the utility of amiodarone (N=32) and of the implantable defibrillator (AICD, N=25) to prevent SCD, and role of dual chamber (DDD) pacemakers to relieve symptoms due to left ventricular obstruction (N=10). Five mechanisms explained SCA in all the HCM patient who presented with this event: 1) VT, 2) severe obstruction to the outflow of blood in the left ventricle, 3) myocardial ischemia induced by moderate increases in heart rate (previously unrecognized syndrome affecting young HCM patients), 4) an atrial tachycardia resulting in severe hypotension, and 5) bradycardia. Factors that were associated with syncope were 1) significant obstruction and inducibility of VT at EP studies. Two variables independently predicted subsequent SCD: 1) a history of previous SCA and 2) inducibilty of VT at EP studies. Empiric amiodarone therapy, reported to prevent SCD in HCM patients with VT Holter, was associated with SCD in 8 patients within 3 months of initiation of drug therapy - nearly all in patients with VT on Holter. EP studies showed that amiodarone frequently aggravated conduction problems and in most HCM patients was proarrhythmic. Notably, symptomatic patients in whom amiodarone facilitated VT induction were more likely to experience SCD subsequently. The AICD device, significantly reduced subsequent SCD (mean follow-up period; 14 months). DDD pacemakers reduced obstruction and resulted in symptomatic improvement in 8/10 patients who would otherwise have needed cardiac surgery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04827-02 CB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Myocardial Viability in Coronary Artery Disease and Left Ventricular Dysfunction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert O. Bonow, M.D., Chief, Nuclear Cardiology Section, CB, NHLBI
Others: Vasken Dilsizian, M.D., Department of Nuclear Medicine, CC
Stephen L. Bacharach, Ph.D., Physicist, DNM, CC
Alberto Cuocolo, M.D., Fogarty Fellow, DNM, CC
Pasquale Perrone-Filardi, M.D., Fogarty Fellow, DNM, CC
Liisa-Maria Voipio Pulkki, M.D., Guest Researcher, CB, NHLBI

COOPERATING UNITS *(if any)*
Imaging Physics Section, Department of Nuclear Medicine, CC

LAB/BRANCH
Cardiology Branch

SECTION
Nuclear Cardiology Section

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
- ☐ (b) Human tissues
- ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In many patients with coronary artery disease (CAD), impaired left ventricular (LV) function arises on the basis of regionally ischemic or hibernating myocardium rather than irreversibly infarcted myocardium. However, the identification of such potentially irreversible LV dysfunction has been problematic. Many exercise-induced thallium-201 defects do not normalize on subsequent resting redistribution images, even when the underlying myocardium is viable. We have demonstrated that reinjection of Tl-201 at rest immediately after the standard 4-hour redistribution image facilitates late uptake of Tl-201 and distinguishes between viable and infarcted myocardium. We studied 100 patients with chronic CAD. Of 260 abnormal myocardial segments during exercise, 85 (33%) were irreversibly abnormal on the redistribution study. However, 42 of these defects (49%) demonstrated improved or normal uptake after Tl-201 reinjection. That the late uptake of Tl-201 after reinjection represents viable myocardium is substantiated in 3 subgroups of patients. 1) In 20 patients restudied 3-6 months after revascularization, improved wall motion and improved blood flow was observed in 87% of segments with "irreversible" defects on redistribution studies identified as viable by Tl-201 reinjection before revascularization; such improvement occurred in no segment identified as scar. 2) In 12 patients studied by magnetic resonance imaging, segments identified as viable by this method had a normal degree of systolic wall thickening, significantly greater than the regions identified as scar. 3) In 16 patients studied by PET imaging with ^{18}F-fluorodeoxyglucose (FDG), segments with Tl-201 defects identified as viable by reinjection had metabolic evidence for myocardial viability. The concordance between the Tl-201 reinjection and FDG uptake data was excellent, with 51% of regions with severe "irreversible" defects identified as viable by both techniques. These data indicate that Tl-201 reinjection is a convenient and relatively inexpensive method to identify viable myocardium in patients with chronic CAD and LV dysfunction.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04828-02 CB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ischemia and Abnormal Diastolic Function in Hypertrophic Cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert O. Bonow, M.D., Chief, Nuclear Cardiology Section, CB, NHLBI
Others: Richard O. Cannon, Senior Investigator, CB, NHLBI
Liisa-Maria Voipio-Pulkki, M.D., Guest Researcher, CC, NHLBI
Vasken Dilsizian, M.D., Department of Nuclear Medicine, CC
Pasquale Perrone-Filardi, M.D., Fogarty Fellow, DNM, CC
Stephen L. Bacharach, Ph.D., Physicist, DNM, CC

COOPERATING UNITS *(if any)*
Imaging Physics Section, Department of Nuclear Medicine, CC

LAB/BRANCH
Cardiology Branch

SECTION
Nuclear Cardiology Section

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Exercise-induced myocardial perfusion defects as assessed by thallium-201 single photon emission computed tomography (SPECT) develop in over 2/3 of patients with hypertrophic cardiomyopathy (HCM), and these abnormalities may be reduced (or prevented entirely) by verapamil therapy. The mechanism responsible for thallium defects during exercise in HCM has been assumed to be myocardial ischemia. An alternative explanation is disturbed cellular active cation uptake across the sarcolemma related to the cardiomyopathic process. Since uptake of technetium-99m methoxybutyl isonitrile (MIBI) is not dependent on the active Na-K ATPase transport system, as is Tl-201, we performed exercise MIBI studies in 10 HCM patients with exercise-induced Tl-201 defects. Of the 50 myocardial regions analyzed, there was concordance of Tl-201 and MIBI uptake in 23/24 (96%) of abnormal and 26/26 (100%) of normal myocardial regions. There was also 100% concordance of exercise-induced cavity dilatation. Thus, these data support the concept that Tl-201 defects in HCM represent true perfusion abnormalities and not a fundamental disturbance of the active Na-K ATPase transport system. Evidence of myocardial ischemia in HCM has also been demonstrated using positron emission tomography (PET). We studied 12 symptomatic HCM patients with severe hypertrophy (septum >20 mm) by exercise Tl-201 SPECT and resting PET, using ^{18}F-deoxyglucose (FDG) to assess regional myocardial glucose utilization (rMGU) and ^{15}O-water to quantitate regional blood flow (rMBF). When assessed relative to rMBF, rMGU demonstrated regional heterogeneity in 7 patients, with a pattern of increased rMGU/rMBF compatible with regional myocardial ischemia. These data at rest were supported by the TL data: in all 7 patients, the same regions with increased rMGU/rMBF at rest developed reversible Tl-201 perfusion defects with exercise. Thus, elevated rMGU relative to rMBF in patients with HCM represents either metabolic evidence of MI at rest or the metabolic sequelae of prior ischemic episodes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04830-02-CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Promotion of Myocardial Angiogenesis via Intracoronary Infusion of Growth Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ellis F. Unger, M.D.	Senior Investigator	CB	NHLBI
Shmuel Banai, M.D.	Visiting Associate	CB	NHLBI
Michael Jaklitsch, M.D.	Visiting Fellow	CB	NHLBI
Matie Shou, M.D.	Visiting Fellow	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

Veterinary Resources Branch, NIH

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several polypeptides with the potential to cause blood vessel growth (angiogenesis) via endothelial cell proliferation and migration have been sequenced and synthesized during the last few years. Our ultimate goal is to utilize these angiogenic agent(s) to facilitate myocardial revascularization in patients with coronary heart disease.

The specific purpose of this investigation is to utilize two of these peptides, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), to effect angiogenesis and ameliorate myocardial ischemia in a canine model. These polypeptides, available to us in large quantities, are potent stimulators of angiogenesis *in vitro*. In our experimental model, the left circumflex coronary artery (LCX) of dogs is occluded gradually over a 2 to 3 week period by an ameroid constrictor applied to the proximal vessel. As a result of LCX occlusion, its perfusion territory becomes dependent on collateral vessels. Myocardial infarction generally does not occur as long as the occlusion is gradual.

Five weeks after placement of the ameroid, regional myocardial blood flow will be quantitated with radiolabeled microspheres under basal conditions, and during pharmacologically induced coronary vasodilatation. Dogs will then be randomized to receive bFGF, VEGF, or placebo directly into the coronary circulation. After 4 weeks, resting and maximal myocardial blood flow will again be quantitated. Thus, collateral function can be compared before and after treatment in the three groups. Vessels will be examined morphometrically to determine the capillary density in the areas exposed to bFGF and VEGF. In addition, proliferating cells will be labeled by incorporation of an analog of thymidine, bromodeoxyuridine, into the DNA of s-phase cells. The bromodeoxyuridine is then detectable using an immunostaining technique employing a monoclonal antibody. Dogs will be monitored for any potential adverse effects of bFGF and VEGF by periodically assessing (through blood tests) various hematologic and biochemical parameters in the three groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04834-02 CB

PERIOD COVERED: October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiologically Induced Left Ventricular Hypertrophy and Screening of Athletes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Barry J. Maron, M.D.	Senior Investigator	CB	NHLBI
Paolo Spirito, M.D.	Senior Investigator	CB	NHLBI
Jannet F. Lewis, M.D.	Guest Researcher	CB	NHLBI
Antonio Pelliccia, M.D.	Guest Researcher	CB	NHLBI

COOPERATING UNITS *(if any)*

Howard University Hospital, Washington, D.C. and Institute of Sport Science, Rome, Italy

LAB/BRANCH: Cardiology Branch

SECTION: Echocardiographic Laboratory

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Reliable clinical distinctions in highly trained competitive athletes between physiologically-induced morphologic changes ("athlete heart") and hypertrophic cardiomyopathy is often difficult. It would appear from recent data that left ventricular wall thicknesses of 16 mm are incompatible with athlete heart. Also Doppler echocardiographic assessment of left ventricular filling may aid in resolving the differential diagnosis of these two conditions, in that filling patterns are virtually always normal in athletes with wall thickening and abnormal in 80% of patients with mild morphologic expressions of hypertrophic cardiomyopathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04835-02 CB

PERIOD COVERED: October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Significance of Left Ventricular Mass in Hypertrophic Cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Barry J. Maron, M.D.	Senior Investigator	NHLBI	CB
Paolo Spirito, M.D.	Senior Investigator	NHLBI	CB
Jannet F. Lewis, M.D.	Guest Researcher	NHLBI	CB

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Cardiology Branch

SECTION
Echocardiographic Laboratory

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Left ventricular hypertrophy is the sine quo non of hypertrophic cardiomyopathy and also responsible for many of the pathophysiologic consequences of the disease. The extent and pattern of left ventricular wall thickening is variable within the disease spectrum and it may show pronounced changes which describe clinical course or be a determinant of clinical events and functional abnormalities. Consequently, contrary to prior exceptions, left ventricular hypertrophy in hypertrophic cardiomyopathy is dynamic and often shows striking changes through the lifetime of the patient.

Significance of Left Ventricular Mass in Hypertrophic Cardiomyopathy

Recent studies have defined the morphologic and clinical expression of hypertrophic cardiomyopathy in older patients >60 years of age in whom the onset of severe symptoms and progressive course is deferred to relatively late in life. Often left ventricular structure is particularly distorted by the accumulation of calcium in the mitral annulus and the mechanism of dynamic subaortic obstruction differs from younger patients with this disease. In elderly patients with hypertrophic cardiomyopathy, obstruction to left ventricular outflow is effected largely by posterior ventricular septal motion in systole and contact with the abnormal-sized mitral valve, rather than only by the sharp anterior excursion of the greatly enlarged mitral valve that is present in younger patients with this disease.

Significance of the magnitude and extent of left ventricular hypertrophy in influencing clinical course has been the subject of a series of investigations. We have shown that striking and dynamic progression of left ventricular hypertrophy is a normal process during childhood and adolescence in patients with hypertrophic cardiomyopathy, and in some predisposed individuals the de novo development of left ventricular hypertrophy may be deferred until as late as age 15. Furthermore, such patients may show electrocardiographic abnormalities before the development of hypertrophy, as a prelude to the development of the morphologic expression of the disease. Later in life some patients 30-50 years of age may show decrease in left ventricular wall thickness, often associated with deteriorating systolic function an enlarging cavity size, and progressive congestive symptoms as well as the development of chronic atrial fibrillation. Indeed, when large populations of patients with hypertrophic cardiomyopathy were analyzed, wall thickness decreased with aging and particularly marked hypertrophy appeared to exist only in those patients <40 years of age. Hence, the morphologic appearance of the left ventricle in hypertrophic cardiomyopathy characteristically differs at the various stages of life and certain patients may show dynamic structural changes throughout life.

Other data suggests certain pathophysiologic significance with regard to the extent of left ventricular hypertrophy in hypertrophic cardiomyopathy. For example, more substantially increased left ventricular mass occurs in those patients who have experienced cardiac arrest or sudden death, as well as patients with nonsustained (asymptomatic) ventricular tachycardia on 24 hour ambulatory electrocardiogram, a proven harbinger of sudden death in this disease. While greater magnitude and more diffuse distribution of left ventricular thickening appear to be related to the likelihood of electrical events and sudden catastrophes in hypertrophic cardiomyopathy, the significance of extent of hypertrophy with respect to certain cardiac functional abnormalities may not be as certain. For example, while left ventricular wall thickening is a sine que non of hypertrophy, and left ventricular diastolic dysfunction is the most common pathophysiologic abnormality responsible for congestive symptoms in hypertrophic cardiomyopathy, the extent of hypertrophy bears little relationship to the presence or magnitude of left ventricular diastolic filling abnormalities, (as assessed by Doppler echocardiography). Hence, substantial left ventricular wall thickening is not required for left ventricular filling, hearts with localized and relatively mild hypertrophy may show diastolic dysfunction, and normal or only mildly thickened portions of the left ventricular wall may contribute to abnormal filling and the cardiomyopathic process in hypertrophic cardiomyopathy.

Project Number: Z01 HL 04835-02 CB

6. NHLBI INTRAMURAL PROJECT CODING SHEET

Instructions: Place a checkmark next to each category that applies to this project. The degree or percentage of relevance to each category may be requested from you at a later date. Please enter additional information where requested.

___ AIDS or related

___ Biotechnology

✓ Clinical study

Ethnicity: Caucasian

Gender: both

Age range: 10 - 75 years

___ Diabetes

___ Diagnostic imaging/radiology

___ Digestive Diseases

___ Endocrinology

___ Fish oil

___ Genetic disorder

(Specify:_____)

___ Genome mapping and sequencing

___ Human fetal tissue

___ Laser technology

___ Neurotoxicology

___ Nutrition

___ Organ transplantation

___ Prevention

___ Toxicology

********* ORPHAN DISEASES *********

✓ Cardiomyopathies

___ Cooley's anemia

___ Cystic fibrosis

___ Epstein-Barr Virus

___ Familial emphysema

___ Hemophilia

___ Kawasaki Disease

___ Lipid disorders

___ Lupus - Systemic Erythematosis

___ Sarcoidosis

___ Sickle cell disease

___ Sjogren's

___ Von Willebrand Factor & related

___ Other

(Specify: _____)

** SEXUALLY-TRANSMITTED DISEASES **

___ Cytomegalovirus (CMV)

___ Hepatitis (Type: _____)

___ Herpes

B/A/D: Basic: ____ + Applied: 100% + Developmental: ____ = 100 Percent

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04836-01 CB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Impact of Operation on Thallium Perfusion Defects in Hypertrophic Cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sec.	CB NHLBI
Vasken Dislizian, M.D.	Clinical Investigator	CB NHLBI
Robert O. Bonow, M.D.	Deputy Chief	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Reversible thallium defects during exercise are common in patients with hypertrophic cardiomyopathy and are believed to indicate myocardial ischemia. In order to assess the impact of operative relief of left ventricular outflow obstruction on thallium defects in symptomatic patients with hypertrophic cardiomyopathy, 11 patients underwent tomographic thallium scintigraphy prior to and 6 months following septal myotomy/myectomy (7) or mitral valve replacement (4). Operation resulted in reduction of basal (39±30 to 3±6 mmHg) and peak provocable (126±27 to 32±30 mmHg) left ventricular outflow gradients, and basal (20±7 to 13±4 mmHg) and post pacing (33±8 to 21±6 mmHg) left ventricular end-diastolic pressures (all $p < 0.01$). Pre-operatively 10 of 11 patients had reversible thallium defects during exercise (7-regional thallium defects, 9-apparent cavity dilatation with increased lung uptake of thallium). Post-operatively, 7 of these 10 patients had significant improvement in exercise thallium results (4 with completely normal scans), compared to pre-op studies. Thus, operative relief of left ventricular outflow obstruction in hypertrophic cardiomyopathy results in substantial improvement in exercise left ventricular perfusion and stress-induced left ventricular filling pressures, suggesting decreased ischemia and its hemodynamic sequelae.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04837-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Exercise in Hypertrophic Cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sec.	CB NHLBI
Vasken Dilsizian, M.D.	Clinical Investigator	CB NHLBI
Robert O. Bonow, M.D.	Deputy Chief	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Apparent cavity dilatation is a common finding during exercise tomographic thallium scintigraphy in patients with hypertrophic cardiomyopathy. To investigate the mechanism of apparent cavity dilatation, 50 patients with hypertrophic cardiomyopathy underwent exercise thallium scintigraphy, catheterization study, and radionuclide angiography. In the 26 patients with apparent cavity dilatation on thallium scintigraphy, left ventricular outflow tract gradients (61 ± 48 vs 27 ± 35 mmHg, $p < 0.01$) and left ventricular end-diastolic pressure (20 ± 7 vs 16 ± 6 mmHg, $p < 0.05$) were higher, and maximum provocable gradients tended to be higher (114 ± 35 vs 89 ± 54 mmHg) compared to the 24 patients without apparent cavity dilatation on thallium scintigraphy. Ejection fractions and indices of diastolic filling at rest were similar between the two groups. However, the 26 hypertrophic cardiomyopathy patients with apparent cavity dilatation on scintigraphy were more likely to have myocardial ischemia (myocardial lactate production) during pacing at 150 beats per minute (21 of 26 vs 10 of 24, $p = .01$), have higher left ventricular end-diastolic pressures post pacing (32 ± 5 vs 24 ± 10 mmHg, $p < 0.01$) and have associated increased lung uptake of thallium with exercise (14 of 26 vs 2 of 24, $p < 0.002$). Thus, stress induced apparent cavity dilatation may result from ischemia-related diastolic dysfunction leading to endocardial compression and elevated pulmonary pressures, and is most likely to occur in patients with high basal left ventricular outflow gradients and left ventricular end-diastolic pressures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04838-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Systemic Alpha-2 Adrenoceptor Blockade and the Human Heart & Forearm

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sec.	CB	NHLBI
Ehud Grossman, M.D.	Visiting Associate	H&E B	NHLBI
Arshed A. Quyyumi, M.D.	Senior Investigator	CB	NHLBI
David S. Goldstein, M.D., Ph.D.	Senior Investigator	H&E B	NHLBI

COOPERATING UNITS *(if any)*: Cardiology Branch and Hypertension & Endocrinology Branch

LAB/BRANCH: Cardiology Branch

SECTION: Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation of the sympathetic nervous system and catecholamine kinetics may provide insight into coronary and systemic blood flow regulation. Although α-adrenoceptor blockade produces vasodilatation, blockade of presynaptic α-2 receptors stimulates release of norepinephrine. To assess the impact of systemic α-2 receptor blockade on the heart, yohimbine was infused intravenously in 12 patients with normal coronary arteries. We measured systemic and coronary hemodynamics and systemic and coronary flow and resistance, as well as norepinephrine spillover into the systemic artery and coronary and femoral veins. Yohimbine increased the spillover of norepinephrine into the great cardiac vein, artery and femoral vein by 117, 157, and 852% respectively. Systemic α-2 blockade increased cardiac norepinephrine spillover with no change in coronary vascular resistance, contrasting with increased resistance and much larger increases in norepinephrine spillover in the forearm. Thus, direct vasodilator effects of α-2 blockade by yohimbine are overwhelmed by vasoconstrictor effects due to a release of norepinephrine, especially in the systemic circulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04839-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Exercise hemodynamics in hypertrophic cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sec.	CB	NHLBI
Jean Diodati, M.D.	Visiting Scientist	CB	NHLBI
Myron A. Waclawiw, Ph.D.	Statistician		NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most, but not all patients with hypertrophic cardiomyopathy, report symptom benefit following operative relief of left ventricular outflow obstruction. In order to determine predictors of exercise benefit, 25 patients underwent pre-operative and 6 month post-operative catheterization and exercise hemodynamic measurements. Increase in oxygen consumption between pre-operative and post-operative treadmill exercise tests (Bruce protocol) was chosen as an index of exercise benefits. Twenty-two different variables were investigated for predictive significance. Univariate analyses indicated pre-operative left ventricular end-diastolic pressure and post-operative reduction in left ventricular end-diastolic pressure as significant predictors of post-operative increase in maximum oxygen consumption during exercise. There was borderline significance for post-operative reduction in left ventricular outflow gradient. Multivariate step-wise regression analysis of pre-operative hemodynamic variables selected pre-operative left ventricular end-diastolic pressure as a significant predictor of post-operative increase in oxygen consumption during exercise. We conclude that elevated left ventricular filling pressures identify patients most likely to demonstrate exercise benefit following relief of outflow obstruction in hypertrophic cardiomyopathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04840-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Airway hyperresponsiveness in microvascular angina

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sec.	CB	NHLBI
Stephen E. Epstein, M.D.	Chief	CB	NHLBI
David B. Peden, M.D.	Staff Fellow, Clinical Allergy Section		NIAID
Michael A. Kaliner, M.D.	Chief, Clinical Allergy Branch		NIAID

COOPERATING UNITS *(if any)*

Clinical Allergy Branch, NIAID

LAB/BRANCH
Cardiology Branch

SECTION
Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Anginal chest pain in patients with angiographically normal coronary arteries may be caused by a limited coronary flow response to stress because of abnormal function of the coronary microcirculation (microvascular angina). Studies of forearm arterial function suggested that patients with microvascular angina may have a diffuse disorder of smooth muscle tone. Because dyspnea is common in these patients and seems disproportionate to the severity of myocardial ischemia, we studied airflow in the basal state and following methacholine inhalation in order to determine whether bronchial smooth muscle is affected in this syndrome. Five of 36 patients with microvascular angina had a basal forced expiratory volume in 1 second (FEV1) less than 70% predicted and did not receive methacholine. Of the remaining 31 patients, 14 (45%) had a greater than 20% reduction in FEV1 following methacholine inhalation (up to 25 mg/ml), a response significantly greater than that of 9 patients with heart disease (0%, $p<0.025$) and 24 normal volunteers of similar age and gender distribution (13%, $p<0.025$). Further, the product of the methacholine dose inhaled and magnitude of decline in FEV1 from baseline (methacholine response score) was significantly lower in microvascular angina patients than in normal volunteers (16 ± 8.6 vs 22.2 ± 3.7, $p<0.025$). We conclude that airway hyperresponsiveness is frequently demonstrable in patients with microvascular angina, findings consistent with our hypothesis that this syndrome may represent a more generalized abnormalality of vascular and nonvascular smooth vessel function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04841-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Coronary Flow Reserve and Esophageal Motility in Patients with Chest Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III — Head, Cardiovascular Diag. Sec. — NHLBI CB

Edward L. Cattau, M. D. — Gastroenterology Division, Georgetown University Hospital

Stanley B. Benjamin, M.D. — Chief, Gastroenterology Division, Georgetown University Hospital

Stephen E. Epstein, M.D. — Chief — NHLBI CB

COOPERATING UNITS *(if any)*

Gastroenterology Division, Georgetown University Hospital

LAB/BRANCH: Cardiology Branch

SECTION: Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To ascertain the relative prevalence of abnormalities of coronary flow reserve and esophageal function in patients with chest pain despite angiographically normal coronary arteries, 87 patients underwent an invasive study of coronary flow reserve, and during the same week, esophageal testing. Twenty of 87 patients (23%) had abnormal esophageal motility, including 16 of 63 patients with abnormal coronary flow reserve (microvascular angina). Seventy-one of 87 patients (82%) with anginal like chest pain and normal epicardial vessels in our series had a disorder of either coronary flow reserve, esophageal motility, and/or reproduction of typical chest pain during acid infusion. Of interest, chest pain was commonly encountered during cardiac and esophageal testing (85% of patients), regardless of the ability to demonstrate an abnormality of coronary flow reserve or abnormal esophageal function. This suggests that pain experienced by these patients may be a consequence of myocardial ischemia, esophageal dysfunction, abnormal visceral nociception, or a combination of any or all of these entities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04842-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Left Ventricular Performance in Asymptomatic Aortic Stenosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sect.	NHLBI CB
Christopher Clyne, M.D.	Research Associate	NHLBI CB
Robert O. Bonow, M.D.	Deputy Chief	NHLBI CB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnostic Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Symptomatic patients with aortic stenosis exhibit abnormal ventricular function during exercise. Using radionuclide angiography, we studied left ventricular systolic and diastolic performance in 11 asymptomatic patients with isolated aortic stenosis (rest gradient by continuous wave Doppler 64±31 mmHg, range 40-144 mmHg) and left ventricular hypertrophy by echo. Patients were compared to 12 aged and gender-matched normal volunteers. Changes from rest to exercise ejection fraction, stroke volume, cardiac output, and end-diastolic and end-systolic volumes were quantified for each group. Peak diastolic filling rate at rest was reduced in patients with aortic stenosis compared to normals. During supine bicycle exercise, patients with asymptomatic aortic stenosis demonstrated significantly less of an increase in left ventricular ejection fraction during supine exercise. This was associated with less of an increase in cardiac output and actual falls in stroke volume and end-diastolic volume during exercise compared to increases in these parameters in the normal volunteers. Thus, abnormal left ventricular systolic and diastolic function limits the cardiac output response to exercise, resulting in effort limitation in patients with aortic stenosis, even when clinically asymptomatic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04843-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Exercise Responses in Asymptomatic Aortic Stenosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard O. Cannon, III, M.D.	Head, Cardiovascular Diag. Sect.	NHLBI CB
Christopher Clyne, M.D.	Research Associate	NHLBI CB

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Cardiology Branch

SECTION: Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Exertional symptoms in symptomatic patients with aortic stenosis have been attributed to abnormal exercise hemodynamics. In order to assess exercise hemodynamics in asymptomatic patients with aortic stenosis, 11 patients (valve gradient 64±31 mmHg by Doppler) underwent symptom limited exercise (Bruce protocol) with intra-arterial blood pressure monitoring. Rest and maximum systolic blood pressure, percent of predicted maximum oxygen consumption, percent of predicted maximum O2 pulse (an index of stroke volume), exercise duration, age, and maximum heart rate were compared to 25 controls of similar age distribution. Compared to the controls, the asymptomatic patients with aortic stenosis achieved a lower systolic blood pressure, a lower percent of predicted oxygen consumption, and a lower percent of predicted oxygen pulse. All but one patient stopped because of fatigue; the remaining patient complained of lightheadedness. No patient developed exercise-induced hypotension. Thus, aortic stenosis patients show impaired hemodynamic responses to upright exercise, even when asymptomatic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04844-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prediction of Ambulatory Myocardial Ischemia in Coronary Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Julio Panza, M.D.	Senior Staff Fellow	CB NHLBI
Arshed A. Quyyumi, M.D.	Senior Investigator	CB NHLBI
Jean Diodati, M.D.	Visiting Associate	CB NHLBI
Timothy Callahan	Machine Technician	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.2	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with stable coronary artery disease may experience episodes of myocardial ischemia during daily life which are often symptomatically silent. The presence of these episodes during ambulatory ECG monitoring is believed to provide prognostic information independent of that provided by exercise testing. However, the relationship between the findings of ambulatory ECG monitoring and the results of exercise testing is still undefined given the dissimilar results of previous reports. To further investigate this issue and, in particular, to establish the importance of the exercise protocol in determining the relationship between exercise testing and ambulatory ECG monitoring, we have studied 70 patients with stable coronary artery disease with 48-hour ambulatory ECG monitoring and treadmill exercise test using two different exercise protocols with slow (NIH Combined protocol) and brisk (Bruce protocol) increments in workload. All studies were carried out after withdrawal of medications. A close inverse correlation between ischemic threshold measured from exercise testing and the frequency and duration of ischemic episodes during ambulatory monitoring was observed using the NIH Combined protocol; however, a significantly weaker inverse correlation was found when using the Bruce protocol. The mean heart rate at the onset of ischemic episodes during monitoring did not differ significantly from the heart rate at the onset of ST segment depression during exercise testing with the NIH combined protocol, but it was significantly higher with the Bruce protocol. These findings indicate that a close relationship exists between ambulatory myocardial ischemia and the results of the exercise test; this relation is critically determined by the exercise protocol and is better observed with protocols that produce slow workload increments. These observations suggest that ambulatory ECG monitoring may not provide independent information in patients with stable ischemic heart disease and, therefore, does not need to be included in the routine evaluation of these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04845-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*: Variation in Ambulatory Myocardial Ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Julio Panza, M.D.	Senior Staff Fellow	CB NHLBI
Arshed A. Quyyumi, M.D.	Senior Investigator	CB NHLBI
Jean Diodati, M.D.	Visiting Associate	CB NHLBI
Timothy Callahan	Machine Technician	CB NHLBI
Stephen E. Epstein, M.D.	Chief	CB NHLBI

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Cardiology Branch

SECTION: Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
.4	.2	.2

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use stenderd unreduced type. Do not exceed the space provided.)*

Patients with stable coronary artery disease may experience episodes of myocardial ischemia during daily life. Circadian and short-term variation in the frequency of these episodes has been demonstrated previously. However, the occurrence of variation over long-time intervals and its relation to variations in ischemic threshold assessed from exercise testing are unknown. We studied 40 patients with ambulatory ECG monitoring and exercise treadmill test at initial evaluation and after a mean follow-up of one year during which no acute events or changes in symptoms occurred. During follow-up, 21 patients (52%) showed a significant change in the magnitude of myocardial ischemia during late in life. Patients who had an increase in the number of ischemic episodes during monitoring showed a significant fall in ischemic threshold (time of exercise at 1 mm of ST segment depression during exercise). Conversely, patients with a reduction in the number of episodes during follow-up had a significantly higher ischemic threshold at their last evaluation. Patients with no change in the number of episodes, also showed no significant changes in the results of exercise testing. These findings indicate that patients with coronary artery disease may experience significant variations in the magnitude of myocardial ischemia in daily life over relatively long periods of time, even without suffering an acute event or changes in symptoms. These changes in ambulatory myocardial ischemia are linked to parallel variations in ischemic threshold, and therefore can be predicted by analysis of the exercise test.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HL 04846-01 CB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms Of Ambulatory Ischemia in Coronary Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Julio Panza, M.D.	Senior Staff Fellow	CB	NHLBI
Arshed A. Quyyumi, M.D.	Senior Investigator	CB	NHLBI
Jean Diodati, M.D.	Visiting Associate	CB	NHLBI
Timothy Callahan	Machine Technician	CB	NHLBI
Stephen E. Epstein, M.D.	Chief	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Cardiology Branch

SECTION
Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
.4	.2	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Patients with stable coronary artery disease may experience episodes of myocardial ischemia during daily life. The mechanisms underlying the occurrence of these episodes are not well understood. To investigate this issue, we performed ambulatory ECG monitoring in 54 patients with stable coronary artery disease and positive exercise test. The ischemic threshold was measured from the exercise test as heart rate at the onset of ST-segment depression. An increase in heart rate preceded the vast majority (89%) of the ischemic episodes during monitoring. The number of episodes significantly correlated with ischemic threshold, and even more strongly with the number of times that the patients achieved ischemic threshold during monitoring. These findings indicate that the most important mechanism in the genesis of episodes of myocardial ischemia during daily life in patients with coronary artery disease is the increase in myocardial oxygen demands as evidenced by the increase in heart rate. The frequency of episodes of myocardial ischemia during daily life is determined by the frequency with which the ischemic threshold is reached during the day.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04847-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Circadian Variation in Vascular Tone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Julio Panza, M.D.	Senior Staff Fellow	CB NHLBI
Arshed A. Quyyumi, M.D.	Senior Investigator	CB NHLBI
Stephen E. Epstein, M.D.	Chief	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The presence of circadian variation in the occurrence of unfavorable events has been demonstrated in several cardiovascular conditions, including elevations in blood pressure, ambulatory myocardial ischemia, onset of myocardial infarction, sudden cardiac death, and stroke. To investigate the mechanisms underlying the circadian variation in cardiovascular disease, we studied 12 normal subjects with determinations of basal forearm vascular resistance and the response to intra-arterial infusion of phentolamine (alpha-adrenergic blocker) and sodium nitroprusside (non-specific vasodilator) at 7:00 AM, 2:00 PM, and 9:00 PM. A circadian variation in basal forearm vascular resistance was observed, resistance being highest in the morning, and lower in the afternoon and evening. Reductions in forearm vascular resistance with phentolamine showed a similar circadian distribution; however, no such variation in the vasodilator response to sodium nitroprusside was observed. These findings indicate that circadian variation exists in vascular tone, with highest tone in the morning and lower in the afternoon and evening. This variation is determined by changes in alpha-sympathetic vasoconstrictor activity and may causally contribute to the higher blood pressure and increased incidence of cardiovascular events observed in the morning hours.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04848-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Obstruction in Hypertrophic Cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Julio A. Panza, M.D.	Senior Staff Fellow	CB NHLBI
Tamara J. Maris	Special Volunteer	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiographic Laboratory

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hypertrophic cardiomyopathy is a primary myocardial disease characterized by a wide spectrum of different clinical and morphologic manifestations, including patients with and without obstruction to left ventricular outflow. To characterize the development of subaortic obstruction during childhood and to define morphologic determinants of this phenomenon, we studied 26 consecutive children who presented with nonobstructive hypertrophic cardiomyopathy at their first evaluation. During a mean follow-up of six years, 7 patients (27%) developed dynamic obstruction to left ventricular outflow as identified by the appearance of systolic anterior motion of the mitral valve on serial echocardiographic examinations. Both groups of patients (with and without development of obstruction) showed progession of left ventricular hypertrophy during follow-up. While patients who remained nonobstructive showed homogeneous progression of all left ventricular segments, the 7 patients with development of obstruction had selective increase in thickness of the basal anterior septum with a consequent reduction of the left ventricular outflow tract. These findings indicate the subaortic obstruction may appear in the hypertrophic cardiomyopathy during childhood. This phenomenon is associated with and is probably a consequence of narrowing of the left ventricular outflow tract secondary to selective progression of hypertrophy in the basal anterior ventricular septum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04849-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Silent Myocardial Ischemia Evaluated During Ambulatory ST Segment Monitoring

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Arshed Quyyumi, M.D.	Senior Investigator	CB	NHLBI
Julio Panza, M.D.	Senior Staff Fellow	CB	NHLBI
Jean Diodati, M.D.	Senior Staff Fellow	CB	NHLBI
Vasken Dilsizian, M.D.	Clinical Investigator	CB	NHLBI
Robert O. Bonow, M.D.	Senior Investigator	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Silent myocardial ischemia (SMI) is a condition which is characterized by an imbalance between the heart's requirement for oxygen (demand) and the supply of oxygen. When transient, this imbalance between supply and demand results in chest pain or angina but is often silent (SMI). Episodes of SMI can be detected by continuous electrocardiographic monitoring of the ST segment using Holter monitoring. Some reports have suggested that presence of SMI on Holter identifies a high risk subgroup of patients with coronary artery disease. We investigated 80 patients with mild coronary artery disease who were not candidates for bypass surgery. Approximately half had SMI during normal daily activities but there was no increase in the frequency of heart attacks, death or unstable angina in these patients compared with those with absence of SMI indicating then the presence of SMI does not necessarily increase the risk in patients who are otherwise in the low risk category..

Several techniques are currently available for detecting transient myocardial ischemia. These include treadmill exercise testing, exercise thallium scintigraphy, exercise radionuclide ventriculography, and ambulatory ST monitoring for SMI. To investigate the relationship between SMI detected by Holter monitoring and other techniques, we performed a multivariate analysis in 100 patients who had all tests including cardiac catheterization. The independent predictors of SMI during daily life were the 1) treadmill exercise time to onset of ischemia, 2) the heart rate during treadmill test at the onset of ischemia, and 3) the R wave height in lead V5 of the resting electrocardiogram. The lack of predictability with nuclear techniques can largely be explained by the poor detection of SMI during ambulatory monitoring in patients with previous heart attack.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04850-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Endothelium-Dependent Coronary Microvascular Vasomotion in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Arshed Quyyumi, M.D.	Senior Investigator	CB NHLBI
Richard O. Cannon, M.D.	Senior Investigator	CB NHLBI
Stephen E. Epstein, M.D.	Chief	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Cardiology Branch

SECTION: Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The lining of blood vessels, called the endothelium, is capable of secreting substances which allow blood vessels to relax in response to a variety of stimuli. One factor released is known as endothelium-derived-relaxant-factor (EDRF). Acetylcholine, a naturally occurring substance, is able to promote release of EDRF from blood vessels. Absence or dysfunction of the endothelium leads to diminished vasodilation or vasoconstriction with acetylcholine. Several previous studies have examined the effect of acetylcholine on large coronary arteries, but little information is available on the role of EDRF in the small blood vessels of the heart. We examined the importance of the dose of acetylcholine administered on the response of the small blood vessels of the heart. It is accepted that the response to acetylcholine of large coronary arteries in older patients and in patients with raised blood cholesterol level is diminished, but we found that the small blood vessel response to acetylcholine was not influenced significantly by age or the blood cholesterol level. We also examined the importance of the endothelium in patients who have chest pain and evidence of myocardial ischemia (angina) despite normal large coronary arteries, by investigating the effects of acetylcholine infusions on the reactivity of the small coronary arteries and comparing it with the response to another vasodilator, nitroprusside. Nitroprusside does not depend on an intact endothelium for its effect. This comparison revealed that a proportion of patients with chest pain despite normal large coronary arteries have dysfunction of their endothelium, with a diminished response to acetylcholine and a normal response to nitroprusside.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04851-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Platelet activation in patients with coronary artery disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jean G. Diodati, M.D.	Visiting Associate	NHLBI CB
Arshed A. Quyyumi, M.D.	Senior Investigator	NHLBI CB
Richard O. Cannon, III, M.D.	Head, Cardiovascular Diagnosis Sec.	NHLBI CB
Stephen E. Epstein, M.D.	Chief	NHLBI CB

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Cardiology Branch

SECTION: Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Platelet activation is believed to play a role in the pathophysiology of coronary artery disease. To determine whether diseased epicardial or small vessel coronary arteries create conditions that activate platelet, we measured platelet aggregation in 25 patients with large vessel coronary artery disease, 14 patients with microvascular angina and 6 normals. We measured platelet hyperreactivity by impedance aggregometry, a technique that looks at the tendency the platelets have that clump together in whole blood.

In our first study we found that platelets that pass through an atherosclerotic bed during rapid atrial pacing become hyperreactive compared to platelets that pass through a normal coronary bed, also during rapid atrial pacing.

In a second study we found that maximum upright exercise causes a systemic increase in platelet reactivity in 10 patients with coronary artery disease and 5 patients with chest pain and normal coronary arteries.

The platelet hyperreactivity in the two situations described above, through platelet induced spasm or platelet plugs in the coronary circulation, both decreasing blood supply to the myocardium, may contribute to ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04852-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A model of arterial smooth muscle cell proliferation to study coronary restenosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ellis F. Unger, M.D.	Senior Investigator	CB	NHLBI
Shmuel Banai, M.D.	Visiting Associate	CB	NHLBI
Michael Jaklitsch, M.D.	Visiting Fellow	CB	NHLBI
Matie Shou, M.D.	Visiting Fellow	CB	NHLBI
Stephen E. Epstein, M.D.	Chief	CB	NHLBI
Ira H. Pastan, M.D.	Chief	LMB	NCI
Clay Siegall, Ph.D.	Staff Fellow	LMB	NCI

COOPERATING UNITS *(if any)*

National Cancer Institute

LAB/BRANCH: Cardiology Branch

SECTION: Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Percutaneous transluminal coronary angioplasty (PTCA) has become a routine mode of therapy to relive arterial luminal stenosis in patients with coronary artery disease. Other trans-catheter interventional techniques, such as laser devices, mechanical atherectomy, and endovascular stents are currently being used in human patients. The "Achilles heel" of all interventional intravascular techniques is the phenomenon of arterial restenosis that occurs within 3 months after the PTCA in about 30% of the patients. It is likely that damage to the vessel wall during the arterial stretch at the time of angioplasty causes smooth muscle cell proliferation and the subsequent development of restenosis. Thus, restenosis is a healing phenomenon resulting from arterial injury and is independent of the atherosclerotic process.

Efforts to gain better understanding of this phenomenon have been confounded by the lack of an appropriate animal restenosis model. By applying localized mechanical pressure to a rabbit artery, disruption of the normal histological architecture of the arterial wall occurs. As a result, striking smooth muscle cell proliferation occurs in the media of the vessel; a histopathologic reaction similar to human restenosis. In a preliminary study, this phenomenon was highly reproducible (75%).

We are now beginning a series of experiments in which we plan to selectively inhibit the process of smooth muscle proliferation with a variety of genetically engineered peptides and toxins. Endpoints of the studies will relate to the degree of smooth muscle cell proliferation and extent of narrowing of the vessel lumen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04853-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Promotion of Myocardial Angiogenesis via Direct Application of aFGF to the Heart

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Ellis F. Unger, M.D.	Senior Investigator	CB	NHLBI
Shmuel Banai, M.D.	Visiting Associate	CB	NHLBI
Michael Jaklitsch, M.D.	Visiting Fellow	CB	NHLBI
Matie Shou, M.D.	Visiting Fellow	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

Veterinary Resources Branch, NIH

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	4.4	PROFESSIONAL:	2.9	OTHER	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We sought to determine the effect of acidic fibroblast growth factor (aFGF) on ischemic and normal myocardium, and to determine whether direct application of aFGF to the heart could promote angiogenesis. Dogs underwent placement of an ameroid constrictor on the left anterior descending coronary artery (LAD). Three weeks later, a left internal mammary artery pedicle (IMA) was positioned over the LAD territory. A gortex or collagen I sponge saturated with aFGF (N=12) or saline (N=4) was interposed between the pedicle and the heart. Weekly angiography of the IMA was performed in all dogs, but significant IMA to coronary collaterals were not demonstrable in any dog. Eight dogs had histologic evidence of non-transmural infarction in the LAD territory (5 aFGF, 3 control). Striking smooth muscle cell (SMC) hyperplasia was present in arterioles and small arteries exclusively in areas of subendocardial infarction in all of the aFGF-treated dogs but in none of the control dogs (p<0.05). Non-infarcted myocardium appeared normal in all dogs. Two additional dogs received a similar aFGF-sponge but no ameroid; no pathology was seen. Thus, aFGF appears to stimulate SMC hyperplasia in areas subjected to infarction. Both injury and exposure to aFGF may be required to cause vascular SMC proliferation in the heart.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04854-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Localization of Basic and Acidic FGFs in the Developing Rat Heart

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paolo Spirito, M. D.	Senior Investigator	NHLBI	CB
Ya-Min Fu, M. D.	Visiting Scientist	NHLBI	CB
Zu-Xi Yu, M.D.	Visiting Scientist	NHLBI	CB
Ward Casscells, M.D.	Senior Investigator	NHLBI	CB
Stephen E. Epstein, M.D.	Chief	NHLBI	CB

COOPERATING UNITS *(if any)*

none

LAB/BRANCH: Cardiology Branch

SECTION: Section on Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1	.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☑ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

In the present study we used Western blot techniques to investigate the expression of aFGF and bFGF in the rat embryo, and we used immunohistochemical techniques to define the cellular and tissue distribution of aFGF and bFGF in embryos at different stages of development (from 11 to 20 days). Forms of 26 to 29 kD for aFGF and bFGF were identified both in crude tissue extracts and heparin-bound material, and showed mitogenic activity for 3T3 fibroblasts. Thus, they appear to be the main natural forms of aFGF and bFGF in the rat embryo. Immunoreactive aFGF and bFGF were identified in the cytoplasm of cells of neuroectodermal and mesodermal origin but not in endoderm-derived cells, and they were ubiquitous in the extracellular matrix. The distribution of aFGF and bFGF also showed changes during development that were associated with the process of cellular and tissue differentiation. For example, intensity and extent of immuno- reactivity for both peptides progressively increased in the middle layer of the spinal cord with increasing differentiation of the neural cells. The immunostaining patterns for aFGF and bFGF were virtually superimposable for each organ and at each stage. In conclusion, the nonuniform but specific tissue distribution of aFGF and bFGF and the changes in their distribution during embryonic development, suggest that these peptides have different regulatory effects in different cells and tissues during organogenesis. Their coexpression implies some form of functional interaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04855-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphology and growth factors density in human coronary atherectomy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Moshe Y Flugelman, M.D.	Special Volunteer	NHLBI CB
Ward Casscells, M.D.	Senior Staff Fellow	NHLBI CB
Martin Leon, M.D.	Senior Investigator	NHLBI CB
Ya-min Fu, M.D.	Special Volunteer	NHLBI CB
Gad Keren, M.D.	Special Volunteer	NHLBI CB
Rosalie Correa, M.D.	Special Volunteer	NHLBI CB
Stephen E. Epstein, M.D.	Chief	NHLBI CB

COOPERATING UNITS *(if any)*

Cardiology, Washington Hospital Center, Washington, D.C.

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Restenosis of the coronary artery develops in up to 40% of the patients who undergo coronary angioplasty. The development of restenosis imposes a major therapeutic and economic disadvantage to the commonly employed coronary angioplasty. The recent introduction of coronary atherectomy enables us to obtain coronary artery specimens for research purposes as a by-product of the therapeutic procedure. Study of the human coronary artery specimens, both of patients with restenosis and of patients with primary atheromata, will provide us with the opportunity to improve our understanding of restenosis and atherosclerosis.

The present study was initiated to test the hypothesis that post angioplasty restenosis is mediated by growth factors and/or their receptors. We hypothesize that the mechanical trauma to the coronary artery and the exposure of vessel wall elements to the different components of the blood induce increased expression of growth factors and /or their receptors: hence, the induction of smooth cell proliferation and migration. Human coronary atherectomy specimen will be stained for different cellular and matrix content. The density of receptors to FGF and TGF Beta will be studied by the use of immunocytochemistry methods. Primary atheromata specimens will be compared to specimens obtained from patients with post angioplasty restenosis and the density of growth factors receptors will be quantitated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04856-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hemodynamics of Coronary Artery Stents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jon A. Peacock, M.D., Ph.D.	Medical Staff Fellow	NHLBI	CB
Robert Lutz, Ph.D.	Chemical Engineer	DRR	BEIB
Travis Jones, Ph.D.	Chemical Engineer	DRR	BEIB

COOPERATING UNITS *(if any)*

Biomedical Engineering and Istrumentation Branch, Division of Research Resources

LAB/BRANCH

Cardiology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	.90	.60

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During coronary angioplasty a balloon-tipped catheter is advanced to the site of an atherosclerotic lesion. By inflating the balloon the artery is opened and the normal flow of blood restored without having to resort to open heart surgery. Unfortunately, in about one third of patients who undergo angioplasty the artery restenoses (or closes down again) within six months of the procedure. In recent years several novel approaches to the problem of restenosis have been undertaken, including coronary stents. These devices are made of stainless steel and form an expandable scaffold around the angioplasty balloon. When the balloon is inflated at the side of the coronary lesion, the stent also expands and remains in place within the artery to hold it open and prevent restenosis.

Preliminary clinical trials with stents have shown promising results. However, there have been some cases of thrombosis (blood clots) within the stent. We were concerned that abnormal flow disturbances induced by the stent could be responsible for such thrombosis. To study this possibility, we built a model heart and used a transparent fluid with the same properties as blood. By mounting electrochemical sensors flush with the walls of our model coronaries, we were able to compare the stability of flow in these arteries before and after the insertion of coronary stents. We discovered that under simulated resting conditions, the stents had no effect on coronary flow patterns. However, under mild exercise conditions, the stents disturbed the flow in their immediate downstream vicinity. If prolonged, the wall shear stress was sufficient in this case to cause platelet aggregation and thrombosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04857-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Transduced Endothelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David Dichek, M.D.	Senior Staff Fellow	MHB NHLBI
Michael Jaklitsch, M.D.	Special Volunteer	CB NHLBI
Ward Casscells, M.D.	Senior Staff Fellow	CB NHLBI
Zu-Xi Yu, M.D.	Special Volunteer	CB NHLBI
Sadatoshi Biro, M.D.	Special Volunteer	CB NHLBI
Moshe Flugelman, M.D.	Special Volunteer	CB NHLBI
Edith Speir, B.S.	Biochemist	CB NHLBI
Stephen Epstein, M.D.	Chief	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☑ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Tissue plasminogen activator (tPA) is a protein that is active in the process of wound healing and angiogenesis. Recombinant human tPA has been produced and packaged into a retroviral vector that can be used to transduce endothelial cells. This opens the possibility of using endothelial cells that over-express tPA in in vivo wound healing and angiogenesis experiments. An initial step in this process is the careful in vitro characterization of transduced cells in regards to normal cellular function and invasiveness. Primary cultures of endothelial cells are obtained from sheep, rabbit, and cows. Pure endothelial cell cultures of the same passage are divided into 3 groups. The first group is transduced with the B2NSt viral vector, which contains the human tPA cDNA and a neomycin resistant gene. The second group is transduced with LBgSN viral vector, which contains the beta-galactosidase gene and a neomycin resistant gene. The second group serves as a transduced cell control. The third group is not transduced and serves as a normal control. Transduced cells are selected in G418, a neomycin analogue toxic to nontransduced eukaryotic cells, for 2 weeks. Cellular functions are tested in assays of cell attachment to gelatin-coated dishes, cell proliferation, migration, invasion of gels, and in vitro tube formation. Differences in the three groups are quantitated and tested for statistical significance. An endothelial cell with increased invasiveness while maintaining differentiated cellular functions may be able to augment natural wound healing and blood vessel growth in areas of injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04858-01 CB

PERIOD COVERED: October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diastolic Filling in "Athlete Heart" and Hypertrophic Cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jannet F. Lewis, M.D.	Guest Researcher	NHLBI	CB
Barry J. Maron, M.D.	Senior Investigator	NHLBI	CB
Antonio Pelliccia, M.D.	Guest Researcher	NHLBI	CB
Paolo Spirito, M.D.	Guest Researcher	NHLBI	CB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Cardiology Branch

SECTION: Echocardiography Laboratory

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In some athletes with a substantial increase in left ventricular wall thickness, it may be difficult to distinguish with certainty physiologic hypertrophy due to athletic training from hypertrophic cardiomyopathy with relatively mild wall thickening. To assess the potential role of Doppler echocardiographic indexes of diastolic filling in differentiating these two conditions, we measured Doppler diastolic indexes in 16 young competitive athletes with increased left ventricular wall thickness (range 13-16 mm) and in 12 young asymptomatic patients with nonobstructive hypertrophic cardiomyopathy and similar magnitude of left ventricular hypertrophy (range 13-18 mm).

In the athlete group, the mean values for each measured diastolic index was not significantly different from the mean values obtained in untrained normal subjects.

Conversely, in the patients with hypertrophic cardiomyopathy, mean values for each of the four Doppler diastolic indexes were significantly different from normal ($p<0.02$), and one or more indexes were abnormal in 10 (83%) of the 12 patients. These findings indicate that Doppler echocardiographic indexes of left ventricular filling may provide additional useful information and aid in distinguishing marked physiologic hypertrophy due to athletic training from pathologic hypertrophy associated with nonobstructive hypertrophic cardiomyopathy

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04859-01 CB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hypertrophic Cardiomyopathy with Marked Hypertrophy of Posterior Wall

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jannet F. Lewis, M.D.	Guest Researcher	CB	NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography Laboratory

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The vast majority of patients with hypertrophic cardiomyopathy (HCM) show asymmetric and predominant thickening of the anterior ventricular septum (VS); the posterior left ventricular free wall (PW) is usually spared. In striking contrast, we describe a select group of 17 patients with HCM, with marked asymmetric hypertrophy of PW. Patients were age 13 to 54 years (mean 31); 9 (53%) were female. PW thickness was 20-42 mm, while VS was only 12-24 mm (mean 17); 13 patients had PW thickness which substantially exceeded that of VS, creating the appearance of "inverted" LV asymmetry. Mitral systolic anterior motion and obstruction to LV outflow occurred in 16/17 patients. Most patients (11/17, 65%) had severe cardiac symptoms which did not improve with drug therapy; 6 of these underwent operation (mitral valve replacement in 5, myotomy-myectomy in only 1), and each improved. This subgroup of patients emphasizes the diverse morphologic spectrum of HCM, which includes patients with marked hypertrophy of PW in the presence of only mild VS hypertrophy. Because of relatively modest septal thickening, mitral valve replacement appears to be the operation of choice rather than myotomy-myectomy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04860-01 CB

PERIOD COVERED: October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Middle-Aged Asymptomatic Patients with Hypertrophic Cardiomyopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Barry J. Maron, M.D.	Senior Investigator	NHLBI	CB
Gabriela M. Hecht, M.D.	Guest Researcher	NHLBI	CB
Julio A. Panza, M.D.	Visiting Associate	NHLBI	CB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography Laboratory

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Patients with hypertrophic cardiomyopathy may present a wide spectrum of clinical and morphologic manifestations. Although some characteristics of the natural history of these patients are well known, the initial presentation and subsequent clinical course of certain subgroups are not yet well defined. To further our understanding of the natural history of hypertrophic cardiomyopathy, we analyzed 31 middle-aged patients (35 to 55 years), who initially presented to our institution with no or minimal symptoms. Another group of 30 age and sex-matched patients, but in this case symptomatic (NYHA functional class II), was utilized as a control group for the purpose of morphologic comparison.

Our data suggest that those patients with hypertrophic cardiomyopathy who do achieve middle-age without developing important symptoms, usually show a relatively benign long-term clinical course, predominantly nonobstructive form, and mild left ventricular hypertrophy in which the majority of patients have a localized distribution of left ventricular hypertrophy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04861-01 CB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mitral Valve Prolapse and Hypertrophic Cardiomyopathy: 500 Consecutive Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Ruth K. Petrone	Echo Technologist	CB	NHLBI
Julio A. Panza, M.D.	Senior Staff Fellow	CB	NHLBI
Heinrich G. Klues, M.D.	Guest Researcher	CB	NHLBI
Elfriede E. Peterson	Echo Technologist	CB	NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography Laboratory

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Hypertrophic cardiomyopathy and mitral valve prolapse are both conditions that may be genetically transmitted and also incur a risk for sudden cardiac death. While the distorted ventricular geometry in hypertrophic cardiomyopathy might suggest a predisposition to mitral valve prolapse, the frequency with which the two entities coexist is not known, nor is the clinical significance of such an association. To further define the relationship of hypertrophic cardiomyopathy to mitral valve prolapse, 529 consecutive patients with hypertrophic cardiomyopathy were studied by echocardiography. Unequivocal systolic prolapse into the left atrium was identified in only 16 (3%) of the 529 patients. Of these 16 patients, 8 also had systolic anterior motion (including 5 with mitral-septal contact and basal outflow obstruction); 10 of the 16 had evidence of substantial mitral regurgitation. Of note, 9 of the 16 patients with hypertrophic cardiomyopathy and mitral valve prolapse had experienced atrial fibrillation associated with marked left atrial enlargement compared to 75 of 513 patients with hypertrophic cardiomyopathy but without prolapse.

Hence, in a large population of patients with hypertrophic cardiomyopathy, prevalence of mitral prolapse was relatively low. This frequency of mitral valve prolapse would appear less than expected, based on the reported prevalence of mitral valve prolapse and hypertrophic cardiomyopathy individually in the general population. However, those patients in whom hypertrophic cardiomyopathy and mitral valve prolapse coexist appear to develop atrial fibrillation with greater frequency than the general population of hypertrophic cardiomyopathy. These data provide a mechanism by which to prospectively identify some patients with hypertrophic cardiomyopathy who are predisposed to develop atrial fibrillation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04862-01 CB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mitral Valve in Hypertrophic Cardiomyopathy: Evidence For Primary Abnormality

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Heinrich G. Klues, M.D.	Special Volunteer	CB NHLBI
Allen L. Dollar, M.D.	Staff Associate	PB NHLBI
William C. Roberts, M.D.	Chief	PB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI

COOPERATING UNITS *(if any)*

Pathology Branch, NHLBI

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0	0.1

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Hypertrophic cardiomyopathy is characterized by a hypertrophied non-dilated left ventricle and has been considered to be largely a disease of cardiac muscle. To assess the possibility that the mitral valve itself may be primarily involved in the cardiomyopathy disease process, we performed gross and histologic analyses on mitral valves from 94 patients with hypertrophic cardiomyopathy and 45 normal controls. Area of the mitral valve was increased in patients with hypertrophic cardiomyopathy compared to controls (12.9 ± 3.7 vs 9.1 ± 2.2 cm2; $p < 0.001$). This increase in mitral valve size was due primarily to an increase in leaflet length (2.2 ± 0.5 for anterior leaflet vs 1.7 ± 0.3 cm for controls; $p < 0.001$), since circumference did not differ between the two groups.

Of the 94 patients, the mitral valve was increased in area $\geq$ 12.0 cm2 in 55 (or 58%). In 12 of these 55 valves, both the anterior and posterior leaflets were enlarged; the other 43 valves with increased overall area showed asymmetric or segmental enlargement of either the anterior leaflet (36 patients) or the mid-scallop of the posterior leaflet (7 patients). Multiple regression analyses showed that variation in mitral valve area was largely independent of other clinical or morphologic components of the disease process. Mitral valve area was mildly but significantly associated only with gender and body height and these relationships accounted for only 20% of the variability identified in valve size. The observations and findings presented here strongly support the concept that the disease process in hypertrophic cardiomyopathy is not confined to the left ventricular myocardium and a primary congenital abnormality of mitral valve structure is characteristic of the majority of patients with this disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04863-01 CB

PERIOD COVERED: October 1, 1989 to September 30, 1990.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In vitro studies of cell physiology of fibroblast growth factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ward Casscells, M.D.	Senior Staff Fellow	NHLBI CB
Edith Speir, B.S.	Biochemist	NHLBI CB
Shashi Shrivastav, Ph.D.	Special Volunteer	NHLBI CB
Zu-Xi Yu, M.D.	Special Volunteer	NHLBI CB
Sadatoshi Biro, M.D.	Special Volunteer	NHLBI CB
Virginia Tanner	Special Volunteer	NHLBI CB
Ya-Min Fu, M.D.	Special Volunteer	NHLBI CB
Stephen E. Epstein, M.D.	Chief	NHLBI CB

COOPERATING UNITS *(if any)*

Section on Ultrastructure, Pathology Branch, NHLBI

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

For the past year we continued to focus on the cell physiology of basic and acidic fibroblast growth factors, using both in vitro and in vivo models.

Because these peptides have no signal sequences, the mechanism of their exit from the cell has been a mystery. We found in cultured endothelial cells that bFGF is localized to intracellular vesicles suggesting regulated (as supposed to continuous) secretion. Thus, cell death and other nonspecific forms of cell rupture are unlikely to be the only mechanism of exit from the cell.

We also found that cultured adult rat cardiac myocytes, endothelial and smooth muscle cells have more acidic and basic FGF and FGF receptors than do the same cells in vivo. The cultured myocytes, endothelial and smooth muscle cells also revealed specific cytoplasmic and nuclear staining. Nuclear staining was confirmed by heparin affinity chromatography, mitogen assays, and Westerns.

In cultured endothelial cells we found that migration in either low or high serum is associated with an increased expression of bFGF. Neutralizing antisera to bFGF caused some inhibition of migration of cells in low or high serum in the absence of added FGFs, suggesting that the FGFs are released by the migrating cells and that this facilitates their migration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02208-16 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Iron Chelation and Transfusional Hemachromatosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Arthur W. Nienhuis, M.D., Chief, Clinical Hematology Branch, NHLBI
Others: Patricia Griffith, R.N., Clinical Nurse Specialist, CHB, NHLBI
Janice Kimball, R.N., Clinical Nurse Specialist, CHB, NHLBI
W. F. Anderson, M.D., Branch Chief, Lab. of Molecular Hematology, NHLBI
Gary Brittenham, M.D., Division of Hematology, Cleveland General Hospital
R.A. Hutcheon, M.D., Dir. Home Care Program, Montreal Children's Hospital
Evan Tucker, M.D., Senior Investigator, Cardiology Branch, NHLBI

COOPERATING UNITS *(if any)*

Molecular Hematology Branch, NHLBI; Division of Hematology, Cleveland General Hospital, Cleveland, Ohio; Home Care Program; Montreal Children's Hospital Montreal, Canada; Cardiology Branch, NHLBI

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	-

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies are designed to evaluate the clinical benefits achieved by iron chelation in patients with chronic iron overload. Desferoxamine is administered by subcutaneous infusion and iron removal is determined by measurement of the serum ferritin and periodic non-invasive measurement of liver-iron concentration. Clinical status is evaluated by standard parameters including non-invasive testing of cardiac and endocrine function as indicated by the patients age and risk category. The study is designed to document the natural history of severe beta thalassemia, treated effectively with regular transfusions and chelation therapy tailored to the patient's clinical status. During the past year we have completed a detailed analysis of our experience with this regimen over the past twelve years. Fifty-nine patients were studied. Liver iron concentration as determined at the end of the study was directly related to the amount of transfusion given and inversely related to the amount of Desferal used during the interval between measurements. The patients were divided into two groups of equal size and clinical characteristics, based on compliance with the chelation regimen. Among patients with a significant iron burden at the time of initiation of chelation, nine of sixteen who were poorly chelated died during the study. There were no deaths among the well-chelated patients. There was also a much higher incidence of impaired glucose tolerance and diabetes mellitus among the poorly chelated patients (60 percent versus 9 percent). These data unequivocally establish the long-term benefits of regular chelation therapy in thalassemic patients requiring blood transfusions. Reversal of cardiac disease has been documented in a total of five patients now on intensive chelation therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02307-11 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retroviral Mediated Gene Transfer Into Hematopoietic Stem Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: David M. Bodine, Ph.D., Senior Staff Fellow, CHB, NHLBI
Others: Donald Orlic, Ph.D., Guest Researcher, CHB, NHLBI
Amanda Cline, Research Assistant, CHB, NHLBI
Stephen J. Brandt, M.D., Guest Researcher, CHB, NHLBI
Kevin McDonagh, M.D., Medical Staff Fellow, CHB, NHLBI
Nancy Seidel, Research Assistant, CHB, NHLBI
Arthur W. Nienhuis, M.D., Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gene replacement therapy has several potential therapeutic applications. Addition of a structurally normal human beta globin gene into bone marrow cells could correct the deficiency of beta globin synthesis in patients with beta thalassemia or replace the defective beta globin in the hemoglobin of patients with sickle cell disease. Retrovirus mediated gene transfer is the most efficient means to introduce new genetic material into eukaryotic cells. We have previously shown that a human beta globin gene transferred via a retrovirus into mouse bone marrow stem cells is expressed in mature erythroid cells, and not in other myeloid or lymphoid cells. We have defined the optimal conditions for gene transfer to mouse bone marrow stem cells. These conditions include pretreatment of the donor animals with 5-fluorouracil (5-FU) and culture of the donor bone marrow cells for 6 days in the presence of interleukin-3 (IL-3) and interleukin-6. Using our extremely high titer ($<10^{10}$ retrovirus particles/ml) amphotropic retrovirus producer clone we have successfully achieved gene transfer in 7 of 7 Rhesus monkeys reconstituted with infected bone marrow cells. The provirus has persisted in 3 of 3 animals analyzed between 95 and 110 days. These results demonstrate reproducible gene transfer to extremely primitive bone marrow progenitor cells in a large animal model, an important first step towards globin gene transfer to human patients. Our current focus is to optimize conditions for gene transfer to primates based on our results in mice, and to include the appropriate regulatory sequences for high level expression of the human beta globin gene in transduced cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02310-10 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterizaton of the Gene for Human Dihydrofolate Reductase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Takashi Shimada, M.D., Ph.D., Visiting Associate, CHB, NHLBI
Others: Hiroyuki Fujii, M.D., Ph.D., Visiting Fellow, CHB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Usa standard unreduced type. Do not exceed the space provided.)*

The functional anatomy of the bidirectional promoter of the human dihydrofolate reductase (DHFR) gene has been studied by an in vivo transient assay using the firefly luciferase gene as a reporter. We have recently identified a gene located immediately upstream from the DHFR gene. This upstream gene encodes a protein highly homologous to a bacterial DNA mismatch repair protein, Mut S, and therefore, we have named it the mismatch repair protein (MRP). The DHFR gene and the MRP gene are arranged in a head-to-head configuration separated by an 88 base pair segment and the expression of these two genes are regulated by a short bidirectional promoter. Deletion and substitution analyses have shown that the 109 base pair fragment is sufficient for bidirectional promoter activity. A sequence CACAAATA, that is the only AT rich segment of the DHFR promoter, is not required for promoter activity. However, the GC box, GGCGGG plays an important role in expression of both divergent genes. All four GC boxes in the promoter are functionally shared and modulate both transcriptional activities. Particularly, on GC box located in the center of the two genes is essential for bidirectional activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02313-08 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Identification of Regulatory Elements that Modulate Human Globin Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI

Others:

D. Bodine, Ph.D., Sr. Staff Fellow, CHB
A. Cline, Research Assistant, CHB
H. Lin, M.D., Med. Staff Fellow, CHB
C. Lowrey, M.D., Med. Staff Fellow, CHB
K. McDonagh, Med. Staff Fellow, CHB
A. Moulton, Research Assistant, CHB
P. Ney, M.D., Med. Staff Fellow, CHB
M. Purucker, M.D., Med. Staff Fellow, CHB
B. Sorrentino, M.D., Med. Staff Fellow CHB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.6	5.6	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Globin genes exhibit tissue, developmental and maturational specificity. It is our purpose to understand the molecular basis of globin gene regulation. Our efforts have focused on the beta-globin gene cluster that contains the epsilon, gamma and beta genes expressed during the embryonic, fetal and adult developmental periods, respectively. These genes are encompassed within a 60 kilobase segment of DNA on human chromosome 11. Within this cluster are several cis-acting regulatory elements that interact with trans-acting factors (proteins) to modulate globin gene expression. Our efforts have focused on three such regulatory elements. The locus activating region (LAR) located upstream from the cluster establishes erythroid specificity of expression. Four separate regulatory elements have been identified within the LAR. We have characterized a 20 base pair segment within one of these elements that functions as a very powerful enhancer. This enhancer is responsible for the increase in globin gene expression that occurs during erythroid maturation. It binds members of the AP-1 family including JUN and FOS species. However, its ability to augment hemoglobin synthesis during differentiation relies on its binding of an erythroid specific nuclear protein, NF-E2. The enhancer 3′ to the gamma globin gene and the upstream region of the gamma globin gene promoter have also been characterized. These sequences are involved in gene regulation during development. A complex of proteins have been shown to bind to related sequences in the two elements. One of these proteins may be fetal stage specific and thus could participate in the developmental switch. Our efforts are now focused on purification of these two proteins that may be particularly relevant to the developmental specificity of globin gene expression in erythroid cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02315-08 CHB

PERIOD COVERED

October 1, 1989 to September 31, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Treatment of Aplastic Anemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Neal S. Young, M.D.

Others: N. Frickhofen, M.D., Guest Researcher, CHB, NHLBI
S. Rosenfeld, M.D., Medical Staff Fellow, CHB, NHLBI
W. Jackson, M.D., Senior Staff Fellow, CHB, NHLBI
S. Anderson, Medical Technologist, CHB, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Infectious Diseases, NIAID (C.J. Lyle)
Mahidol University, Bangkok, Thailand (Dr. Surapol Issaragrisil)

LAB/BRANCH

Clinical Hematology Branch

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.55	2.25	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Aplastic anemia and other forms of bone marrow failure have clinical and laboratory features consistent with a possible viral etiology followed by immunological pathophysiology. Aplastic anemia may follow on a viral infection, especially non-A non-B hepatitis or infectious mononucleosis. Patients have evidence of activation of their immune system similar to that observed in many viral infections, including activation of cytotoxic lymphocytes, excessive lymphokine production, and decreased natural killer cell activity and number. We have previously reported the presence of Epstein-Barr virus in the bone marrow of some patients with aplastic anemia. With the recent cloning of the etiologic agent for non-A non-B hepatitis, termed hepatitis C, we have searched for such viral sequences in the bone marrow and blood of these patients. We have sought evidence of virus infection using a commercial assay for antibody to a nonstructural protein of hepatitis C, and in our own laboratory we have developed a sensitive and accurate polymerase chain reaction method for detection of hepatitis C sequences. Based on our previous work with dengue, also a flavivirus, in which viral propagation was demonstrated in hematopoietic cells, we have attempted to inoculate hepatitis C into hematopoietic cell lines and explanted bone marrow cultures. Hepatitis C is present in the bone marrow and blood of a subset of patients with aplastic anemia, but in most cases the presence of the virus is correlated with the development of transfusion-associated hepatitis and not present at outset.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02319-07 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

B19 (Human) Parvovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Neal S. Young, M.D.

Others: N. Frickhofen, M.D., Guest Researcher, CHB, NHLBI
S. Kajigaya, Ph.D., Fogarty Visiting Fellow, CHB, NHLBI
J. Liu, M.D., Medical Staff Fellow, CHB, NHLBI
S. Rosenfeld, M.D., Medical Staff Fellow, CHB, NHLBI
S. Anderson, Medical Technologist, CHB, NHLBI
K. Mishler, Biologist, CHB, NHLBI
S. Green, Chemist, CHB, NHLBI
T. Shimada, M.D., Visiting Associate, CHB, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Respiratory Diseases
Communicable Disease Center, Atlanta, Georgia (Dr. Larry Anderson)
Central Public Health Laboratory, London, England (Dr. Bernard Cohen)
Purdue University, West Lafayette, Indiana (Dr. Michael Rossman)

LAB/BRANCH

Clinical Hematology Branch

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.5	.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory performs basic and clinical studies of the B19 parvovirus, the only member of the Parvoviradae family pathogenic in humans. Acute infection causes fifth disease, a childhood rash illness and a polyarthralgia syndrome in adults. In patients with underlying hemolysis, acute infection results in transient aplastic crisis. In patients with underlying immunodeficiency, virus infection persists and causes chronic anemia. The virus is extraordinarily trophic for erythroid progenitor cells and has been propagated only in suspension cultures of human bone marrow. Current advances in our laboratory in the last year have centered around use of the baculovirus expression system to produce large quantities of both the capsid viral proteins and the single nonstructural protein. The self-assembled capsids made in infected insect cells will be useful as antigen in clinical immunoassays and as a vaccine to prevent the disease. The putative oncosuppressive effects of the nonstructural protein gene may be characterized for the first time using large quantities of recombinant protein. We have also shown that B19 parvovirus can enter cells like macrophages, granulocytes, and some lymphocytes, particularly in the presence of antibody, but that it does not replicate in these cells. The macrophage may be a natural reservoir of virus in persistently infected patients. Clinically, we have continued to accrue patients with acquired immunodeficiency syndrome (AIDS) who presented with pure red cell aplasia secondary to persistent parvovirus infection. These patients uniformly respond to immunoglobulin therapy, but in contrast to patients with congenital immunodeficiency, immunoglobulin therapy is not permanently curative. Relapse can be predicted by the level of CD4 cells on presentation and, more immediately, by recurrence of high-level viremia. Patients with AIDS probably are very infectious because of the high concentration of virus in their blood, and they represent a threat to other patients and to health care workers, especially pregnant nurses and physicians.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02320-07 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacologic Manipulation of HbF Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Arthur W. Nienhuis, M.D, Branch Chief, CHB, NHLBI
Others: Kevin T. McDonagh, M.D., Medical Staff Fellow, CHB, NHLBI
Brian Agricola, Research Technician, NHLBI
Ellen Byrne, Research Technician, NHLBI
Griffin Rodgers, M.D., Senior Investigator, LCB, NIDDK
Alan Schechter, M.D., Laboratory Chief, NIDDK
George Dover, M.D., Professor of Pediatrics, Johns Hopkins Medical School

COOPERATING UNITS *(if any)*

Laboratory of Chemical Biology, NIDDK; Division of Medical Genetics, Department of Pediatrics, Johns Hopkins Medical School

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with severe beta thalassemia or sickle cell anemia would benefit significantly if HbF production could be consistently augmented. The imbalance of globin synthesis characteristic of thalassemia could be partially corrected by increased gamma globin synthesis. Reduction of intracellular HbS concentration by replacement with HbF reduces the polymerization potential of intracellular sickle hemoglobin decreasing the sickling "propensity" of red cells from such individuals. Several classes of substances stimulate HbF synthesis including cytotoxic agents (e.g. hydroxyurea), hematopoietic growth factors (erythropoietin) and agents that modify DNA or chromatin structure (e.g. 5-azacytidine or sodium butyrate, respectively). Our studies have shown a high frequency of response to hydroxyurea in patients with sickle cell disease. This increment in HbF synthesis is accompanied by macrocytosis, an enrichment in HbF containing cells during maturation and a significant reduction in polymer fraction within circulating red cells. We are currently testing the combination of hydroxyurea and erythropoietin. One patient has completed this study and has shown an approximately two-fold increase in HbF synthesis to approximately twenty percent, the threshold level thought to be necessary to significantly reduce sickling complications. A similar trial of hydroxyurea in patients with thalassemia has documented a low incidence of response. Untransfused patients with thalassemia intermedia have shown an increase in Hb concentration and one transfusion dependent patient has experienced a lengthening of her transfusion interval. Three patients with severe beta thalassemia with end-stage disease have begun a trial of intravenous 5-Azacytidine given at two or three week intervals. Significant increases in hemoglobin concentration have been experienced and the transfusion requirement eliminated. Our experience with pharmacological agents demonstrate the potential for modification of HbF synthesis with significant therapeutic benefit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02330-04 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mapping of Hypertrophic Cardiomyopathy Locus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Neal D. Epstein, M.D., Senior Investigator, CHB, NHLBI

Others:

H. Lin, M.D., Med. Staff Fellow, CHB
B. Maron, M.D., CB, NHLBI
L. Fananapazir, M.D., CB, NHLBI
S. Epstein, M.D., Chief, CB, NHLBI
J. Mulvihill, M.D., Clin. Epid. Br., NCI
M. Leppert, Ph.D., HHMI, Univ. of Utah S.L.C., Utah
R. White, Ph.D., HHMI, Univ. of Utah S.L.C., Utah

COOPERATING UNITS *(if any)*

Howard Hughs Medical Institute, University of Utah School of Medicine, Salt Lake City, Utah; Cardiology Branch, NHLBI; Clinical Epidemiology Branch, NCI

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the chromosomal location of the genes responsible for hypertrophic cardiomyopathy (HCM). This type of heart disease is diagnosed by echocardiography. Its clinical manifestations are highly variable including anatomical abnormalities only, cardiac failure, left ventricular outflow obstruction and/or sudden death. Fifty percent of cases appear to be sporadic whereas the remainder are familial. We have identified five large families in which the disease is clearly transmitted as an autosomal dominant characteristic. For this study, the disease status has been ascertained by echocardiography and DNA was collected from all relevant members of each family. Using DNA probes that detect polymorphic differences among individuals, we have tested for linkage of individual polymorphisms to the HCM gene. Approximately 40 percent of the human genome has been excluded from containing the HCM locus. Recent results have shown that the two largest and most informative families are genetically heterogenous, that is, the genes responsible for the disease in each family localize to two different chromosomes. In one family the gene responsible for HCM maps to the long arm of chromosome 14, corroborating a previous finding in a French Canadian family by another group. In the other family the gene responsible for HCM maps to chromosome 2p. Our clinical studies have verified that many sporadic cases are likely to have a genetic basis. For example, we have identified a pair of identical twins, one of whom has inherited severe HCM, while the other has normal cardiac function and anatomy. Two other families demonstrate obligate carriers of the HCM gene without ventricular hypertrophy but with electrical abnormality. These individuals underscore the importance of a genetic marker for HCM to detect the variable forms of the disease. The ultimate goal is to identify the actual genes that cause the disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02331-04 CHB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inhibition of HIV Replication by Anti-sense RNA Sequences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Takashi Shimada, M.D., Visiting Associate, CHB, NHLBI
Others: Hiroyuki Fujii, M.D., Visiting Fellow, CHB, NHLBI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI
Hiroaki Mitsuya, M.D., COP, DCT, NCI
Samuel Broder, M.D., COP, DCT, NCI

COOPERATING UNITS *(if any)*

Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	-

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV) that infects and destroys helper T-lymphocytes. The general objective of this work is to devise strategies for introducing new genes into lymphocytes that will render them resistant to HIV infection. This approach has been described "intracellular immunization". We have first attempted antisense RNA inhibition of HIV replication. Although we have established a highly efficient retroviral mediated gene transfer system into $CD4^+$ T-cells and a sensitive assay system, the antisense strategy has proved ineffective in modifying HIV replication. We are currently examining several other possible antiviral gene products for this protocol. Rev and Gag are encoded by the HIV genome and are essential components for viral replication. It has been recently shown that mutants forms of these viral protein molecules interfere with HIV replication. The feasibility of using these mutant molecules in the intracellular immunization protocol will be tested in our assay system.

We have established a stable cell line that express HIV envelope protein on cell surface at a very high level. This cell line will be very useful for studying the interaction between Env and CD4 molecules on cell surface and also can serve as a useful indicator for testing anti-HIV molecules targeting env, tat, and rev functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02333-03 CHB

PERIOD COVERED

October 1, 1989 to September 31, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of the Murine CSF-1 Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Angel W. Lee, M.D., Ph.D., Senior Staff Fellow, CHB, NHLBI
Others: Arthur W. Nienhuis, M.D., Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
2.0	2.0	-

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The CSF-1 receptor is the cell surface receptor for the macrophage colony stimulating factor. It is found on cells of the monocyte-macrophage lineage and placental trophoblasts. The interaction of CSF-1 with its receptor mediates all its biological actions. We have investigated the mechanism whereby ligand binding activates the tyrosine kinase function of the murine receptor. Using a hybrid receptor composed of the external domain of glycophorin A, an erythrocyte structural protein, and the transmembrane and cytoplasmic domains of the CSF-1 receptor, we have shown that it is possible to activate the kinase domain by the cross-linking properties of anti-glycophorin antibodies. Once activated as a kinase, this hybrid receptor is able to support mitogenesis and associate with a known intracellular substrate for the wildtype receptor (phosphatidyl inositol-3-kinase). However, unlike the wildtype receptor, it is not downregulated, indicating that the external domain must contain signals that target the CSF-1 receptor for lysosomal degradation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02335-03 CHB

PERIOD COVERED

October 1, 1989 to September 31, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Expression of Growth Factor Genes and Oncogenes in Primary Hematopoietic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: David Bodine, Ph.D., Senior Staff Fellow, CHB, NHLBI
Cynthia Dunbar, M.D., Medical Staff Fellow, CHB, NHLBI
Others: Stephen Brandt, M.D., Guest Worker, CHB, NHLBI
Chris Walsh, M.D., Medical Staff Fellow, CHB, NHLBI
Debra Cockayne, M.D., Staff Fellow, CHB, NHLBI
Don Orlic, Ph.D., Guest Worker, CHB, NHLBI
Nancy Seidel, B.A., Research Assistant, CHB,NHLBI
Arthur W. Nienhuis, M.D., Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	-

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Malignant transformation of normal cells occurs as a consequence of dysregulated expression of proto-oncogenes or mutations in such genes that result in the synthesis of an abnormal product. Retroviral mediated gene transfer provides a highly efficient mechanism to modify the genetic dowry of primary hematopoietic stem and progenitor cells. Genes for hematopoietic growth factors, their receptors or mutated forms of normal cellular genes can be introduced, singly or in combination. The hematopoietic syndromes or neoplasms that result bear witness to the transforming capacity of the introduced genes and provide models for specific therapeutic intervention targeted to a dysregulated genes or an abnormal gene product. Moreover, dysregulated expression of a growth factor gene provides a unique opportunity to characterize its spectrum of biological activity. Previously, we have characterized the myeloproliferative syndrome induced by IL-3 and a lymphoproliferative process resembling the human disease - Castleman's Syndrome - produced by IL-6. An activated form of the RAS oncogene causes thymic lymphomas in mice. Retroviral vectors containing the IL-4, IL-7, IL-9 and the M-CSF coding sequences have been constructed and used for gene transfer into hematopoietic stem cells. IL-4 and IL-7 act predominantly on lymphoid progenitors, M-CSF is specific late stage monocytes and macrophages and IL-9 is a newly discovered growth factor that seems to act predominantly on primitive erythroid progenitors. Current efforts are focused on characterizing the effects of overexpression of these genes during regeneration of the hematopoietic and lymphoid systems in transplanted mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02336-03 CHB

PERIOD COVERED

October 1, 1989 to September 31, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Production and Mechanism of Action of Hematopoietic Growth Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Arthur W. Nienhuis, M.D., Chief, CHB, NHLBI
Robert Redner, M.D., Senior Staff Fellow, CHB, NHLBI

Others: Debra A. Cockayne, Ph.D., Staff Fellow, CHB, NHLBI
Elise Feingold, Ph.D., Staff Fellow, CHB, NHLBI
Angel Lee, M.D., Ph.D., Staff Fellow, CHB, NHLBI
Gail Osawa, Research Assistant, CHB, NHLBI
David Smith, M.D., Medical Staff Fellow, CHB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The proliferation and differentiation of hematopoietic cells is under the control of hematopoietic growth factors. A variety of cell types are capable of producing these growth factors including endothelial cells, fibroblasts, stromal cells, monocytes and lymphocytes. Growth factors stimulate both proliferation and/or differentiation of hematopoietic cells. There is substantial redundancy in the spectrum of activity of hematopoietic growth factors in that several individual factors may act on mature and immature cells of several lineages and there are several factors that have overlapping spectra of activity. One goal is to devise strategies to determine the in vivo role of particular factors in both hematopoiesis and lymphoid cell differentiation. We have devised an expression vector that allows an overproduction of antisense RNA sequences in T-lymphoid cells. Our in vitro data indicate that such antisense sequences will inhibit growth factor production. Transgenic animals expressing the antisense sequences in T-lympocytes should be deficient in the production of a particular growth factor, providing an opportunity to determine, by analysis of the deficient phenotype, the role of that factor in hematopoiesis and lymphoid cell proliferation. The second series of experiments is directed at the role of particular early response gene products in the response to hematopoietic growth factors. The AP-1 family of proteins is composed of JUN and FOS species that form various homo- and heterodimers and act as transcriptional modulators of gene expression. We have found that the pattern of early response gene expression in two closely related cell lines is specific for the cell line and unrelated to the growth factor used to activate cell proliferation. A third series of experiments involved introduction of the M-CSF receptor in a primitive hematopoietic cell line to determine whether it would influence the pattern of differentiation. We found that the receptor stimulated with M-CSF induced a mitogenic response, but that the pattern of early response gene expression was identical to that invoked by IL-3 and that no change in the differentiation pattern of the cells was observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03574-03 HE

PERIOD COVERED
Oct. 1, 1989 to Sept. 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular mechanisms of hypertension and atherosclerosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry R. Keiser	Chief	HE NHLBI
Others:	Margaret Hill	Medical Technician	HE NHLBI
	Reuben Brown	Phys. Sci. Tech.	HE NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied in culture vascular smooth muscle cells (VSMC) obtained from either spontaneously hypertensive rats (SHR) or Wistar-Kyoto rats (WKR). VSMC from SHR proliferated more rapidly than cells from WKR regardless of the medium used. The incorporation of radioactive thymidine into DNA was also greater in SHR VSMC than for cells from WKR. To determine if this increased growth was due to the production of growth factors, medium was taken from the cells and incubated with quiescent SHR or WKR VSMC for 24 hours. However, none of the media tested would promote DNA synthesis in either type of cells with or without added l% fetal bovine serum (FBS). Thus the increased growth response was not due to the production of growth factors by the cells. Platelet derived growth factor (PDGF) did not promote DNA synthesis in these cells. However, both epidermal growth factor (EGF) and fibroblast growth factor (FGF) stimulated DNA synthesis of both SHR and WKY, but in each case, produced greater stimulation in SHR than in WKR VSMC. Of the growth factors tested, EGF produced the greatest response and its effects were either additive or synergistic when it was added to either FGF or PDGF. Specific ligand binding studies of 125-I- EGF to quiescent VSMC showed that the VSMC from SHR had both a greater affinity and a greater number of binding sites for EGF than did the cells from WKR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03583-02 HE

PERIOD COVERED

Oct. 1, 1989 to Sept. 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dopa-dopamine system in genetically salt-sensitive rats.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ehud Grossman	Visiting Associate	HE NHLBI
	Aaron Hoffman	Visiting Associate	HE NHLBI
	David S. Goldstein	Senior Investigator	HE NHLBI
	Harry R. Keiser	Chief	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We measured daily urinary excretion rates of dopamine (DA) and dopa during dietary salt loading and natriuretic responses to exogenous DA in Dahl salt-sensitive (DS) and salt-resistant (DR) and Sprague-Dawley rats. Excretion rates of dopa increased by about 6-fold during salt loading in all rat strains. Maximal urinary dopa responses were attained within 1 day of salt loading. Daily excretion rates of DA also increased by about 5-6-fold in DS and DR rats and about 2-fold in Sprague-Dawley rats, with maximal DA responses attained by day 5. DA infusion (3 μg/kg/min) increased urinary sodium excretion by 406% in Sprague-Dawley rats but only 267% and 147% in DS and DR rats ($p<0.05$ for Sprague-Dawley vs Dahl rats). The results demonstrate that salt loading markedly and rapidly increases dopa excretion in rats. Considering values for DA excretion in other rat strains, the results suggest that Dahl rats have increased formation of DA for a given amount of dopa delivery to the kidney and that this abnormality is unrelated to salt-sensitive hypertension in DS rats. The results also provide in vivo support for the view that the responsiveness of renal DA receptors mediating natriuresis is related to production of endogenous DA in the kidney.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03584-01 HE

PERIOD COVERED

Oct. 1, 1989 to Sept. 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sympathoadrenal function in health and disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: David S. Goldstein, Senior Investigator, HE, NHLBI

Others: Ines Armando,HE,NHLBI; John Bacher,VRB,DRS; Richard O.CannonIII,CB,NHLBI; Peter C.Chang,Visiting Scientist,DIR,NINDS; Bert Chidakel,BEIB,DRS; Anna Deka-Starosta,Visiting Associate,HE,NHLBI; Graeme Eisenhofer, Baker Med.Res.Inst., Victoria,Australia; Ronald Finn,Memorial-Sloan Kettering Hosp.,NewYork,NY; Carol J. Folio,R.N.,HE,NHLBI;Moshe Garty,Chief,Dept.of Med.,Beilinson Med.Ctr., Petach Tikve,Israel;John R. Gill,Jr.,HE,NHLBI; Ehud Grossman,Visiting Associate, HE,NHLBI; Peter Herscovitch,Chief,PET Sect.,NMD,CC; Courtney Holmes,Med.Tech., HE, NHLBI; David Hovevey-Sion,Visiting Fellow,DIR,NINDS;' Arshad Quyyumi,CB,NHLBI; Harry R. Keiser,Chief,HE,NHLBI; Kenneth L. Kirk,LC,NIDDK; Irwin J. Kopin,Chief,CNB, NINDS; Robert Miletich,Senior Staff Fellow,DIR,NINDS; Katalin Szemeredi,Visiting Associate,HE,NHLBI

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The sympathoadrenal system is one of the most powerful and rapidly-acting of the body's "stress" systems. Our goals have been to understand better how this system is regulated, how its function is integrated with that of other systems contributing to the maintenance of the internal environment, and whether and how it plays a pathophysiologic role in neurocardiologic diseases. During the past year we developed positron emission tomographic (PET) scanning to provide the first non-invasive, in vivo means to examine cardiac sympathetic innervation and function. We also developed in vivo microdialysis to examine relationships between concentrations of neurotransmitters in the brain and sympathoadrenal outflow. The microdialysates will be analyzed by a new multi-electrode array detector (neurochemical analyzer). We obtained further evidence suggesting a relationship between endogenous plasma dopa and biosynthesis of the sympathetic neurotransmitter, norepinephrine (NE), and noted abnormally increased urinary excretion of dopa in patients with salt-sensitive hypertension. We used a method including direct sympathetic nerve recording to examine pre-synaptic actions of anti-hypertensive drugs in vivo. The close relationship between sympathetic nerve activity and regional release of NE into the bloodstream was confirmed in humans and rats. We obtained evidence of sympathoadrenal hyperreactivity, as indicated by a yohimbine challenge test, in some patients with hypertension; demonstrated by microdialysis that yohimbine releases NE in the brain in rats; and showed by direct nerve recording that yohimbine increases sympathetic nerve traffic and augments regional release of NE in the heart and limbs in humans. PET scanning, neurochemical analysis, and direct sympathetic nerve recording can be used to answer long-standing questions about the role of the sympathoadrenal system in health, stress, and disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03585-01 HE

PERIOD COVERED
Oct. 1, 1989 to Sept. 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Sodium-dependent secretion and retention of NE in Adrenergic terminals.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D.F. Bogdanski Pharmacologist HE NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms for both Na^+-dependent hypertension and the therapeutic effect of Li^+ are not known. This report supports the hypothesis that Na regulates the secretion and recapture of NE from a pool of mobile amine in adrenergic terminals in rat heart slices. Lithium produces a functional deficiency of Na. Mobilized NE was accumulated in terminals as a result of metabolic deficiencies during the overnight dialysis of terminals in solutions of either Na, K, Li or choline at 0^o. The axolemma was the dialysis membrane. The mobilized NE was depleted during a subsequent in cubation of terminals in Krebs medium (KRB) at 37^o. The rapidly induced depletion was independent of the primary dialysis cation, and, the presence of Ca in the KRB. Depletion was inhibited by either dialyzed (intraterminal) ATP or import export blockers such as cocaine. These agents in the incubation medium were not inhibitory. The depletion of mobile NE differed from the secretion of vesicular NE. Forty percent of the remaining NE was stably retained in synaptic vesicles. Secretion from vesicles was evoked by the incubation of terminals in a Na-deprived (Choline) KRB (Ch-Ca). Secretion was delayed, dependent upon intraterminal Na, and, Ca in the medium. Secretion was mediated by export from a previously described vesicular-axolemmal secretory and transmitter recovery unit comprised of vesicles whose membranes had fused with the axolemma. Bound intravesicular NE was mobillized in the process then exported. The vesicle membrane was accessible to ATP in the medium. In non-dialyzed terminals, secretion was inhibited by ATP only when Na was present. Thus, Na was required for the translocation of NE into and out of terminals. Lithium, in a therapeutic concentration of I mM prevented the vesicle from recapturing released or mobilized NE. This effect was indicated by an increased deamination of NE. Thus, Li appeared to interfere with a requirement for Na in the recapture process in an active unit. On the basis of published reports, Li may act by inhibiting Mg-ATPase activity and dependent NE uptake in isolated synaptic vesicles. The present report adds the important information that Li can act in therapeutic concentrations on vesicles in situ. Since Li stimulates deamination in rat brain in vivo, the postulated role for Na may be relevant therapeutically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03586-01 H

PERIOD COVERED

Oct. 1, 1989 to Sept. 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Endothelin in the Regulation of Blood Pressure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI:	Aaron Hoffman	Visiting Associate	HE NHLBI
Others:	Harry R. Keiser	Chief	HE NHLBI
	Ehud Grossman	Visiting Associate	HE NHLBI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The endothelins (ET) are a new family of vasoactive peptides produced and released by vascular endothelial cells upon various physical and chemical stimuli.

We investigated: 1) The pharmacological effects of exogenously administered ET on blood pressure, heart rate, cardiac output, regional blood flow and peripheral resistance in rats under several experimental conditions. 2) the presence of immunoreactive ET in plasma and in other body fluids, by radioimmunoassay and high performance liquid chromatography. 3) The possible involvement of ET in the pathogenesis of hypertension in man.

ET had a unique biphasic cardiovascular effect with an initial decrease in blood pressure followed by a prolonged hypertensive response. The pressor phase could be blocked by verapamil but not by other agents. The depressor phase could not be blocked by anything tested. Cardiac output initially increased and later markedly decreased. There was a selective effect on individual vascular beds with a maximal increase in resistance in the renal bed. The renal vascular effect, but not the systemic effect, was enhanced in rats on a high salt diet. ET induced an increase in circulating atrial natriuretic factor. The cardiovascular effects were markedly diminished in rats with experimental CHF. Whereas ET was antidiuretic and antinatriuretic, its' precursor, Big-ET, had a substantial natriuretic effect.

The levels of ET in the plasma were very low both in man and in rats. Other body fluids such as urine, cerebrospinal fluid, saliva and synovial fluid contained much higher concentrations of the peptide.

In hypertensive patients plasma levels of ET were normal but urinary levels were markedly decreased, especially in a subgroup of salt sensitive hypertensives.

We conclude that : 1) ET is a potent vasoactive peptide. 2) This activity is mediated by calcium channels and not by other hormonal systems. 3) ET may be involved in nonvascular physiologic events. 4) Renal ET may have a role in the pathogenesis of hypertension.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03587-01 HE

PERIOD COVERED
Oct. 1, 1989 to Sept. 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Release and actions of NPY in the rat cardiovascular system.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Anna Deka-Starosta	Visiting Associate	HE NHLBI
	Zofia Zukowska-Grojec	Special Volunteer	HE NHLBI
Others:	Gregory Shen	Special Volunteer	HE NHLBI
	Richard Kvetnansky	Visiting Scientist	CND NINDS
	Adam Myers	Assistant Prof. Dept. of Physiol.&Biophysics	Georgetown Univ.
	Harry R. Keiser	Chief	HE NHLBI

COOPERATING UNITS (if any)
Dept. of Physiology and Biophysics, Georgetown University, Washington, DC

LAB/BRANCH
Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neuropeptide Y (NPY) is a 36 amino-acid peptide which co-exists with norepinephrine (NE) in sympathetic postganglionic nerves innervating the cardiovascular system and in platelets. NPY causes vasoconstriction when infused into the systemic circulation. Our previous studies have determined that NPY-immunoreactivity (-ir) is released from both these sources and that the magnitude and pattern of NPY release differed from that of NE. To study the kinetics of NPY release from sympathoneural terminals, left renal nerves were stimulated in adrenal-demedullated anesthetized rats. Significant, frequency-dependent increases in renal spillover of NE were accompanied by moderate (25-30%) elevations in renal venous NPY-ir (authenticity confirmed by HPLC) which were independent of frequency or mode of stimulation. There was no renal extraction of NPY as assessed by extraction of [^{125}I]NPY. In conscious rats, stress of immobilization which increased plasma catecholamine levels, failed to elevate plasma NPY-ir, however, clearance of NPY increased. In contrast, in adrenal-demedullated rats immobilization evoked marked increases of plasma NPY-ir without affecting NPY clearance, indicating that sympathoneuronal release of NPY increased. Adrenal medulla (or adrenaline) seems to play a role in plasma clearance of NPY during stress but is insignificant as a source of NPY released into circulation.

NPY exerts multiple bioactivities in the rat cardiovascular system: 1/ vasoconstriction and potentiation of NE actions (Y1 receptors, primary action); 2/ vasodilation via histamine release from mast cells and inhibition of NE release (Y2 receptors, secondary actions); 3/ potentiation of collagen-induced platelet aggregation (Y2 ?, inhibition of adenyl cyclase), and 4/ stimulation of proliferation of vascular smooth muscle cells in culture. Thus, NPY may be released from sympathetic nerves and platelets in states such as stress and influence vascular and platelet actions and interactions promoting vasoconstriction, thrombosis and vascular growth. Using specific NPY antagonists which will be available soon, we will be able to determine whether these actions of NPY contribute to vascular derangements in experimental models of hypertension and chronic stress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03588-01 HE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Plasma and Brain Extracellular Fluid Levels of Catechols in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Katalin Szemeredi, Visiting Associate, HEB, NHLBI; Gyorgy Bagdy, Professional Consultant, CNB, NIMH; David S. Goldstein, Senior investigator, HEB, NHLBI; Harry R. Keiser, Chief, HEB, NHLBI; Samuel Komoly, Visiting Fellow, LEN, NINDS; Irwin J.Kopin, Scientific Director, DIR, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The present study examined whether systemic injection of the alpha-2 adrenoceptor blocker, yohimbine, affects concentrations of norepinephrine (NE) and its metabolites in extracellular fluid in the brain and in blood. Microdialysis probes were inserted into the posterior hypothalamus, medulla, and caudate/putamen in rats. Microdialysate and arterial blood were sampled after intravenous administration of yohimbine. In the hypothalamus yohimbine produced significant increases in extracellular fluid concentrations of NE, its intraneuronal metabolite, dihydroxyphenylglycol (DHPG), and methoxyhydroxyphenylglycol (MHPG), a major neuronal and extraneuronal metabolite of NE. The increases in these levels were small or absent in the caudate/putamen, where dopamine is the primary catecholamine transmitter. During systemic infusion of tracer amounts of [3H]-NE, little if any radioactive NE or DHPG appeared in the microdialysate, whereas significant levels of [3H]-MHPG were present and increased as plasma [3H]-MHPG levels rose. The results support the view that alpha-2 adrenoceptor blockade in the brain increases hypothalamic and medullary release, reuptake, and metabolism of NE. The findings cannot be explained by disruption of the blood-brain barrier for catecholamines by insertion of the microdialysis probes. Enhanced sympathetic outflow and peripheral release of NE when alpha-2 adrenoceptors are blocked appears to be attended by enhanced central NE release, presumably as a result of presynaptic alpha-2 adrenoceptor blockade at noradrenergic terminals in the brain. This is consistent with the hypothesis that central noradrenergic NE release is regulated by presynaptic alpha-2 adrenoceptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03589-01 HE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Origin of Plasma Dopa in Rats with Destroyed Central Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Katalin Szemeredi, Visiting Associate, HEB, NHLBI; David S. Goldstein, Senior investigator, HEB, NHLBI; Irwin J.Kopin, Scientific Director, DIR, NINDS; Karel Pacak, Visiting Fellow, CNB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Plasma dopa has been thought to originate from sympathetic nerve endings and to represent the rate of catecholamine synthesis because plasma dihydroxyphenylalanine (dopa) levels in various experimens followed the changes in norepinephrine (NE) synthesis. However, recent studiesof the effect of sympathectomy on the dopa content of skeletal muscle, raised the possibility of skeletal muscle as an additional source of circulating dopa. In this study, we examined the neuronal and skeletal muscle contributions to dopa in arterial plasma.

Electrical stimulaton of the spinal cord of pithed rats has been applied to initiate discharges both of sympathetic neurones resulting in NE release from sympathetic nerve endings and of spinal motoneurones evoking diffuse contraction of skeletal muscle. This stimulation caused marked elevations in arterial plasma NE, dihydroxyphenylglycol (DHPG), and dopa concentrations. Pretreatment with curare, a skeletal muscle relaxant, did not affect NE and DHPG responses but decreased dopa responses by about 50%. Chlorisondamine, a ganglionic blocker, inhibited all NE, DHPG responses, and dopa responses also by 90%. Adrenal-demedullation did not affect electrical stimulation induced dopa responses in pithed rats.

In conclusion, dopa is released into the blood stream during sympathetic stimulation and this response can be inhibited by ganglionic blockade. In addition, these results show, that dopa can be released from a non-neural pool, during skeletal muscle contraction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 02010-19 MDB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Plasma Lipoproteins and Apolipoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

H. Bryan Brewer, Jr., Chief, MDB, NHLBI
F. Thomas, Research Chemist, MDB, NHLBI
J. Hoeg, Senior Investigator, MDB, NHLBI
R. Ronan, B.A., Chemist, MDB, NHLBI
M. Meng, M.S., Chemist, MDB, NHLBI

COOPERATING UNITS *(if any)*

Dr. Dubo Bojanovski, Zentrum Innere Medizine, Medizinische Hochschule Hannover, Hannover, West Germany

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.9	2.9	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The elucidation of the molecular defects in patients with dyslipoproteinemias provides the unique opportunity to acquire new information on lipoprotein metabolism in normal subjects as well as patients with specific dyslipoproteinemias. Knowledge of the precise genetic disease in dyslipoproteinemic patients enables more effective approaches to screening potential affected individuals and establishing diagnosis in patients at an early age permitting treatment to be initiated during the initial course of the disease.

The present studies identified the molecular defect in a kindred with hereditary systemic amyloidosis A mutant form of apoA-I, designated apoA-I_{Iowa}, contained a single amino acid substitution of a glycine->arginine at residue 26 and was the molecular defect in this family. The mutant A-I apolipoprotein was catabolized at a rapid rate leading to low levels of plasma HDL. In addition, the mutation resulted in a change in the molecular properties of the apolipoprotein leading to its accumulation in tissues. The accumulation of the mutant apolipoprotein was responsible for the amyloid accumulation in the disease.

A severe form of type III hyperlipoproteinemia was identified in a young female, and was shown to be due to a mutant apoE designated apoE-$4_{Phil.}$ ApoE-$4_{Philadelphia}$ had two separate mutation, arginine->cysteine at residue 145 and lysine to glutamic acid at amino acid 13. Kinetic studies established that the mutant apoE-$4_{Phil.}$ isoprotein had delayed catabolism and was responsible for the hyperlipidemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02012-15 MDB

PERIOD COVERED

October 1 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Zafarul H. Beg, Ph.D., Research Chemist, MDB, NHLBI
J.A. Stonik, Chemist, MDB, NHLBI
H.B. Brewer, Jr., M.D., Chief, MDB NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Peptide Chemistry, Molecular Disease Branch

SECTION

Molecular Disease Branch

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews

☒ (b) Human tissues

☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously established that rat and human hepatic HMG-CoA reductase activity is modulated *in vitro* and *in vivo* in a bicyclic cascade system involving reversible phosphorylation of both HMG-CoA reductase and reductase kinase. Recently we have identified two additional kinase systems for the regulation of HMG-CoA reductase activity by short-term covalent modification, involving a Ca_{2+}/calmodulin-dependent kinase and protein kinase C. In order to understand the coordinate regulation of HMG-CoA reductase, cholesterol synthesis, and the role of apolipoproteins such as apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB-100) in the transport and regulation of cellular cholesterol, a systematic investigation of their role in plasma lipid and lipoprotein transport and metabolism has been undertaken. Recently we have shown the post-transnational modification of human plasma apoA-I involving reversible phosphorylation. Plasma LDL (apoB-100) have been correlated directly with the development of premature cardiovascular disease. During the last year we have established that both secreted and cellular apoB-100 from Hep G-2 cells were phosphorylated. We have also demonstrated the phosphorylation of human plasma apoB-100 (LDL) using protein kinase C, a cAMP dependent protein kinase and a Ca^{2+}/calmodulin-dependent kinase. We have also shown that both secreted and cellular phospho-apoB-100 do respond to increases in the levels of the above three intracellular protein kinases when cells were challenged with phorbol ester, glucagon and a Ca^{2+}ionophore, respectively. The phosphorylation of apoB-100 may play an important role in the intracellular transport of hepatic VLDL during lipid assembly and secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02019-12 MDB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Peptide Chemistry Section

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel Rader, M.D., Medical Staff Fellow, MDB, NHLBI
Juergen Schaefer, M.D., Visiting Fellow, MDB, NHLBI
Katsunori Ikewaki, M.D., Visiting Fellow, MDB, NHLBI
Marie Kindt, Chemist, MDB, NHLBI
Yoshiko Dogherty, Chemist, MDB, NHLBI
Martha Meng, Chemist, MDB, NHLBI
H. Bryan Brewer, Jr., M.D., MDB, NHLBI

COOPERATING UNITS *(if any)*

Dr. Loren Zech, Senior Investigator, Office of the Dir., NHLBI

LAB/BRANCH: Molecular Disease Branch

SECTION: Peptide Chemistry

INSTITUTE AND LOCATION: NHBLI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	4.0	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The metabolic and functional heterogeneity of high density lipoproteins were investigated in a series of in vivo kinetic studies. The two major classes of HDL particles are known as LpA-I and LpA-I,A-II. A method to isolate these particles was established and then utilized to study their in vivo metabolism. It was demonstrated that the LpA-I particle is more rapidly catabolized from plasma and also undergoes a net conversion to LpA-I,A-II. Two patients with normotriglyceridemic hypoalphalipoproteinemia were also studied and were found to have more rapid catabolism of both types of particles. These studies enhance our understanding of HDL metabolism.

A possible homozygote for familial hyperalphalipoproteinemia has been identified with dramatic elevation in HDL and a family history of longevity on both sides. An in vivo kinetic study in this subject revealed markedly elevated apoA-I production rates with normal apoA-II production. This is the first individual identified with apoA-I overproduction and may lead to the identification of a gene or mutation responsible for regulating apoA-I synthesis.

Amino acids labeled with stable isotopes have been used to investigate the in vivo kinetics of a mutant apolipoprotein, apoA-I_{Iowa}, in a a patient heterozygous for the mutation by endogenous labeling of both normal and mutant proteins and then directly comparing their kinetics. It was found that the apoA-I_{Iowa} has a substantially faster fractional catabolism, consistent with the hereditary amyloidosis and hypoalphalipoproteinemia found in the kindred. This study demonstrates that the stable isotope method has great potential for the study of the in vivo kinetics of mutant proteins, particularly in heterozygotes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02022-10 MDB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Lipid and Lipoprotein Biochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Jeffrey M. Hoeg, M.D., Senior Investigator, MDB, NHLBI
Thomas Eggerman, M.D., Ph.D., Medical Staff Fellow, MDB, NHLBI
Amy P. Patterson, M.D., Medical Staff Fellow, MDB, NHLBI
Stephen J. Demosky, Jr., Chemist, MDB, NHLBI
Douglas Wood, Clinical Chemist, MDB, NHLBI
Barbara Winterrowd, Medical Technician, MDB, NHLBI
Uwe K. Schumacher, Biologist, MDB, NHLBI

COOPERATING UNITS *(if any)*

Drs. Repin, Sviridov, Kosykh, and Smirnov, Cardiocenter of the USSR, Moscow

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHBLI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	3	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cholesterol and other fats are carried in the bloodstream within lipoprotein particles. These particles distribute these fatty substances to the various tissues under the direction of the apolipoproteins. Our primary interest is to understand both the biosynthesis and the catabolism of the apolipoproteins at the cellular level. We have previously demonstrated that the apolipoproteins are recognized by specific membrane-associated receptors within the human liver. These receptors can effect the removal of apolipoproteins from the extracellular environment and can also affect the synthesis of nascent apolipoproteins. Our studies indicate that the regulation of hepatic apolipoprotein output is primarily posttranslational. We have demonstrated that apolipoproteins A-I and B undergo a variety of posttranslational modifications including glycosylation, phosphorylation, and fatty acid acylation. These prosthetic side chains may play roles in lipoprotein particle assembly and secretion. Studies utilizing tissues from patients with inborn errors of lipoprotein metabolism have been crucial in dissecting the pathophysiologic relevance of these aspects of hepatic apolipoprotein metabolism. We have been utilizing the conceptual framework derived from our basic research of cellular apolipoprotein metabolism to applied research. We evaluate and treat patients with a variety of inborn errors of apolipoprotein and lipid metabolism including familial hypercholesterolemia, cholesteryl ester storage disease, type III hyperlipoproteinemia, abetalipoproteinemia, and hypobetalipoproteinemia. These studies include patients aged 5-70 years, both male and female, and of caucasian, black and hispanic backgrounds. The insights derived from the application of these concepts may have broad implications for the treatment and prevention of cardiovascular disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02028-06 MDB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of the ApoC-II and Lipoprotein Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Silvia S. Fojo, M.D., Ph.D.	Senior Investigator	MDB NHLBI
Pia Lohse	Visiting Associate	MDB NHLBI
Obaidullah Beg, Ph.D.	Visiting Fellow	MDB NHLBI
Lorenzo Previato, M.D.	Guest Researcher	MDB NHLBI
Helen Dichek, M.D.	Staff Fellow	DEB NICHD
Cathy Parrott	Chemist	MDB NHLBI

COOPERATING UNITS *(if any)*

John D. Brunzell, University of WA, Div. of Metabolism, Dept. of Medicine, Seattle, WA
John Chapman, Inserm Lipoprotein Lab., Group Hospitalier PITIE -Salpetriere, Paris, France

LAB/BRANCH

Molecular Disease Branch

SECTION

Molecular Disease Branch

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 6.5	PROFESSIONAL: 4.0	OTHER: 2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lipoprotein lipase (LPL) is the key enzyme involved in normal triglyceride metabolism. In the presence of its cofactor, apoC-II, LPL hydrolyzes triglycerides present in chylomicrons and VLDL to mono-and di-glycerides and free fatty acids. The genetic defects that lead to a deficiency of apoC-II have been elucidated in 3 different kindreds with the familial hyperchylomicronemia syndrome. A single base pair mutation results in the introduction of a stop codon in the signal peptide of the apoC-II$_{paris2}$ gene. Two independent mutations have been identified in the apoC-II and apoE genes of the proband from a Spanish kindred which result in a combined functional deficiency of apoC-II and apoE. The identification of 2 independent mutations occurring in 2 genes which are closely linked is unique. The proband from the third kindred is a compound heterozygote with 2 different allelic mutations in the LPL gene that lead to the synthesis of non-functional LPL. Expression studies have established the functional significance of these mutations.

We have previously described a mutant LPL (LPL$_{Bethesda}$) with an ala$_{176}$ to thr substitution near the interfacial recognition binding site of LPL that leads to both abnormal LPL activity and heparin binding. In order to understand the role that ala$_{176}$ plays in maintaining normal LPL function we have modified the residue by site-directed mutagenesis. Substitution of ala$_{176}$ by any residue except for gly results in the loss of LPL activity suggesting that stearic hindrance plays a role in the loss of activity of LPL$_{Bethesda}$. Substitution of ser$_{132}$ by gly leads to a total loss of hydrolytic activity consistent with its role as the catalytic serine.

The luciferase reporter gene was used to analyse the regulatory elements which modulate the expression of apoC-II in liver. A 5' flanking 542 bp fragment contains the control elements that direct tissue specific expression of apoC-II. Deletion studies established that apoC-II expression is modulated by proximal and distal cis-acting elements and that the region from -130 to +10 is sufficient to achieve maximal levels of gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02030-03 MDB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

RNA Editing in Mammalian Systems in Vivo and In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gregory E. Tennyson, Senior Staff Fellow, MDB:NHLBI
Amy P. Patterson, Medical Staff Fellow, MDB:NHLBI
Charles A. Sabatos, Biologist, MDB:NHLBI
H. Bryan Brewer, Jr., Chief, MDB:NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI:NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.75	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous work has shown that two B apolipoproteins circulate in higher mammals, apoB-100 and apoB-48, with the latter isoprotein generated by an RNA editing process which alters the genomically encoded CAA (gln) at nucleotide 6666 to UAA. This mechanism effectively truncates the nascent protein at exactly apoB-48 size. Two projects in this laboratory have focused on apoB RNA editing. First, we have studied apoB RNA editing in the developing rat. The emergence of RNA editing occurred at 21 days in rat intestine, and 14 days in rat liver, both parallelling isoprotein data. These studies underscore the dependence of apoB-48 isoprotein secretion on the emergence of apoB RNA editing in utero. Second, we have cloned apoB cDNAs spanning 7.5 kb from the liver of an avian, Gallus domesticus, known to secrete only the apoB-100 isoprotein. Overall, humans and avians have 60% homology in the apoB cDNAs over the regions cloned. In particular, 70% homology exists between the chicken and the 23 nucleotide sequence conserved in 4 other species. In addition, the CAA (gln) codon was present at the predicted location where RNA editing may occur.

Finally, we have characterized the molecular defect in a kindred from Padova, Italy, with the syndrome of familial hypobetalipoproteinemia. A Padova proband was identified with very low levels of LDL cholesterol and three discrete apoB species. An abnormal, truncated apoB protein, termed apoB-87$_{Padova}$, and normally migrating apoB-48 and apoB-100 were all detected in lipoprotein samples from the proband. A single nucleotide G deletion at the immediate 5' end of exon 28 of the apoB gene was found in the homozygous proband, resulting in a shifted reading frame which prematurely terminates translation after amino acid 3978, providing the mechanism for apoB-87 synthesis. The mechanism for production of an apparently normally sized apoB-100 in the homozygous apoB-87$_{Padova}$ proband is unknown. This is the first report of a homozygous apoB gene defect in familial hypobeta- lipoproteinemia with detectable circulating apoB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02826-09 MDB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Unesterified cholesterol-rich Lipid Particles in Atherosclerotic Lesions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Fei-Fei Chao	Visiting Associate	MDB, NHLBI
Others:	Frances Carter	Biol. Lab. Technician	MDB, NHLBI
	Ya-Jun Chen	Guest Worker	MDB, NHLBI
	Martha Meng	Chemist	MDB, NHLBI
	H. Bryan Brewer	Chief	MDB, NHLBI
	Howard S. Kruth	Chief, Sect. Exp. Ather.	MDB, NHLBI

COOPERATING UNITS *(if any)* Lab. of Cell. & Devel. Biology, NIDDK (E.J. Blanchette-Mackie and N. Dwyer); Dept. of Physiology, George Washington University (B.F. Dickens); Dept. of Nutrition, USDA (E. Berlin); Dept. of Path., Univ. of Md., Sch. of Med. (J. Resau and W.T. Mergner); Dept. Biochem. Biophys., Oregon St. Univ. (Wilbert Gamble)

LAB/BRANCH

Molecular Disease Branch

SECTION

Section of Experimental Atherosclerosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously isolated and characterized unique unesterified cholesterol (UC)-rich lipid particles that accumulate in atherosclerotic lesions of humans. Because human low density lipoprotein (LDL) is a cholesterol-rich lipid particle in the blood and has a molar proportion of cholesterol to phospholipid (3:1) similar to aortic UC-rich lipid particles, we investigated a possible precursor-product relationship between LDL and UC-rich lipid particles.

We carried out enzymatic hydrolysis of LDL cholesteryl ester (CE) to determine if we could transform LDL to a particle similar to UC-rich lipid particles. No CE hydrolysis occurred when LDL was incubated with cholesterol esterase (CEase). However, when LDL was first incubated with trypsin and then incubated with CEase, LDL CE was hydrolyzed. This suggests that the protein moiety of LDL (apoB) acts as a barrier to block the susceptibility of CE to CEase. Trypsin pretreatment was not required for CE hydrolysis when LDL apoB was alternatively fragmentated by oxidation. When the same trypsin and CEase treatment was applied to high density lipoprotein (HDL), very little HDL CE hydrolyzed, suggesting that the structure and orientation of lipid within LDL and HDL result in a different susceptibility of their CE to hydrolysis.

After complete hydrolysis of LDL CE, the degraded LDL particle transformed to uni- and multilamellar liposomal structures with sizes approximately 5 times larger than native LDL. The completely hydrolyzed LDL had structural and physical properties similar to those of UC-rich lipid particles that accumulate in human atherosclerotic lesions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02831-03 MDB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cholesterol Metabolism in Human Monocyte-derived Macrophages

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Sonia I. Skarlatos	Staff Fellow	MDB, NHLBI
Others:	Rani Rao	Chemist	MDB, NHLBI
	George Johnson	Biol. Lab. Technician	MDB, NHLBI
	Daniel Rader	Med. Staff Fellow	MDB, NHLBI
	Silvia S. Fojo	Senior Investigator	MDB, NHLBI
	H. Bryan Brewer	Chief	MDB, NHLBI
	Howard S. Kruth	Chief, Sect. Exp. Ather.	MDB, NHLBI

COOPERATING UNITS *(if any)*

Department of Transfusion Medicine, CC

LAB/BRANCH

Molecular Disease Branch

SECTION

Section of Experimental Atherosclerosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study cholesterol metabolism in human monocyte-macrophages(MACS). Because MACS are one of the major cell types which accumulate lipid in atherosclerotic lesions, it is of great significance to study lipid metabolism in normal MACS as well as in MACS from patients demonstrating abnormal processing of lipoproteins.

MACS accumulated substantial amounts of cholesterol when incubated with nonlipoprotein cholesterol. Interestingly, only a subpopulation of MACS accumulated cholesteryl ester. Cholesteryl ester accumulated in typical lipid droplets but unesterified cholesterol accumulated in a unique cytoplasmic compartment that remains to be identified. Cholesterol-enriched MACS secreted accumulated cholesterol even in the absence of an added cholesterol acceptor by a process that we are now characterizing.

We also studied lipid metabolism in MACS from patients with various lipoprotein disorders. ApoE-deficient MACS accumulated nonlipoprotein cholesterol to a much greater extent than normal MACS and showed less efflux than normal MACS, demonstrating that apoE is necessary for normal regulation of MACS cholesterol metabolism. Tangier MACS degrade significantly more HDL in contrast to normal MACS suggesting that these cells could very well be one of the sites where enhanced catabolism of HDL occurs in vivo. Lipoprotein lipase-deficient MACS do not increase their triglyceride content when incubated with VLDL in contrast to normal MACS which do.

These studies should provide insight as to how MACS contribute to the metabolism of lipoproteins and cholesterol within human atherosclerotic lesions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02213-13-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Elements Regulating Expression of the Adenovirus 2 Major Late Promoter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Brian Safer, Section Head, MHB, NHLBI
Susan Farfinkel, Biologist, MHB, NHLBI
William F. Jacob, Staff Fellow, MHB, NHLBI
Linda Yang, Biologist, MHB, NHLBI

COOPERATING UNITS *(if any)*

Roger Cohen, Senior Staff Fellow, CBER, FDA

LAB/BRANCH

Molecular Hematology Branch

SECTION

RNA and Protein Biosynthesis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	.8	.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The adenovirus 2 major late promoter (MLP) is among the most active promoters transcribed in vitro using nuclear extracts from uninfected cells. Mutational analysis of the Ad2 MLP previously determined that sequences at and proximal to the TATA box (-31 to -25) were essential for a basal level of transcriptional activity. Basal activity could be increased 10-20 fold by binding of a trans-acting factor to an upstream promoter sequence. The two factors binding to the TATA and UPS, TFIID and MLTF respectively, appear to bind cooperatively to the Ad2 MLP.

The region of the Ad2 MLP surrounding the CAP or transcription start site (+1) has not been as well characterized. However, mutation of sequences at and downstream of the CAP site lowers the efficiency of transcription. These studies suggest, therefore that the sequences at and surrounding the CAP site might define a cis-acting regulatory sequence required for accurate regulation of transcription. In this study, the protein DNA interactions at the Ad2 MLP CAP site were investigated by DNase I footprint analysis and mobility gel shift analysis. These studies showed that distinct polypeptide factors bind to the TATA box and CAP site sequences. Mutation of the CAP sequence does not affect the interaction of TFIID with the TATA box nor is binding of the factor recognizing the CAP site affected by depletion of TFIID. When the CAP sequence is mutated, transcriptional activity of the Ad2 MLP is reduced both in vitro and in vivo. Depletion of nuclear extracts of CAP binding activity also reduces Ad2 MLP transcriptional activity. DNA affinity-purified CAP binding factor, when added back to the depleted extract, is able to restore activity to control levels. Efforts are currently underway to purify the CAP site binding factor to homogeneity and obtain a cDNA clone. Expression of the factor will then allow biochemical studies to define its mechanism of action during establishment of the transcription complex at the Ad2 MLP as well as host cell genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02216-11-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Correction of Genetic Defects by Gene Transfer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

W. French Anderson, Chief, MHB, NHLBI
William Polvino, PRAT Fellow, MHB, NHLBI
Leon Baltrucki, Medical Staff Fellow, MHB, NHLBI
Nga Nugyen, Medical Technologist, MHB, NHLBI
Theresa Wang, Biologist, MHB, NHLBI
Evelyn Karson, Medical Staff Fellow, MHB, NHLBI

COOPERATING UNITS *(if any)*

S. Rosenberg, Chief, SB, NCI
R.M. BLAESE, Chief, MET, NCI
Genetic Therapy, Inc., Gaithersburg, MD

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.3	.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A highly efficient procedure for transferring functional genes into mammalian cells has been developed using retroviral vectors as a delivery system. Retroviral vectors have been built which contain one of a number of different human (or other) genes. Clinical protocols have been prepared using retroviral-mediated gene transfer as a means of studying or treating human diseases. A human gene transfer protocol using NeoR-gene marked TIL to study adoptive immunotherapy for malignant melanoma is being successfully carried out. Two gene therapy protocols have been approved: one for the treatment of patients suffering from ADA Deficiency by inserting the human ADA gene into the patient's own T lymphocytes and the other for inserting a TNF gene into TIL cells as an addition to TIL adoptive immunotherapy for the treatment of advanced cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02218-02-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of gene therapy for the treatment of AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R. Morgan, Adjunct Scientist, MHB, NHLBI
W.French Anderson, Chief, MHB, NHLBI
L. Couture, Staff Fellow, MHB, NHLBI
J. Mason, Staff Fellow, MHB, NHLBI
C. Lassy, Technician, MHB, NHLBI
J. Ragheb, NRCS Fellow, MHB, NHLBI

COOPERATING UNITS *(if any)*

R.C. Gallo, Chief, NCI
D. Mosier, Medical Biology Institute, La Jolla, CA
K. Fong, Smith Kline & Beecham, King of Prussia
F. Wong-Staal, UCSD
Genetic Therapy,Inc.
Gaithersburg, MD

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.35	1.85	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Retroviral vectors have been developed which produce a secreted form of the receptor for the HIV virus, the human CD4 T-cell antigen. Amphotropic retroviral packaging cell lines were used to produce viral-vector particles which were then used to transduce a wide variety of cell types (including primary human cells). The production of the secreted CD4 molecule (sCD4) by transduced cells was demonstrated by immunoprecipitation and radioimmunoassay. Co-culture protection experiments were initiated in which human T-cell lines were grown in the presence of transduced cells secreting low levels of sCD4. The co-culture was then challenged with HIV-1 and productive infection assayed by syncytia formation and p24 production. Significant protection from HIV infection was observed in co-cultures producing sCD4. sCD4 retroviral vectors could potentially be used to engineer the cells of an HIV infected individual, and data indicate this strategy may be a potential gene therapy approach for the treatment of AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HL-02219-02-MH

PERIOD COVERED: October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Transfer for Cardiovascular Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David Dichek, Medical Staff Fellow, MHB, NHLBI
W. French Anderson, Chief, MHB, NHLBI
Kathryn Anderson, Adjunct Scientist, MBH, NHLBI
Kurt Newman, Adjunct Scientist, MHB, NHLBI
Ofer Nussbaum, Staff Fellow, MHB, NHLBI
Nga Nguyen, Research Biologist, MHB, NHLBI
Theresa Chen, Technician, MHB, NHLBI
Mark Kahn, Medical Staff Fellow, MHB, NHLBI and Sung Lee, Medical Staff Fellow,MHB

COOPERATING UNITS *(if any)*

Ted Smith, Genetic Therapy, Inc.
J. Hoeg, Senior Investigator, MDB, NHLBI
Z. Beg, Senior Investigator, MDB, NHLBI
M. Flugelman, Visiting Fellow,CB,NHLBI
R. Vermani,Armed Forces Institute of Pathology,Washington,DC
Genetic Therapy Inc.,Gaithersburg, MD

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
2.2	1.45	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major goal of this project is to develop improved therapies for hypercholesterolemia and intravascular thrombosis, two clinical problems which underlie a significant percentage of the cardiovascular morbidity and mortality in the Western world. Much recent work has focused on the genetic basis of these clinical problems, due either to inborn mutations or to inappropriate gene expression. Accordingly, we are attempting to design and implement genetic therapies for these two disease processes.

The clearest example of the genetic contribution to hypercholesterolemia is the disease Familial Hypercholesterolemia, in which mutations in the low density lipoprotein receptor gene result in severe hypercholesterolemia. An animal model exists of this disease, the Watanabe rabbit. This rabbit strain also lacks normal LDL receptors and has hypercholesterolemia. We have succeeded in introducing a normal LDL receptor gene into cultured Watanabe rabbit cells, and have reintroduced the cells into the donor rabbits. The receptor bearing cells have been found in tissue sections up to 4 weeks after implantation, demonstrating that *in vivo* expression of a normal LDL receptor is feasible in this important animal model.

A second project involves the overexpression of the tissue plasminogen activator (t-PA) gene in endothelial cells, a potential therapy for intravascular thrombosis. We have determined that overexpression of t-PA results in a net increase in fibrinolytic activity of the cells, and are currently designing means to achieve t-PA overexpression in endothelial cells *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02220-01-MH

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Transfer into Liver and Hematopoietic/Thymic Stem Cells.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L. Baltrucki, Med. Staff Fellow, MHB, NHLBI
W.F. Anderson, Chief, MHB, NHLBI
Kathy Anderson, M.D., Associate Scientist, Children's Hospital
Ramachandra Reddy, Medical Staff Fellow, MHB, NHLBI
Theresa Wang, Biologist, MHB NHLBI

COOPERATING UNITS *(if any)*

Lola Reid, M.D., Albert Einstein Medical Center, New York, N.Y.
Genetic Therapy Inc., Gaithersburg, MD.

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
1.35	1.1	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Retroviral vectors have been developed as an efficient system for delivering genes into many types of eukaryotic cells, in vitro. Success with in vivo gene transfer, however, has been limited by the lack of an effective delivery system. To address this problem, we are; 1. developing methods for introducing vectors or transduced hepatocytes/stem cells, directly into regenerating liver, initially in the rat animal model, and 2. attempting to create implantable bioartificial devices, which will function as substitutes for liver tissue, capable of expressing genes with therapeutic potential, indefinitely.

The efficiency of gene transfer into the self-renewing hematopoietic/lymphoid stem cells is too low for retroviral-mediated gene transfer to be applied successfully to human gene therapy. In an effort to improve the efficiency of gene transfer into stem cells, a three-dimensional culture system, based on a hollow-fiber/microcarrier perfusion bioreactor, is under development. In this system, bone marrow stromal cells or thymic epithelial cells will be cultured with the CD34+ marrow cell population, to high cell densities. These co-cultures will serve as "affinity columns" for pluripotent and T-lymphoid stem cells respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02221-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tissue Specific and Inducible Vectors for Use in Human Gene Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Larry Couture, Staff Fellow, MHB, NHLBI
W. French Anderson, M.D., Chief, MHB, NHLBI

COOPERATING UNITS *(if any)*

K. Culver, M.D., NCI
Y. Chiang, Ph.D., Genetic Therapy Inc.
S. Rosenberg, M.D., NCI

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

New retroviral vectors are being developed for potential use in human gene therapy. Two major directions in vector development are being pursued. The first direction is the enhancement of expression and/or expansion of tropism for current retroviral vectors by replacing Moloney LTR U3 sequences with those from various other retroviral LTR's. This battery of chimeric LTR vectors will allow the selection of a vector backbone suitable for a variety of gene therapy protocols.

The second major direction is the development of retroviral vectors with inducible and tissue specific expression. Recombinant LTR's containing the enhancer elements from inducible cellular genes or the TAT responsive element of HIV have been constructed. Intact Moloney LTR vectors have been built with the reporter gene CAT whose expression is driven off various cellular enhancer/promotor sequences.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02223-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Targeting of Retroviral Envelopes for Gene Gransfer to Specific Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ofer Nussbaum, Visiting Fellow, MHB, NHLBI
W. French Anderson, M.D., Chief, MHB, NHLBI
Richard Morgan, Adjunct Scientist, MHB, NHLBI

COOPERATING UNITS *(if any)*

V. Ferrans, Genetic Therapy Inc, Gaithersburg, MD

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Retroviral vectors, based on Mo-MuLV, are successfully being used as a gene delivery system, for gene transfer into mammalian cells, while host range is determined by the viral envelope glycoprotein gp-70. Although the viral envelope is species specific (ecotropic type, amphotropic type or xenotropic type), it is not tissue specific, and host cell range is limited. We are trying to change the Mo-Mulv envelope glycoprotein, in order to target the virus particle (containing recombinant vector) to specific cells, and/or to increase target cell range. These goals might be achieved by the identification and modification of the viral receptor-recognition site, which is located on the viral envelope (package) glycoprotein - the gp-70. We have demonstrated that by exchanging specific DNA sequences of the viral gp-70, between ecotropic and amphotropic types of Mo-MuLV, the host range of these retroviral packages can be interconverted. In addition, a large switch is being made, between Mo-MuLV gp-70 (and part of the gp-15), and the outer membrane domain of the Influenza virus binding and fusion envelope glycoprotein - the HA. This might allow to target the retroviral vector to any mammalian cells having on their surface gangliosides carrying N-acetyl sialic acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02224-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endothelial Cell Seeding of Intravascular Prostheses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David Dichek, Medical Staff Fellow, MHB, NHLBI
Kurt Newman, Adjunct Scientist, MHB, NHLBI
W. French Anderson, Chief, MHB, NHLBI
Kathryn Anderson, Adjunct Scientist, MHB, NHLBI
Nga Nguyen, Research Biologist, MHB, NHLBI
Theresa Chen, Technician, MHB, NHLBI
Ramachandra Reddy, Medical Staff Fellow, MHB, NHLBI

COOPERATING UNITS *(if any)*

R. Bowman, Emeritus Scientist, NHLBI
M. Flugelman, Visiting Fellow, CB, NHLBI
Genetic Therapy, Inc., Gaithersburg, MD

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major goal of this project is to improve the performance of intravascular prosthetic devices by seeding these devices with genetically altered endothelial cells. Failure of intravascular prostheses due to thrombosis and neointimal hyperplasia is a significant clinical problem for which current therapeutic approaches are largely ineffective.

We are currently working with two types of intravascular devices: stents and grafts. We have succeeded in seeding both of these devices with endothelial cells in which we have inserted a marker gene. We developed a pulsatile flow apparatus into which both seeded grafts and stents can be inserted. We are using this apparatus to develop protocols to optimize cell retention under physiologic flow conditions.

A computerized image analysis system has also been developed, to aid in the quantitation of the coverage of grafts with seeded cells. This system is automated, easy to use, and gives quantitative, reproducible results. We expect that it will be useful in continuing studies aimed at the optimization of cell seeding and retention.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02225-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Analysis of Murine Retroviral Envelope Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Morgan, Adjunct Scientist, MHB, NHLBI
W. French Anderson, Chief, MHB, NHLBI
Ofer Nussbaum, Staff Fellow, MHB, NHLBI
James Mason, Staff Fellow, MHB, NHLBI
Craig Lassy, MHB, NHLBI
Daryl Muenchau, Medical Technologist, MHB, NHLBI
Sabine Sturm, Visiting Associate, MHB, NHLBI

COOPERATING UNITS *(if any)*

S. Ruscetti, NCI
A. Khan, NIAID
Genetic Therapy Inc, Gaithersburg, MD

LAB/BRANCH

Molecular Hematology Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.6	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Optimal transduction of a particular subset of cells in an individual may require targeting of retroviral vectors to a specific cell type. To achieve this goal, we have begun to study the structure-to-function properties of the envelope protein of murine retroviruses (it is the envelope protein which directs the retrovirus to a particular cell type). Several approaches are being pursued. One analysis centers on a study of chimeric envelope genes containing defined regions of the MMLV ecotropic envelope substituted for the corresponding 4070A amphotropic envelope regions. Additional studies underway include investigating the properties of chimeric MMLV/AKV ecotropic envelopes, and analysis of the resulting host range potentials of hybrid MCF/xenotropic envelope viruses. Results of these studies may eventually help in the construction of systems for the direct delivery of gene therapy agents to specific cells in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02226-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Translation Factors and T-cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas R. Boal, Chemist, MHB, NHLBI
Brian Safer, Section Head, MHB, NHLBI
John A. Chiorini, Biologist, MHB, NHLBI

COOPERATING UNITS *(if any)*

C. Carter, Department of Transfusion Medicine, Blood Bank, NIH

LAB/BRANCH

Molecular Hematology Branch

SECTION

RNA and Protein Biosynthesis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.1	.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary T-cells are metabolically quiescent, with little DNA, RNA or protein synthesis. We are studying the role of eIF-2 and eIF-4E expression in the mechanism of translational activation upon mitogenic stimulation. mRNA levels of eIF-2α, eIF-2β and eIF-4E all increase greatly in the 1st 24 hour of stimulation. Nuclear run-on analysis shows that the increase in eIF-2α mRNA is not due to a significant increase in the relative level of transcriptional initiation, nor is it due to an increase in mRNA half-life. Dot-blot analysis of nuclear RNA shows that eIF-2α hnRNA increases dramatically, using either intron or exon-specific probe. This suggests that the increase in eIF-2α hnRNA is regulated at a very early point, but subsequent to transcriptional initiation. In activated cells these mRNAs are on large polysomes, indicating their efficient translation. Nonetheless, by 24 hours the number of eIF-2α, eIF-2β and eIF-4E molecules per cell only increase 2-3-fold, similar to the increase in ribosome nubmer per cell. This suggests a mechanism involving modification of pre-existing factors. We have looked at changes in the extent of phosphorylation, and we find that neither eIF-2α nor eIF-2β undergo significant changes in phosphorylation. There is, however, a dramatic increase in the phosphorylation of eIF-4E, which has been shown by other workers to correlate with increases in translational activity. It thus appears that part of translational activation of quiescent T-cells during mitogenic stimulation is due to the phosphorylation of eIF-4E.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02227-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification of Regulatory Elements that Modulate the Translational Efficiency

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John A. Chiorini, Biologist, MHB, NHLBI
Brian Safer, Section Head, MHB, NHLBI
Thomas Boal, Chemist, MHB, NHLBI
Lindy Yang, Biologist, MHB, NHLBI

COOPERATING UNITS *(if any)*

Roger B. Cohen, Senior Staff Fellow, CBER, FDA

LAB/BRANCH

Molecular Hematology Branch

SECTION

RNA and Protein Biosynthesis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.9	.1	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Eukaryotic translation factor 2 consists of three subunits alpha, beta, and gamma which are found in equal molar amounts in the cell. We are interested in studying the mechanism of regulation of these housekeeping genes. Northern analysis of the messages for the alpha and beta subunits revealed that the message for beta was five times more abundant than the message for alpha. An analysis of the translation efficiently showed that alpha message was translated more efficiently than the message for beta and thus allowing the proteins to be present in equal molar amounts. In order to identify the element responsible for this differential translation full length message for both subunits were required. While a full length clone existed for alpha only a partial cDNA was available for beta, therefore we have undertaken the cloning of the beta subunit. eIF-2β is a single copy gene with at least four pseudo genes. The expressed gene contained in a 25 KB segment which is divided into 9 exons. By screening Lamda phage libraries and PCR generated libraries we have cloned 19 KB of the loci representing 8 of the 9 exons. Several tries to clone the last exon which contains the 5' UTR and the promoter region have been unsuccessful and we are currently using our genomic map to generate a specific sub-genomic library. By determining the mechanism (s) which control the differential translation of these two messages we may gain a better understanding of the effect of cis elements regulating the efficiency of translation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02228-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Regulatory Elements that Control Expression of the Human eIF-2 Alpha Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Toby Silverman, M.D., Staff Fellow, MHB, NHLBI
Brian Safer, M.D., Section Head, MHB, NHLBI
William F. Jacob, Ph.D., Staff Fellow, MHB, NHLBI
Linda Yang, Biologist, MHB, NHLBI

COOPERATING UNITS *(if any)*

Roger Cohen, M.D., Senior Staff Fellow, CBER, FDA

LAB/BRANCH

Molecular Hematology Branch

SECTION

RNA and Protein Biosynthesis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.7	1.0	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Initiation of protein synthesis requires the formation of a ternary complex among eIF-2, GTP, and the initiator met tRNA. The formation of this ternary complex represents the first regulated step in protein translation. While much is known about the regulation of protein translation through posttranslational modification of the alpha subunit of eIF-2, little is known about the transcriptional regulation of the three subunits of eIF-2. eIF-2 alpha is a housekeeping gene whose activity is essential for all cells. The promoter structure of eIF-2 alpha is similar to that of most housekeeping genes in that it is G+C rich, has multiple transcription start sites for known transcription factors. Basal transcription shows a two-fold dependence on the presence of a palindromic sequence immediately upstream of and overlapping the transcription start sites. The promoter of eIF-2 alpha is complex and contains at least eight in vitro footprints. The overall goal of this project is to understand in detail the mechanism by which eIF-2 alpha is regulated. Although no human disease is known to be associated with abnormalities in the regulation of eIF-2 alpha, an understanding of the regulation of this gene will be useful in elucidating the regulatory mechanisms utilized by housekeeping genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-02229-01-MH

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and Cloning of Transacting Factors that Regulate Human eIF-2 Alpha

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute* Expression

William F. Jacob, Staff Fellow, MHB, NHLBI
Brian Safer, Section Head, MHB, NHLBI
Toby A. Silverman, Staff Fellow, MHB, NHLBI
John A. Chiroini, Biologist, MHB, NHLBI

COOPERATING UNITS *(if any)*

Roger Cohen, Senior Staff Fellow, CBER, FDA
Paul M. Aebersold, Expert, NCI

LAB/BRANCH

Molecular Hematology Branch

SECTION

RNA and Protein Biosynthesis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eukaryotic translation initiation factor 2 (eIF-2) catalyzes the first step in the initiation of protein synthesis through the formation of a ternary complex between the 40S subunit of the ribosome, the initiator tRNA, and GTP. Regulation of the activity of eIF-2 is a common final step in a number of pathways that regulate the overall rate of translation with the metabolic needs of the cell. Increased phosphorylation of eIF-2α and translational repression have been described in viral infection, growth and differentiation, and metabolic alterations. To examine the regulation of eIF-2 expression at the transcriptional level, we have cloned and characterized the promoter for the gene for the α subunit of eIF-2. We are interested in determining which transcription factors interact with this gene and RNA polymerase II. Analysis of the promoter using a variety of techniques has revealed evidence for a number of specific protein-DNA interactions. We have identified a cluster of five DNase I hypersensitive sites within a 25 kb region surrounding the promoter. These sites are found in all cells types examined. Associated with these sites are a number of cis elements identified by in vitro DNase I footprint experiments which interact with proteins present in nuclear extracts of K562 cells and TIL cells. None of these elements shares sequence homology with the binding sites of known regulatory factors, suggesting that eIF-2α transcription may be regulated through a novel series of regulatory elements and factors. The most prominent element consists of two adjacent protein binding sites composed of palindromic sequences. Mutation of this sites reduces expression of eIF-2α. The protein binding to this element, termed the α palindrome binding protein or α-PAL, has been purified to near homogeneity from K562 and TIL nuclear extracts using conventional and DNA affinity chromatography. This study may ultimately have implications for human disease therapy. Successful gene therapy requires proper regulation of the transferred gene, and the work described in this study is directly aimed at understanding transcriptional regulation. In addition, eIF-2α is an essential housekeeping gene; as a group, housekeeping genes have been relatively little studied. A detailed understanding of transcriptional regulation may also facilitate the identification of therapeutic agents which act through alterations in specific gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03969-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Composition of Coronary Plaques in Isolated Unstable Angina Pectoris

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Others: Amy H. Kragel, MD., Pathology Branch, NHLBI; Shanthasundari G. Reddy, MD., Special Volunteer, NHLBI; Janet T. Wittes, Ph.D., Biostatistics Research Branch, NHLBI.

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Coronary artery plaque morphology was studied in 354 five-mm segments of the 4 major (left main, left anterior descending, left circumflex, and right) epicardial coronary arteries in 10 patients with isolated unstable angina pectoris with pain at rest. The 4 major coronary arteries were sectioned at 5-mm intervals and a drawing of each of the resulting 354 movat-stained histologic sections was analyzed using a computerized morphometry system. The major component of plaque was a combination of dense acellular and cellular fibrous tissue with much smaller portions of plaque being composed of pultaceous debris, calcium, foam cells with and without inflammatory infiltrates, and inflammatory infiltrates without foam cells. There were no differences in plaque composition among any of the 4 major epicardial coronary arteries. Plaque composition varied as a function of the degree of luminal narrowing. Multi-luminal channels were seen in all 10 patients (28 [19%]) of the 146 sections narrowed >75% in cross sectional area and in 36 (10%) of all 354 segments. The high frequency of multi-luminal channels and a high percent of plaques composed of fibrous tissue suggest that a large portion of plaques in patients with angina at rest develops as a consequence of recurrent thrombosis.
Key words: coronary artery disease, thrombus, multiluminal channels, plaque rupture

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03970-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Myocarditis and Acute Myocardial Infarction Associated With Interleukin-2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Others: Amy H. Kragel, MD., Pathology Branch, NHLBI; William D. Travis, MD., Laboratory of Pathology, National Cancer Institute, NIH; Ronald G. Steis, MD., Clinical Research Branch, Biological Research Modifiers Program, National Cancer Institute, NIH; Steven A. Rosenberg, MD., Surgery Branch, National Cancer Institute, NIH

COOPERATING UNITS *(if any)*

National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The hearts of 8 patients aged 22 to 67 years (mean 41) who died during or within 4 days of interleukin-2 (IL-2) based immunotherapy for treatment of renal cell carcinoma or melanoma were studied at necropsy. Death resulted from combined cardiorespiratory failure in 2 patients, sepsis in 4 patients, acute myocardial infarction in 1, and myocarditis in 1 patient. Transmural left ventricular necrosis was present in 1 of the 2 patients with significant atherosclerotic coronary artery narrowing. Noninfectious myocarditis was present in 5 patients: the inflammatory infiltrate was lymphocytic in 4 and composed of a mixture of eosinophils and lymphocytes in 1. Though treatment related mortality associated with high dose IL-2 therapy is uncommon (1.5% in 652 consecutive patients) the potential for significant myocardial ischemia and/or myocarditis exists and, careful monitoring for arrhythmias and/or myocardial failure is warranted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03971-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphologic Findings in Saphenous Veins Used For Coronary Bypass

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Other: Jay M. Kalan, MD., Pathology Branch, NHLBI, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Certain clinical and necropsy findings are described in 53 patients who died from 13 to 185 months (mean 58) after a single aorto-coronary bypass operation. Of the 53 patients, 32 (60%) died from a cardiac cause and of their 73 saphenous vein aorto-coronary conduits 36 (49%) were narrowed at some point more than 75% in cross-sectional area by atherosclerotic plaque; the remaining 21 patients (40%) died from a non-cardiac cause and of their 50 saphenous vein conduits 10 (20%) were narrowed at some point more than 75% in cross-sectional area by plaque. Thus, the non-cardiac mode of death in a large percent of the patients suggests that the bypass operation prolonged life to a degree sufficient for another condition to develop. The 123 saphenous vein conduits were divided into 5-mm segments and a histologic section was prepared from each. Of the 1104 five-mm segments in the 32 patients dying as a consequence of myocardial ischemia, 291 (26%) were narrowed over 75% in cross-sectional area by plaque; in contrast, of the 761 five-mm segments of veins in the 21 patients with a non-cardiac mode of death, 86 (11%) were narrowed over 75% by plaque. Of the total 1865 five-mm segments of vein, only 395 (21%) were narrowed 25% or less in cross-sectional area by plaque. Thus, in patients dying late after coronary bypass the atherosclerotic process continues in all segments of the saphenous veins used as aorto-coronary conduits.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03972-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Coronary and Myocardial Findings With and Without Thrombolytic Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Others: S. David Gertz, Ph.D., MD., Pathology Branch, NHLBI, NIH; Amy H. Kragel, MD., Pathology Branch, NHLBI, NIH; Jay M. Kalan, MD., Pathology Branch, NHLBI, NIH; Eugene Braunwald, MD., Department of Medicine, Brigham and Women's Hospital, Boston, MA; and The TIMI Investigators

COOPERATING UNITS *(if any)*

Department of Medicine, Brigham and Women's Hospital, Boston, MA

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The hearts of 61 patients (age 64 plus or minus 11 years, 39 men) who died from 5 hours to 42 days (median 3 days) following a first fatal acute myocardial infarction (AMI) without having had percutaneous transluminal coronary angioplasty of coronary bypass surgery were studied to compare clinical and cardiac morphologic features of patients receiving thrombolytic therapy with tissue plasminogen activator (t-PA) to those not receiving thrombolytic therapy. Comparison of findings in the 23 patients who received t-PA intravenously 3 plus or minus hours after onset of symptoms to the 38 patients who did not showed: similar baseline characteristics with respect to: age, gender, history of hypertension; location of the AMI; heart weight; severity and numbers of coronary arteries narrowed; and frequencies of plaque rupture, plaque hemorrhage and coronary thrombi. Among the patients receiving t-PA, however, there was a greater frequency of platelet-rich (fibrin-poor) thrombi in the infarct-related coronary arteries (6 of 11 -vs- 4 of 25; p=.02), and a lower frequency of myocardial rupture (left ventricular free wall or ventricular septum) (5[22%] of 25 -vs- 18[46%] of 38; p=.045).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03973-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphologic Findings in Patients Dying Suddenly From Mitral Valve Prolapse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Other: Allen L. Dollar, MD., Pathology Branch, NHLBI, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Certain clinical and necropsy findings are described in 56 patients with mitral valve prolapse (MVP): 15 patients, aged 16 to 69 years (mean 39) died suddenly from MVP, and the remaining 41 patients died from other causes. Of the latter 41 patients, 7, aged 17 to 59 (mean 45), had associated congenital heart disease, and 34 patients, aged 17 to 70 (mean 52), had no associated congenital cardiac abnormalities. Compared to the 34 MVP patients without associated congenital heart disease and not dying suddenly from MVP, the 15 patients who died suddenly from MVP were younger (mean age 39 plus or minus 17 years -vs- mean age 52 plus or minus 15 years [p=0.01], were more often women (10/15 [67%] -vs- 9/34 [26%] [p=0.008]), and had a lower frequency of mitral regurgitation (1/15 [7%] -vs- 13/34 [38%] [p=0.02]). The patients dying suddenly from MVP also were less likely to have evidence of ruptured chordae tendineae (4/14 [29%] -vs- 10/15 [67%] [p=0.04]). The frequency of an increased heart weight (10/15 [67%] -vs- 17/29 [59%]), a dilated mitral valve anulus (12/15 [80%] -vs- 17/21 [81%]), a dilated tricuspid valve anulus (1/14 [7%] -vs- 4/23 [17%]), an elongated anterior mitral leaflet (12/14 [86%] -vs- 7/13 [54%]), an elongated posterior mitral leaflet (11/14 [79%] -vs- 10/13 [77%]), and a fibrous endocardial plaque under the posterior mitral leaflet (11/15 [73%] -vs- 12/19 [63%] were similar between the 2 groups. The severity of the prolapse (mild, 3/15 [20%] -vs- 2/19 [11%]; moderate 4/15 [27%] -vs- 11/19 [58%], and severe 8/15 [53%] -vs- 6/19 [32%]) also was similar between the 2 groups. Thus, persons with MVP who die suddenly without another recognized cause tend to be relatively young women with associated mitral regurgitation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03974-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Frequency and Types of Acute Coronary Lesions in Unstable Angina Pectoris

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Others: Amy H. Kragel, MD., Pathology Branch, NHLBI, NIH; S. David Gertz, Ph.D., MD., Pathology Branch, NHLBI, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The frequency and types of acute lesions in the epicardial coronary arteries of 14 patients with unstable angina pectoris (UAP) not complicated by acute myocardial infarction were studied and compared to those in 32 patients with fatal acute myocardial infarction (AMI) and in 21 patients with sudden coronary death (SCD) in whom myocardial necrosis was absent. The frequency of intraluminal thrombi was significantly lower in the UAP group than in the AMI group (29% vs 69%, p=.02), and identical to that which was observed in the SCD group (29%). The frequency of plaque rupture was less in the UAP than in the AMI group (36% vs 75%, p=.02) and similar to that in the SCD group (36% vs 19%, p-0.4). Similarly the frequency of plaque hemorrhage in the UAP group was less than that in the AMI group (64% vs 90%, p=0.04) and not significantly different from that in the SCD group (64% vs 38%, p=0.2). Segments of artery containing multiluminal channels was greatest in the UAP group (12% in UAP vs 7% in AMI [p=.001] vs 7% in the SCD group [p=.004]). Thus, the types and frequency of acute coronary lesions were similar in patients with UAP and SCD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03975-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Association of Cardiac Myxomas and Epithelial Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Others: Michael T. Longaker, MD., Department of Surgery, University of California, San Francisco, School of Medicine; Benjamin Hendin, BS., Department of Pathology, University of California, San Francisco, School of Medicine; Walter E. Finkbeiner, MD., PH.D., University of California, San Francisco, School of Medicine; Robert Stern, MD., University of California, San Francisco, School of Medicine

COOPERATING UNITS *(if any)*

Departments of Surgery and Pathology, University of California, San Francisco, School of Medicine, San Francisco, CA 94143

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We reviewed the records of 25 cases of cardiac myxomas at 2 referral centers. Six patients, or 24%, had a history of malignancy. An association between prior malignancy and cardiac myxoma has not been suspected previously. The gelatinous stroma of myxomas is composed predominantly of hyaluromic acid. We propose that elevated levels of hyaluronic acid-stimulating activity occur in the circulation of some patients with an epithelial malignancy. A persistent rest of fetal-like cardiac mesenchyme cells in the area of the fossa ovalis exists in a small cadre of cancer patients. Stimulation of hyaluronic acid production and cell proliferation in the fetal cardiac rest by the cancer-derived factor may underlie the subsequent development of cardiac myxomas in such individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03976-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Quantitative Amounts of Coronary Narrowing in Cocaine Addicts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Others: Frederick A. Dressler, MD., Pathology Branch, NHLBI, NIH, Fellow, Division of Cardiology, Department of Medicine, St. Louis University School of Medicine; Sonya Malekzadeh, BA., Pathology Branch, NHLBI, NIH, Freshwoman, George Washington University School of Medicine, Washington, D.C.

COOPERATING UNITS *(if any)*

George Washington University School of Medicine, Washington, DC

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

From January 1979 to February 1989, we studied at necropsy 22 cocaine addicts. The 22 patients were divided into 2 groups: death due to cocaine overdose (13 patients, aged 23 to 45 years[mean 32], and mean total blood cocaine level, 0.36 mg/dl) and non-cocaine related death (9 patients, aged 15 to 50 years [mean 32]). Of the 22 patients, 17 were men and 5 were women; 19 were black and 3 were white. Gross examination in the 22 patients disclosed that 8 patients (36%) had 1 or more of the 4 major (left main, left anterior descending, left circumflex, and right) coronary arteries narrowed at some point >75% in cross-sectional area by atherosclerotic plaque. In 17 cases, the 4 major epicardial coronary arteries were divided into 805 five-mm long patients with toxic blood concentrations of cocaine at necropsy, 41 (8%) of 544 five-mm coronary segments were narrowed 76 to 100% and 106 segments (19%) were narrowed 51 to 75% in cross-sectional area by plaque. Of the 5 cocaine addicts in whom death was not related to cocaine use, 8 (3%) of 261 five-mm coronary segments were narrowed 76 to 100% and 19 segments (7%) were narrowed 51 to 75% in cross-sectional area by plaque. These findings suggest that the frequency of coronary artery narrowing is greater in patients who die shortly after taking cocaine compared to those with death not due to cocaine overdose. Also the frequency of severe coronary arterial narrowing is considerably greater than expected for the entire group of patients whose mean age was only 32 years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03977-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cardiac Findings After Recombinant Tissue Plasminogen Activator Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Others: S. David Gertz, MD., Ph.D., Pathology Branch, NHLBI, NIH; Jay M. Kalan, MD., Pathology Branch, NHLBI, NIH; Amy H. Kragel, MD., Pathology Branch, NHLBI, NIH; Eugene Braunwald, MD., Department of Medicine, Brigham and Women's Hospital, Boston, MA.; and The TIMI Investigators

COOPERATING UNITS *(if any)*

Department of Medicine, Brigham and Women's Hospital, Boston, MA

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The hearts of 53 patients (aged 61 + or - 11 years, 34 men) who participated in the Thrombolysis in Myocardial Infarction Study (TIMI) and died from 5 hours to 260 days (median 2.6 days) after onset of chest pain were studied. Of the 53 patients, 38 received recombinant tissue plasminogen activator (rt-PA) without percutaneous transluminal coronary angioplasty (PTCA) (9 patients) or coronary artery bypass grafting (CABG) (6 patients). Comparisons between the 24 patients with hemorrhagic infarcts and the 20 patients with non-hemorrhagic infarcts showed: 1) similar frequencies of myocardial rupture (left ventricular free wall or ventricular septum) [6(25%) of 24 -vs- 5(25%) of 20], cardiogenic shock [9(41%) of 22 -vs- 7(44%) of 16), and fatal hemorrhage [2(10%) of 21 -vs- 3(19%) of 16]; 2) similar frequencies of thrombi in the infarct-related arteries [7(30% -vs- 7(37%)], but all thrombi in patients with hemorrhagic infarcts were non-occlusive, and all thrombi in those with non-hemorrhagic infarcts were occlusive (p=.0002);4) similar degrees of luminal narrowing (0-25%, 26-50%, 51-75%, 76-95%, 96-100%) in all 5-mm segments of the 4 major (left main, left anterior descending, left circumflex and right) epicardial coronary arteries in 27 patients receiving rt-PA alone; 5) similar mean percent reduction in cross-sectional area narrowing by plaque of the infarct-related arteries calculated by planimetry (67 + or -10% -vs- 68 + or -9%); 6) similar frequencies of plaque rupture [13(57%) - vs- 15(77%); p=.12]; 7) similar frequencies of hemorrhage into a plaque [(16(80%) -vs- 14(74%)]; 8) fewer right ventricular infarcts in patients with hemorrhagic infarcts [2 of 11 posterior hemorrhagic infarcts -vs- 6 of 9 posterior non-hemorrhagic infarcts; p=.03].9) similar percents of plaque with pultaceous debris (13 + or - 11% -vs- 18 + or - 9%; p=.18), calcific deposits (14 + or - 12% -vs- 20 + or - 14%; p=.25), and acellular fibrous tissue (49 + or - 14% -vs- 53 + or - 11%; p=.39).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03978-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rupture of Left Ventricular Free Wall During Acute Myocardial Infarction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rupture of the left ventricular free wall is a major complication of acute myocardial infarction, and it usually leads to hemopericardium with tamponade. During the last 16 years, 138 patients with left ventricular free wall rupture during acute myocardial infarction have been studied in the Pathology Branch, NHLBI: 131 (95%) had associated hemopericardium with probable or definite tamponade, and 7 (5%) had no blood in the pericardial sac. The 7 patients without hemopericardium ranged in age from 46 to 93 years. The interval from onset of chest pain typical of myocardial infarction to death was 4 days or less in the 6 patients and 31 days in one patient. No previous reports have appeared describing complete left ventricular myocardial wall rupture without hemopericardium. All 7 patients had a great deal of subepicardial adipose tissue and the presence of that adipose tissue may have helped to prevent extravasation of blood into the pericardial space. Clinically, all 7 patients had features suggesting through and through rupture of both myocardium and epicardium with fatal hemopericardium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03979-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hypertrophic Cardiomyopathy As A Cause Of Massive Cardiomegaly (> 1000 g)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Charles Stewart Roberts, MD., Surgery Branch, NHLBI, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The normal adult human heart in women weighs < or = 350 g and in men, < or = 400 g. The occurrence of heart weights in humans >1000 g is exceedingly rare. During the past 30 years, approximately 10,000 human adult hearts with various types of cardiovascular diseases have been examined in this laboratory. Of that number, 30 weighed >1000 g. The most common (15 patients) cause of the massive cardiomegaly was aortic regurgitation with or without other volume lesions, such as mitral regurgitation or ventricular septal defect. Pressure lesions as the cause of massive cardiomegaly was far less common: 4 patients had aortic valve stenosis, but all also had associated aortic regurgitation or mitral regurgitation. Cardiomyopathy is a rare cause of massive cariodmegaly. Of >200 patients with idiopathic dilated cardiomyopathy studied at necropsy in this laboratory, none had hearts weighing >1000 g. Of 70 patients with cardiac amyloidosis extensive enough to cause cardiac dysfunction, none had massive cardiomegaly. During the past 30 years, we have studied at necropsy 220 patients with hyypertrophic cardiomyopathy (HC) and 200 of them were >20 years of age. Of this latter number, 8 had hearts weighing >1000 g. Thus, HC needs to be added to the list of causes of massive cardiomegaly. What led to the massive cardiomegaly in our 8 patients is unclear.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03980-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth Rate of Left Atrial Myxoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Sonya Malekzadeh, BA., Pathology Branch, NHLBI, NIH, Freshwoman, George Washington University School of Medicine, Washington, D.C.

COOPERATING UNITS *(if any)*

George Washington University School of Medicine, Washington, D.C.

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

How fast do left atrial (LA) myxomas grow? To attempt to answer this question we examined publications describing patients in whom myxoma recurred in the left atrium after earlier excision of a similar tumor from this chamber.

Although many publications have described recurrence of myxoma in the left atrium after previous operative excision, most did not provide either the weight or the dimensions of the myxoma excised at the second operation (1-14). At least 10 publications (9 patients) have described either the dimensions or weight of a recurrent myxoma in the left atrium and these 9 cases are summarized in Table I (15-24). Of the 7 patients where the information was provided, the largest dimension of the recurrent LA myxoma ranged from 1.5 to 10.0 cm (mean 5.1), and the interval from the first operative excision to the operation for excision of the recurrent LA myxoma ranged from 11 to 76 months (mean 33). Thus, the recurrent myxoma in these 7 patients increased in size an average of 0.15 cm a month or 1.8 cm a year between the 2 operations. In 3 of the 9 patients with recurrent LA myxoma, the weight of the recurrent tumor was provided: the recurrent tumor weighed 19, 30 and 58 g and the interval between the 2 operations was 24, 30 and 36 months, respectively. Thus, the recurrent LA myxoma in these 2 patients grew an average of 1.2 g a month or 14 g a year.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03981-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aortic Dissection With The Entrance Tear in Descending Thoracic Aorta

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Charles S. Roberts, MD., Surgery Branch, NHLBI, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical and necropsy findings are described in 40 patients who had aortic dissection with the entrance tear in the descending thoracic aorta. Their ages at death ranged from 39 to 91 years (mean 66); 24 (60%) were men and 16 (40%) were women. Systemic hypertension was present by history in 33 patients (83%) and the hearts were of increased weight in 78%. Of the 40 patients, 31 (78%) had no operative intervention, while 9 (22%) underwent operation for aortic dissection. Of the 31 patients without operative therapy, the diagnosis of aortic dissection was established in life in 9 (29%). The interval from aortic dissection to death was $\leq$ 30 days in 13 patients (42%); rupture of the false channel was the cause of death in 9 (69%), renal failure in 2 (15%), and the cause was unclear in 2 (15%). The interval from aortic dissection to death was > 30 days in 18 (58%) of the 31 patients without operative therapy. The cause of death in these 18 patients was related to the dissection in 11 (61%) (rupture of the false channel in 5; renal failure from dissection in 3, and rupture of the false channel of a second acute dissection in 3); death in the other 7 patients (39%) was unrelated to the dissection but a non-fatal complication, namely stenosis of the true channel from compression by a thrombus-filled false channel, occurred in 4 of these 7 patients. Thus, only 3 (10%) of the 31 patients without operative therapy had no complications of aortic dissection. All 9 patients who underwent operation had had an aortic dissection within 30 days, and the operation was performed because of a major complication of the dissection. Four patients survived 8 to 84 months after operation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03982-01 PA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aortic Dissection With The Entrance Tear in Transverse Aorta

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William C. Roberts, MD., Chief, Pathology Branch, NHLBI

Charles S. Roberts, MD., Surgery Branch, NHLBI, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

None

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical and necropsy findings are described in 12 patients who had fatal aortic dissection with the entrance tear in the transverse aorta. The 12 patients represent 7% of the 181 cases of spontaneous aortic dissection seen at necropsy in the Pathology Branch, National Heart, Lung, and Blood Institute, during a 30-year period. The ages of the 12 patients at death ranged from 37 to 87 years (mean 67); 8 were men; 8 had a history of systemic hypertension, and 10 had hearts of increased weight. Diagnosis of aortic dissection was made during life in only 4 of the 12 patients. All 12 patients died of rupture of the false channel within 2 weeks of onset of signs or symptoms compatible with aortic dissection. The direction of aortic dissection from the entrance tear was entirely retrograde in 4 patients, entirely antegrade in 4 patients, and in both directions in 4 patients. Hemopericardium occurred in the first group, left hemothorax in the second group, and either in the last group. Of the 8 patients in whom the ascending aorta was involved, the retrograde dissection in each extended into the wall of aorta behind the sinuses of Valsalva, 6 had pulmonary adventitial hemorrhage, and 4 had involvement of the arch arteries by dissection. In the 4 patients who had strictly antegrade dissection, none had dissection involving the arch arteries. Thus, tear in the transverse aorta causes a dissection which is usually fatal, which often dissects retrogradely, and which may mimic dissection from a tear in ascending aorta. Aortic dissection from a tear in transverse aorta requires immediate operative intervention.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03983-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Changes in Vein Grafts in a Nonhuman Primate Animal Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Other: L.E. Boerboom, Medical College of Wisconsin
G.N. Olinger, Medical College of Wisconsin
L. Tie-Zhu, Medical College of Wisconsin
E.R. Rodriguez, Pathology Branch, NHLBI
A.H. Kissebah, Medical College of Wisconsin

COOPERATING UNITS *(if any)*

Medical College of Wisconsin, Milwaukee, Wisconsin

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Morphologic and biochemical studies were made of cephalic vein bypass grafts that were interposed in the femoral artery in stump-tailed macaque monkeys. These animals were maintained on a diet that sustained plasma cholesterol levels of 225 mg/dl. Comparisons were made of findings in control animals and in animals treated with dipyridamole and aspirin for as long as 18 months. Results obtained showed that cholesterol content of implanted grafts increased considerably compared to that of unimplanted veins, and that such an increase was much less pronounced in animals treated with dipyridamole and aspirin than in untreated animals; however, intimal thickening and medial fibrosis in vein grafts was not affected by treatment with antiplatelet agents.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03984-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Myocardial and Vascular Lesions Produced By Minoxidil

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI

Other: E.H. Herman, Food and Drug Administration
R.S.K. Young, Food and Drug Administration
T. Balazs, Food and Drug Administration

COOPERATING UNITS *(if any)*

Food and Drug Administration, Washington, D.C.

LAB/BRANCH

Pathology Branch

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Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myocardial and vascular lesions were found in dogs and in miniature swine treated with high doses of minoxidil, a vasodilating antihypertensive agent. The myocardial lesions consisted of focal areas of necrosis and were localized mainly to the regions of the left ventricular papillary muscles. These lesions were considered to be hypoxic in origin and to reflect minoxidil-induced changes in the amount and distribution of regional coronary blood flow, together with increased demands for oxygen consumption. The vascular lesions mainly affected the small arterioles. They were characterized by endothelial damage, intramural accumulation of red blood cells and platelets and by a perivascular inflammatory reaction. They were thought to result from overstretching of dilated arterioles.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03985-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cardiac Morphologic and Functional Changes Induced by Epirubicin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI

Other: M.D. Dardir, Pathology, Y.S. Mikhael, Cairo University, Egypt M.S. El-Guindy, Cairo University, Egypt, A-B. El-Aasar, Cairo University, Egypt, D.W. Alling, National Institutes of Allergy and Infectious Diseases, NIH, S.M. Banks, National Institutes of Allergy and Infectious Diseases, and N.G. El-Mowla, Cairo University, Cairo, Egypt

COOPERATING UNITS *(if any)*

Cairo University, Cairo, Egypt
National Institute of Allergy and Infectious Diseases, NIH

LAB/BRANCH

Pathology Branch

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Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cardiac functional studies were made and endomyocardial biopsies were obtained on 24 patients who received epirubicin in total cummulative doses arranging from 180 mg/m^2 to 918 mg/m^2 for the treatment of neoplasm. A total of 20 biopsies were evaluable. Histologic and ultrastructural changes were similar to those caused by other anthracycline agents. A strong correlation was demonstrated between total dose of epirubicin and pathologic change as quantified using the Billingham scale ($r = 0.7$, $p = 0.0006$). Patients who are expected to receive epirubicin in excess of 450 mg/m^2 should be based on the results of monitoring.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03986-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of ICRF-187 on Epirubicin Toxicity In Hypertensive Rats (SHR)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI

Others: M. Dardir, Pathology Branch
E.H. Herman, Food and Drug Administration

COOPERATING UNITS *(if any)*

Food and Drug Administration, Washington, D.C.

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Spontaneously hypertensive rats treated with cumulative doses of 18 mg/kg of epirubicin developed severe cardiomyopathy and severe nephropathy. Pretreatment with ICRF-187 resulted in marked reduction in the severity of the cardiomyopathy and in moderate reduction in the severity of the nephropathy. These results were similar to those previously obtained with respect to the toxic effects produced by doxorubicin in hypertensive rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03987-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Tissue-Derived Biomaterial in Cardiovascular Prosthetic Devices

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Others: S.L. Hilbert, Food and Drug Administration
M. Jones, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Food and Drug Administration, Washington, D.C.
Surgery Branch, NHLBI

LAB/BRANCH

Pathology Branch

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Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A variety of tissues and tissue components, ranging from allograft and xenograft tissues to albumin-and collagen-coated synthetic materials, have been used to fabricate cardiovascular prosthetic devices. Tissue-derived biomaterials include both viable and non-viable tissues as well as individual tissue components which have undergone some degree of preimplantation processing.

A review of the biochemistry, immunogenicity, mechanical properties, physiochemical properties, preimplantation processing and the morphology of the following cardiovascular prostheses are discussed: heart valve bioprostheses and allografts; blood vessel allografts; biologic vascular grafts; and, protein-coated vascular prostheses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03988-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preimplantation Processing and The Structure of Biologic Heart Valves

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Others: S. L. Hilbert, Food and Drug Administration
M. Jones, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Food and Drug Administration, Washington, D.C.
Surgery Branch, NHLBI

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NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prosthetic cardiac valves composed of biologic materials, including porcine aortic valves, bovine parietal pericardium, human dura mater, and human pulmonary and aortic valves, have been used extensively for the surgical replacement of diseased human native cardiac valves. An extensive review is presented of the techniques employed in the pre-implantation processing necessary to prepare each of these types of prosthetic valves for clinical use, and a description is given of the structural changes produced in the valvular biomaterials by such processing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03989-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Effects of Fixation Pressure On Morphology of Aortic Bioprotheses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Other: S.L. Hilbert, Food and Drug Administration
M.K. Barrick, Food and Drug Administration

COOPERATING UNITS *(if any)*

Food and Drug Administration, Washington, D.C.

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Pathology Branch

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Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bioprostheses manufactured from porcine aortic valves fixed with glutaraldehyde under various conditions of pressure were examined by polarized light microscopy to evaluate the degree of waviness or "crimping" of the collagen in the leaflets. Results obtained showed that the collagen crimping was present in all valves fixed at very low pressure but disappeared with increasing sensation pressure. Preservation of the crimping of collagen is considered important in maximizing the durability of bioprosthetic valves.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03990-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neutrophil Elastase Gene Expression During Bone Marrow Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI: Pierre Fouret, Ultrastructure Section, Pathology Branch, NHLBI
Other: V.J. Ferrans, Pathology Branch, NHLBI
R.M. DuBois, Pulmonary Branch, NHLBI
J-F. Bernaudin, Pulmonary Branch, NHLBI
H. Takahashi, Pulmonary Branch, NHLBI
R.G. Crystal, Pulmonary Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nucleic acid hybridization techniques were used to study the expression of the elastase gene during different stages of development of neutrophil leukocytes in human bone marrow. These studies demonstrated that the elastase gene is expressed only during a short period of time during neutrophil development, at the promyelocyte stage. Thus, the expression of the neutrophil elastase gene is likely under very tight control, and is different than that for other constituents of the neutrophil granules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03991-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alveolar Squamous Metaplasia in Paraquat-Induced Pulmonary Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Other: Y. Fukuda, Pathology Branch, T. Takemura, Pathology Branch

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sequential morphologic studies revealed that transient squamous metaplasia of alveolar epithelial cells developes during the healing phase of alveolar epithelial injury produced by the pulmonary toxicity of paraquat. This transient metaplasia represents an early effort to reline denuded alveolar epithelial surfaces and is followed by a more permanent phase of bronchiolization of alveolar epithelium as further healing of the injury takes place.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03992-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphogenesis of Abnormal Elastic Fibers In Patients With Emphysema

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Pathology Branch, NHLBI
Others: Y. Fukuda, Dept. of Pathology, Nippon Medical School, Tokyo, Japan, Y. Masuda, Dept. of Pathology, Nippon Medical School, Tokyo, Japan, M. Ishizaki, Dept. of Pathology, Nippon Medical School, Tokyo, Japan, Y. Masugi, Dept. of Pathology, Nippon Medical School, Tokyo, Japan.

COOPERATING UNITS *(if any)*

Department of Pathology, Nippon Medical School, Tokyo, Japan

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Morphologic and immunohistochemical studies at the electron microscopic level were made of elastic fibers in lung of four patients with pulmonary emphysema. Abnormal elastic fibers were observed which were interpreted as resulting from: 1) hydrolytic damage by elastase and 2) abnormal synthesis of new elastic fibers in areas of pulmonary remodeling. These observations emphasize the critical importance of alterations of elastic fibers in the pathogenesis of pulmonary emphysema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03993-01 PA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alveolar Macrophages from Individuals Exposed to Inorganic Dusts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Other: T. Takemura, Pathology Branch, NHLBI
W.N. Rom, Pulmonary Branch, NHLBI
R.G. Crystal, Pulmonary Branch, NHLBI

COOPERATING UNITS *(if any)*

Pulmonary Branch, NHLBI

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI-NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alveolar macrophages recovered by bronchoalveolar lavage from individuals chronically exposed to inorganic dust were evaluated by light microscopy and by transmission and scanning electron microscopy. Alveolar macrophages from dust-exposed individuals contained a higher proportion of particles than did those of normal unexposed subjects. In addition, the surfaces of these macrophages showed features of activation, including increased numbers of rufflings, filopodia, pinocytotic vesicles, subplasmalemmal linear densities, and increased frequency of macrophage-macrophage and macrophage-lymphocyte interactions. These changes, which were associated with increased numbers of lysosomes, emphasize the important phagocytic role of macrophages in defending the lower respiratory tract.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 02407-16 PB

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hereditary Lung Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.G. Crystal	Chief	Pulmonary Branch, NHLBI
Others:	T. Abe	Visiting Fellow	Pulmonary Branch, NHLBI
	M. Brantly	Senior Staff Fellow	Pulmonary Branch, NHLBI
	R.C. Hubbard	Senior Staff Fellow	Pulmonary Branch, NHLBI
	N. McElvaney	Visiting Associate	Pulmonary Branch, NHLBI
	H. Okayama	Visiting Fellow	Pulmonary Branch, NHLBI
	C. Vogelmeier	Guest Worker	Pulmonary Branch, NHLBI

(Continued on next page)

COOPERATING UNITS *(if any)*
Andrea Pavirani, Transgene, Strasbourg, France; Michel Perricaudet, Institut Gustave Roussy, Villejuif, France; J.-F. Bernaudin, INSERM U.139, Paris, France; Victor Ferrans and Paavo Paako, Pathology Branch, DIR, NHLBI, NIH, Bethesda, MD.

LAB/BRANCH
Pulmonary Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
20	16	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

α1-antitrypsin (α1AT) deficiency and cystic fibrosis (CF) are the two most common lethal hereditary disorders in the USA and Europe. The major clinical manifestations of both are in the lung. α1AT deficiency results from mutations in the α1AT gene (a 7 exon gene occupying 12 kb of chromosome 14) causing a systemic deficiency of α1AT, the major inhibitor of the destructive proteolytic enzyme, neutrophil elastase (NE). In the context of a deficiency of α1AT, NE released by neutrophils in the lung goes uninhibited, resulting in progressive destruction of the lung. CF results from mutations of the CF gene, a 27 exon gene occupying 250 kb of chromosome 7. All organs with exocrine glands are affected, but the major clinical manifestations are in the lung, with impacted mucus, chronic infection, inflammation, and airway and parenchymal lung derangements. Studies over the past year regarding α1AT deficiency have focused on continuing to identify new mutations in the α1AT gene, evaluating strategies for augmenting lung anti-NE defenses by aerosolization of proteins capable of inhibiting NE and gene therapy. Studies regarding CF have centered on understanding the expression of the CF gene, devising strategies for protecting the respiratory epithelium from the chronic neutrophil dominated inflammation that characterizes CF, and gene therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02533-06 PB

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interstitial Lung Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.G. Crystal	Chief	Pulmonary Branch, NHLBI
Others:	Z. Borok	Staff Fellow	Pulmonary Branch, NHLBI
	R. Buhl	Guest Worker	Pulmonary Branch, NHLBI
	H.A. Jaffe	Senior Staff Fellow	Pulmonary Branch, NHLBI
	I. Nagaoka	Visiting Associate	Pulmonary Branch, NHLBI
	W.N. Rom	Senior Staff Fellow	Pulmonary Branch, NHLBI

(Continued on next page)

COOPERATING UNITS *(if any)*
Victor Ferrans, Pathology Branch, DIR, NHLBI, NIH, Bethesda, MD

LAB/BRANCH
Pulmonary Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
15	12	3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The fibrotic lung disorders represent 15% of the non-infectious, non-malignant lung diseases; they are usually progressive and often fatal. The fibrosis results from damage caused by inflammatory cells and subsequent proliferation of mesenchymal cells, driven by mediators released by alveolar macrophage. The primary group of mediators causing the damage are oxidants. The major growth factor is platelet-derived growth factor, with the knowledge of the specific processes involved in the release of these mediators by inflammatory cells such as alveolar macrophage, strategies can be developed to modulate the expression of the genes coding for these mediators as therapy for these disorders. The granulomatous lung disorders occur in 20 to 50 per 100,000 of the USA population. The "model" disorder of this group is sarcoidosis, a disease characterized by the accumulation of activated helper/inducer T-lymphocytes at sites of disease. Evaluation of T-cells at sites of disease in these individuals demonstrates a marked bias in the populations of T-lymphocytes with similarities of the T-cell antigen receptor, including evidence for exaggerated numbers of T-lymphocytes with identical T-cell antigen receptor β-chains. One subgroup of individuals with sarcoidosis have exaggerated numbers of alternative T-cell antigen receptor containing a $\gamma\delta$ chains. Together the studies strongly suggest that sarcoidosis is caused by an exaggerated response to a subclass of antigens or self-antigens. On this basis, strategies are being devised to understand the specific etiologies and develop appropriate therapies for this disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02535-01 PB

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pulmonary Host Defense

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.G. Crystal	Chief	Pulmonary Branch, NHLBI
Others:	H.A. Jaffe	Senior Staff Fellow	Pulmonary Branch, NHLBI
	R. Buhl	Guest Worker	Pulmonary Branch, NHLBI
	A. Mastrangeli	Visiting Fellow	Pulmonary Branch, NHLBI
	K. Holroyd	Senior Staff Fellow	Pulmonary Branch, NHLBI
	G. Longenecker	Biologist	Pulmonary Branch, NHLBI
	C. Saltini	Visiting Fellow	Pulmonary Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pulmonary Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Through its role as the organ of gas exchange, the lung is constantly exposed to microorganisms impacting the respiratory epithelial surface. The alveolar macrophages play a critical role in host defense through their ability to ingest and kill microorganisms. The ability of the alveolar macrophage to carry out this task can be enhanced by interferon-γ, a mediator normally released by activated T-lymphocytes. Studies are ongoing in normals and asymptomatic individuals infected with the human immunodeficiency virus (HIV) to enhance lung host defense by administration of recombinant interferon-γ by inhalation of an aerosol containing the interferon-γ. In vitro studies are investigating the possibility of using techniques of gene transfer to modify the production of interferon-γ autologous T-lymphocytes. Quantification of the levels of glutathione in HIV-seropositive individuals demonstrated a deficiency of this tripeptide in blood and lung, an observation relevant to the role of glutathione as an antioxidant, and in modulating immune function. Strategies have been developed to augment lung levels of glutathione by aerosol administration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02714-10 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluations of Cardiac Valves: In Vitro Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Jones, M.D., Senior Investigator, Surgery Branch, NHLBI

Victor J. Ferrans, M.D., Pathologist & Senior Investigator, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

Pathology Branch, NHLBI

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	1	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The studies of this project are those of the ongoing use of juvenile sheep for evaluating cardiac valve pathology and pathophysiology. Previous years' studies have concentrated upon conventional hydraulic/hemodynamic evaluations of native and prosthetic cardiac valves, and upon the morphologic and physical characterization of calcific degeneration of bioprosthetic valves. Efforts during the current year explored the following areas: (1) studies of homograft mitral valve substitutes; and (2) studies of Doppler determined intracardiac flow dynamics. The first studies have demonstrated the feasibility of homograft mitral valve replacement, providing a unique mode for investigating mechanisms of bioprosthetic valve leaflet degeneration and mineralization. The current studies have added credence to the hypothesis that preimplantation glutaraldehyde treatment facilitates mineralization. Two-dimensional, color-encoded Doppler evaluations of intracardiac flow dynamics using fundamental physical theories of jet flux and momentum continuity preservation have provided quantitative volumetric information regarding valvular regurgitation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02731-08 SU

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Operative Treatment of Patients with Obstructive IHSS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Charles L. McIntosh, M.D., Senior Investigator & Surgeon, Surgery Branch, NHLBI

Julie A. Swain, M.D., Senior Investigator & Surgeon, Surgery Branch, NHLBI
Barry J. Maron, M.D., Senior Investigator, Cardiology Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Cardiology Branch

LAB/BRANCH
Surgery Branch

SECTION

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The surgical treatment of obstructive hypertrophic cardiomyopathy has been a major area of clinical research in the Surgery Branch for nearly thirty years. The number of patients receiving surgical treatment has gradually increased in the past five years. Currently three procedures are performed: left ventricular myotomy and myectomy (LVMM), mitral valve replacement, LVMM + plication of the anterior leaflet of the mitral valve. The standard procedure was performed in 3 patients, MVR in 3 and the combined operation in 13, in one patient a LVMM and MVR was performed. An additional program has been the use of the automatic internal cardiac defibrillator for patients with a history of sudden death and who are refractory to all anti-arrhythmic agents during electrophysiologic testing.

The postoperative data has shown that excellent relief of obstruction at rest is obtained by all three procedures. Mitral valve replacement appears to provide a more predictable relief of obstruction with provocation. There are too few patients who have had long-term studies with the new procedure for comparison to the other groups.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02777-04 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Monoclonal Antibody and IL-2 as Immunosuppression for Cardiac Allografts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul S. Brown, Jr., M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Gary L. Parenteau, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Thomas Waldmann, M.D., Chief, Metabolism Branch, NCI
Otto Gansow, Ph.D., Head, Inorganic and Radioimmunochemistry Section, Radiation Oncology Branch, NCI
Ira Pastin, M.D., Molecular Biology Laboratory, NCI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Molecular Biology, NCI
Metabolism Branch, NCI
Radiation Oncology Branch, NHLBI

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is now in its fourth year to test the hypothesis that monoclonal antibody binding to IL-2 receptors on activated T-cells will influence graft survival especially when chelated to toxins or radioactive substances. Anti-Tac, a mouse derived monoclonal antibody to human IL-2 receptors has been used alone, with a modified pseudomonas exotoxin and to a beta emitter Yttrium-90 in allograft orthotopic subhuman cardiac transplants. The data show that Anti-Tac prolongs graft survival. Chelation to a pseudomonas exotoxin PE40 caused increased graft rejection and chelation to PE66 a modified pseudomonas exotoxin significantly prolonged graft survival, but the animals died with functioning grafts secondary to drug toxicity. Yttrium-90 chelation also caused significant prolongation of graft survival at moderate and low doses, however high dose caused significant bone marrow suppression. This graft survival was shown to be independent of radiation. A new modified (humanized) form of anti-Tac has been developed and caused significant prolongation of graft survival over regular anti-Tac. This humanized form has been made with recombinant DNA techniques and contains human constant regions and only the variable regions are of mouse origin. This is hypothesized to be less immunogenic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02781-03 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Low Flow Hypothermic Bypass Protects the Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Julie A. Swain, M.D., Senior Investigator and Surgeon, Surgery Branch, NHLBI

Patrick K. Griffith, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Toni Ceckler, Ph.D., IRTA Fellow, Laboratory of Cardiac Energetics, NHLBI
Robert S. Balaban, Ph.D., Chief, Laboratory of Cardiac Energetics, NHLBI
Richard E. Clark, M.D,, Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Cardiac Energetics

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cerebral protection during surgical procedures requiring circulatory arrest of low flow remains the factor that most limits the critical time for repair of lesions. In-vivo ^{31}p-nuclear magnetic resonance spectroscopy (NMR) was used to assess the metabolic state of the brain during circulatory arrest by measuring the concentration of high energy phosphate compounds (HEP) and the intracellular pH (pHi). The degree of cerebral protection during deep hypothermic cardiopulmonary bypass (CPB) at low flow rates was compared to that obtained with a period of circulatory arrest interrupted by intermittent systemic perfusion. Sheep were instrumented with cannulae for CPB and a radiofrequency coil was positioned ont the skull. Animals were placed in the bore of a 4.7T magnet, cooled on CPB to 15°C, and had either circulatory arrest (n=5) or continuous low flow rates of 5ml/kg/min (n=6) or 10ml/kg/min (n=7) for 2 hours. A fourth group (n=5) underwent one hour of circulatory arrest, systemic perfusion for 30 minutes, then another hour of circulatory arrest. Both circulatory arrest and a flow of 5ml/kg/min resulted in severe intracellular acidosis and depletion of HEP. A flow of 10ml/kg/min preserved HEP and pHi. Therefore, deep hypothermia with CPB flows as low as 10ml/kg/min can maintain brain high energy phosphate concentrations and intracellular pH for 2 hours in sheep, whereas flows of 5 ml/kg/min or intermittent full-flow systemic perfusion between periods of circulatory arrest offer minimal benefit. Previous studies from our laboratory have shown that these NMR findings positively correlate with improved survival and preservation of neurological function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02784-03 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Myocardial Preservation with Nicardipine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul S. Brown, Jr., M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Gary L. Parenteau, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Fred W. Holland, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The hypothesis tested in this study was that a new calcium channel blocker of the light, stable dihydropyridine series, nicardipine confers additional myocardial protection to a standard cardioplegic solution. Isolated working left hearts were used from rats at 37 and 10°C, and dose response curves were developed. 27 and 210 minutes of ischemia were used, respectively. The data showed that a normothermia, a twofold increase in survival of the heart was achieved and performance of the hearts was doubled by use of the drug. No differences were found under cold conditions. It is postulated that the binding sites for this agent were minimized by hypothermia. These data confirm the efficacy of certain calcium channel blocking agents to ameliorate the ischemia reperfusion injury at normal temperatures but not a low temperatures.

It was then postulated that nicardipine might work under hypothermic conditions if the animals were pretreated with the drug prior to the induction of hypothermic arrest. When the rats were pretreated with nicardipine 15 minutes prior to excision of their hearts significant improvement in post ischemic performance and conservation of high energy phosphates was demonstrated. This was dose dependent up to 25 micrograms/kg, with decreasing performance above that dose. Nicardipine is not clinically useful when added to cold cardioplegia but is useful when it is given prior to hypothermic ischemic arrest.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02795-03 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thyroid Function and Cardiopulmonary Bypass: A Euthyroid Sick Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fred W. Holland, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Bruce D. Weintraub, M.D., Senior Investigator, NIDDK
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

NIDDK

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this prospective study was to define the effect of cardiopulmonary bypass (CPBP) on thyroid function. Blood samples were obtained from 14 patients the day of admission, postanesthetic induction, post-heparination, following institution of CPBP, the nadir of hypothermia, before termination of CPBP, and 2, 8, and 24 hrs postoperatively. TSH, TBG, Total T-4, Total T-3 and reverse T-3 (rT-3), an inactive metabolite of T-4, heretofore never reported with CPBP, were assayed by radioimmunoassay. Free T-3 (ft-3) was assayed by equilibrium dialysis. Data were compared by paired T-tests for all time intervals against preoperative values. Total T-3 (T-3), the most active thyroid hormone, and fT-3 values were significantly depressed (75 and 50% respectively) following CPBP for 24 hrs ($p<0.05$). Reverse T-3 demonstrated a four-fold rise at 8 and 24 hrs postoperatively ($p<0.05$), but no change at 2 hrs postoperatively. TBG was decreased at all sampling time ($p<0.)5$). TSH, T-4 and fT-4 remained within normal ranges at all sampling times. These results indicate that CPBP simulates the :"Euthyroid Sick Syndrome" as seen in severe burn and critically ill patients. There was marked postoperative depression of T-3 and fT-3 with increased rT-3, while TSH, T-4 and fT-4 remained within normal ranges. These data indicate utilization of fT-3 with concomitant abnormal deiodination of T-4 to rT-3 or abnormal deiodination of rR-3 to T2 (the breakdown product of rT-3). It is concluded that CPBP produces a blunted response of TSH to low T-3 and T-4. The deleterious hemodynamic effects of hypothyroidism are well established. These data provide a basis for intravenous administration of T-3 in the treatment of low cardiac output syndrome following cardiopulmonary bypass.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02799-02 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Right Ventricular Hypertrophy and Cardiac Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Isabella Y.S. Liang, Ph.D., Senior Staff Fellow, Surgery Branch, NHLBI

Joseph E. Flack, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Chahine J. Yamine, M.D., Research Fellow, Surgery Branch, NHLBI
Sandy F.C. Stewart, Ph.D., Senior Staff Fellow, Surgery Branch, NHLBI
Julie A. Swain, M.D., Senior Investigator and Surgeon, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Right ventricular dysfunction after intracardiac repair of congenital heart defects is common and is frequently responsible for the perioperative morbidity and mortality associated with repair. Animals undergoing pulmonary artery banding develop pure right ventricular hypertrophy (RVH), similar to that seen in many congenital cardiac defects. It was the purpose of this study to observe the change in right and left ventricular performance in the heart with RVH before and after global ischemia. In addition, it was hypothesized that removal of obstruction to RV outflow in chronic RVH animals would protect the postischemic performance of both ventricles. RVH was induced by pulmonary artery banding in puppies. After 6 months, the RVH group and an aged matched control group were subjected to global ischemia on 28°C cardiopulmonary bypass with cold crystalloid cardioplegia for 2 hours. In the RVH group, the PA outflow tract was repaired with a pericardial patch. Myocardial function and metabolism were determined for 4 hours after reperfusion. In both control and RVH groups, postischemic cardiac output and ventricular function were significantly lower than their preischemic values in both ventricles. However, no difference in these values was observed between control and RVH group. Aortic and LV pressure and heart rate remained unchanged throughout the study. There were no changes in RV MVO_2 and lactate consumption either in baseline measurement or during reperfusion in both groups. Therefore, postischemic RV and LV performance can be preserved during hypothermic cardioplegic arrest when RV obstruction is relieved in chronic RVH dogs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05001-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Barbiturates on Cerebral Metabolism During Hypothermic Circulatory Arrest

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael G. Siegman, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Julie A. Swain, M.D., Senior Investigator and Surgeon, Surgery Branch, NHLBI
Richard P. Anderson, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Patrick K. Griffith, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Toni Ceckler, Ph.D., Senior Research Fellow, Laboratory of Cardiac Energetics,NHLBI
Robert S. Balaban, Ph.D., Chief, Laboratory of Cardiac Energetics, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Cardiac Energetics

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurologic complications following open heart surgery occur regularly. Barbiturates have been demonstrated to have protective effects in reducing the size of ischemia induced cerebral infarcts in animals. It has also shown protective effects in reducing the neuropsychiatric complications of intracardiac surgery requiring cardiopulmonary bypass in humans.

This project represents an attempt to study the effects of barbiturates on cerebral metabolism and ATP hydrolysis kinetics with the use of 31P NMR spectroscopy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 05002-01 SU

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Lodoxomide on Global Myocardial Ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gary L. Parenteau, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lodoxamide is a potent preventer of mast cell degranulation as well as an inhibitor of xanthine oxidase. Global ischemia of the heart results in impaired return of function. This is due to many factors, however, oxygen free radicals and mast cell degranulation and oxygen free radical production by xanthine oxidase may play significant roles. Study of Lodoxamide was accomplished by using isolated rat hearts subjected to global ischemia on the Langendorf apparatus. Lodoxamide was given upon reperfusion only or at selected concentrations in the buffer solution given continuously to the hearts. Initial data indicate that benefit is gained only when Lodoxamide is given continuously in buffer solution. Additional data demonstrate that there is increasing return of post ischemic function with increasing concentration of Lodoxamide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05003-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Ultrasound Characterization of A Polyurethane Trileaflet Valve

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sandy F.C. Stewart, Ph.D., Senior Staff Fellow, Surgery Branch, NHLBI

Francoise Burte, M.D., Senior Guest Researcher, Surgery Branch, NHLBI
L.S. Yu, Ph.D., Department of Surgery, University of Utah
William J. Kolff, M.D., Ph.D., Department of Surgery, University of Utah
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

University of Utah

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Synthetic trileaflet prosthetic heart valves (STV) intended for total artificial hearts (TAHs) duplicate the design of natural valve using flexible manmade materials. The pressure drop (P)-flow behavior, closing volume, and closed valve leakage of polyurethane film STVs were compared at three flow rates against two mechanical (St. Jude Medical and Bjork-Shiley) and two bioprosthetic valves (Carpentier and Ionescu-Shiley) in an in vitro pulse duplicator. As in other valves, the peak P varied with distance from the STV and was at a maximum 1-3 cm from the valve annulus, falling off asymptotically. Maximum Ps were higher than the Ionescu pericardial, St. Jude, and at the lowest flow rate, the Bjork Shiley, but lower than the Carpentier porcine valve. Like the pericardial, the STV's pressure drop/flow behavior was flatter than the others thus mitigating high P at high flow rates. This may be due to elastic expansion of leaflet orifice area at higher flow rates. STV closing volumes were lower than all the other valves. The Carpentier porcine was the only valve with lower closed valve leakage volumes and lower leakage volumes as a percent of stroke volume. Overall, the STV was judged comparable in performance to other prosthetic valves in current clinical use. STV flow patterns and velocities were also studied with a Corometrics 880 color Doppler ultrasound system. A 20 hz oscillation was occasionally present in the pulsed Doppler spectral envelope during systole on the aortic side of the STV. Continuous wave Doppler spectra showed the same phenomenon, as did color Doppler flow imaging. This phenomenon, not seen in other prosthetic valves, suggested leaflet flutter of the open valve, which may cause durability problems since polymers are sensitive to the frequency and magnitude of cyclic stress. A mid-diastolic regurgitant jet was visible in the color Doppler images on the ventricular side of the STV, originating at a commissural fold near the strut. Though the jet size increased with flow rate, the leakage volumes were nevertheless fairly low.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05004-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Computer Assisted Color Doppler Quantification of Valve Regurgitation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sandy F.C. Stewart, Ph.D., Senior Staff Fellow, Surgery Branch, NHLBI

Francoise Burte, Ph.D., Senior Guest Researcher, Surgery Branch, NHLBI
Richard E. Clark, M.D. Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Color Doppler flow imaging can provide a qualitative noninvasive diagnosis of valvular regurgitation, but quantifying regurgitant volumes is still being developed from first principles of fluid dynamics. Several analytical methods have been reported and are under development here. The regurgitant flow rate can be calculated by the conservation of momentum method, where the momentum at the orifice is equated to that in the body of the jet. In the proximal isovelocity surface area method, the flow rate through the orifice is calculated by multiplying the velocity normal to a spherical or ellipsoidal isovelocity surface by the surface area (assuming conservation of mass). Preliminary testing of the conservation of momentum method has been performed, in an in vitro model of regurgitant flow. The flow through the orifice was directly measured by a graduated cylinder and stopwatch method, in preliminary experiments performed using two orifice sizes. A simple form of the conservation of momentum analysis, which required a single velocity determination at one point rather than the entire image, underestimated or overestimated the flow rate by three times depending on the orifice size, and had a low correlation coefficients under 0.8. Color flow images were acquired with an image processing board, and a computer program was written to calculate the momentum by integrating the velocities across the entire cross section of the jet. Flow rates so calculated provided a better estimate of the measured flow than in the simpler analysis, underestimating the true flow by 40% with a correlation coefficient of 0.95. Further experiments are planned using a more accurate ultrasound machine to measure flows in a new in vitro model that allows placement of the ultrasound transducer on either side of the orifice. Steady flow experiments will be extended to pulsatile flow. A chamber has been built for the pulse duplicator which allows ultrasound measurement of regurgitant flow through precision holes punched in bioprosthetic valves in the mitral position. The conservation of momentum method will be compared to the proximal isovelocity surface area method.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05005-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Myocardial Preservation with Magnesium Cardioplegia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

None

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Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The importance of the Mg^{+2}/Ca^{+2} relation in an oxygenated crystalloid solution with a pH of 7.45, pCO_2 40, pO_2 >750 containing 142mM Na^{+} is demonstrated. Significant augmentation of ventricular recovery is produced at 37°C and 24°C with high magnesium and low calcium containing cardioplegia. Specifically aortic flow, systolic and mean pressures, ATP, stroke volume and work return toward pre-injury values along with beneficial reductions in heart rate, diastolic pressure and overall mortality. Existing clinical formulae for various crystalloid CPS should be altered to include magnesium (15mM) and avoid calcium concentrations greater than 50μMoles/L.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05006-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pattern Matching in Cardiovascular Waveform Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sandy F.C. Stewart, Ph.D., Senior Fellow, Surgery Branch, NHLBI

Isabella Y.S. Liang, Ph.D., Senior Fellow, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Computer analysis of cardiovascular waveforms is faster than manual methods. One task commonly not automated is setting landmarks (e.g. at start systole) for analysis. Such waveforms have beat to beat variations in frequency, DC offset, and shape, which limit simple methods for automatically finding landmarks. Thus users must pick landmarks by eye, a tedious process. A pattern matcher was developed for automatically finding landmarks, programmed within a general analysis package for an IBM PC. The user sets two cursors, at similar landmarks in the waveform's first and second cycle. The program copies a template from the elements around the first landmark. At each point in the waveform the sum of the squared differences between the template and a local subset of the waveform is calculated and stored in a sum array. Next, the minimum sum of squared differences is found among points near the second landmark chosen by the user. The second landmark is reset to the position of the minimum sum, where the template best matches the waveform. The program finds subsequent landmarks by finding the minimum sum at each following cycle, using the distance between the first two landmarks as the period. The implementation was tested with n=11 canine ventricular function data sets with 8 ± 3 (mean ± SD) beats per set (sample rate = 250/s). Landmarks were set 20 ms before the minimum left ventricular dp/dt, manually and by the pattern matcher. The pattern matcher found all landmarks but for two that were too near the data set end. In 22 cases of 88, the pattern matcher was one sample point (5 ms) off. One case was two sample points (10 ms) off. The average error was 1.4 ms compared to the average period of 560 ± 20 ms, or 0.24%. Manual placement took 72 ± 24 s per data set, or 8.6 ± 2.9 s/beat, while automatic placement took 29 ± 4 s/set, or 4.0 ± 1.3 s/beat, a savings of over 100% (p=0.01 by t test). This method allowed matching despite variations in local shape. The template and local data subset were normalized by subtracting out a local mean offset, allowing matching despite variations in local DC offset. The width of the search area allowed matching despite changes in beat frequency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05007-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thromboxane A2 Receptor Specific Antagonism in Hypothermic Cardiopulmonary Bypass

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Eric N. Mendeloff, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Isabella Y.S. Liang, Ph.D., Senior Staff Fellow, Surgery Branch, NHLBI
Julie A. Swain, M.D., Senior Investigator and Surgeon, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies performed on awake sheep have implicated thromboxane A_2 (TxA_2) as a central and effector in the "heparin-protamine reaction". In addition, recent investigations in models of regional ischemia/infarction have demonstrated an important role for TxA_2 in reperfusion pathophysiology. The purpose of this study was to design a model in sheep which demonstrated 2 separate phenomena after hypothermic cardiopulmonary bypass (CPB). These phenomena are: 1) global myocardial injury after global ischemia/reperfusion, and 2) a "heparin protamine reaction" characterized by marked elevation in pulmonary vascular resistance (PVR) and decreased myocardial performance. A receptor specific TxA_2 antagonist, SQ 30,741, was used to determine the role of TxA_2 in these phenomena.

The data showed that SQ 30,741 was moderately effective in decreasing left ventricular function and had a marked effect in preventing post-protamine pulmonary hypertension. The effects were most marked when the agent was given as a continuous infusion starting before CPB. Less effect was seen when the agent was given after CPB and prior to protamine administration.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05008-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolated Lobor Pulmonary Hypertension in the Dog

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Sheel K. Vatsia, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

S. Y. Kang, M.D., Visiting Fellow, Surgery Branch, NHLBI
Peter Ramwell, Ph.D., Professor of Physiology, Georgetown University Medical School
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Department of Physiology and Biophysics, Georgetown University Medical School

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Pulmonary hypertension and concommitant pulmonary vascular disease in patients with congenital heart defects with left to right shunts are the major impediments to a successful surgical result. The mechanisms underlying the pathologic vascular changes in the lung are unknown. The purpose of this study is to create a reliable and reproducible animal model of pulmonary hypertension, and then to study evolving hemodynamic and pathologic changes, with a view of endothelial mechanisms and mediators. Specifically, bioassay of endothelium-derived relaxing factor (EDRF), thromboxane A-2 prostacyclin, endothelins, platelet activating factor and heparin binding growth factors will be performed.

Creation of the model is being performed in 20kg mongrel dogs. The left subclavian artery is anastomosed to the left pulmonary artery followed by ligation of the proximal left pulmonary artery and the inferior lobor artery. Thirty dogs will be used. Currently, we have completed the operative procedure on 2/3 of the animals. The 10 remaining dogs will be operated during August.

Subsequently, they will be divided into 3 groups for the terminal experimental procedures at 3, 6 and 12 month intervals. At the terminal experiment, complete invasive hemodynamic monitoring will be performed, along with angiography, followed by retrieval of lung specimens for biochemical analysis of blood and tissue and pathologic studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05009-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurologic Outcome After Prolonged Circulatory Arrest

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Julie A. Swain, M.D., Senior Investigator, Surgery Branch, NHLBI

Charles S. Roberts, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
David Katz, M.D., Medical Staff Fellow, Surgical Neurology, NINCDS
Louis Rosa, M.D., Senior Investigator, Surgical Neurology, NINCDS
Sheel K. Vatsia, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Surgical Neurology, NINCDS

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous NMR studies in this laboratory have shown a beneficial biochemical effect of antegrade cerebroplegia (CP-A) during hypothermic circulatory arrest. This study compared CP-A with other methods of cerebral protection during hypothermic circulatory arrest to assess the clinical utility of this technique.

Twenty-three sheep were divided into four groups: systemic hypothermia alone (SYST), and systemic hypothermia combined with external cranial cooling (EXTNL), retrograde CP (CP-R) or antegrade CP (CP-A). Cardiopulmonary bypass (CPB) was started, the sheep were cooled to 15°C, and subjected to two hours of circulatory arrest. CPB was restarted, the animals were rewarmed and weaned from CPB. Serial neurologic exams were performed and hourly scores assigned until the animals were extubated.

Postanesthetic neurologic scores improved in all groups throughout the six hour recovery period except the CP-R group. The improvement over time for these scores was similar for the EXTNL and CP-A groups and significantly better than the SYST or CP-R groups ($p=0.004$). The CP-A group had 5 of 7 animals with deficit-free survival despite the similarity in recovery of baseline brain stem function.

We conclude that both antegrade infusion of cerebroplegia and external cranial cooling confer distinct cerebroprotective effects following a protracted period of hypothermic circulatory arrest when compared with the other methods studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05010-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Protection During Hypothermic Circulatory Arrest: Effect of Hypoglycemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard V. Anderson, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Michael G. Siegman, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Toni Ceckler, Ph.D, Senior Research Fellow, Laboratory of Cardiac Energetics,NHLBI
Robert S. Balaban, Ph.D., Chief, Laboratory of Cardiac Energetics, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Cardiac Energetics

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Profound hypothermia combined with either total circulatory arrest or low flow cardiopulmonary bypass (CPB) is often used to facilitate total aortic arch replacement and repair of complex congenital heart defects. Even though CPB and hypothermic circulatory arrest (HCA) have undergone major advances, these techniques continue to be associated with neurologic sequelae ranging from mild developmental abnormalities in children, to major stroke, coma, and even death. Work done in other laboratories using models of normothermic, focal or hemispheric ischemia has demonstrated that hyperglycemia in the presence of ischemia results in increased lactic acidosis, decreased intracellular pH, failure of enzyme systems and cell membranes, and disruption of metabolism ultimately resulting in cell death. CPB in human patients frequently induces hyperglycemia and this often goes untreated in non diabetic patients. During CPB and HCA the brainmay sustain focal or global ischemia.

Investigators in the Surgery Branch of NHLBI have developed animal models for the study of low flow CPB and HCA. Cerebral metabolism, specifically high energy phosphate metabolism and intracellular pH, have been studied in vivo using Phosphorous-31 Nuclear Magnetic Resonance Spectroscopy. Studies using sheep have elucidated the kinetics of high energy phosphate metabolism and intracellular pH during low flow CPB and HCA.

Using the above model, a study is presently underway examining the effects of hyperglycemia during low flow CPB and HCA. Four groups of seven sheep are subjected to one of four conditions: 1) normoglycemic HCA, 2) hyperglycemic HCA, 3)normoglycemic low flow CPB, 4) hyperglycemic low flow CPB. Phosphorous-31 NMR Spectroscopy is being used to study energy metabolism and intracellular pH. Histologic studies are being performed on the brains.
At the present time, four animals from each of the two HCA groups have been completed. Analysis of these data is currently in progress.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05011-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cerebral Metabolism in the Cyanotic Animal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Patrick K. Griffith, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Michael G. Siegman, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Toni Ceckler, Ph.D., Senior Research Fellow, Laboratory of Cardiac Energetics, NHLBI
Robert S. Balaban, Ph.D., Chief, Laboratory of Cardiac Energetics, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Cardiac Energetics

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Circulatory arrest (CA) during deep hypothermia and cardiopulmonary bypass (CPB) is often used during correction of cardiac defects in cyanotic children. The purpose of this study was to determine the effects of chronic cyanosis, hypothermic CPB, and CA on cerebral energy metabolism. This was accomplished by measuring cerebral adenosine triphosphate (ATP) and creatine phosphate (CrP) prior to, during, and after hypothermic CPB and CA in normal and cyanotic dogs using 31-Phosphorus nuclear magnetic resonance. Dogs (6-8 weeks old) were made cyanotic (n=6) by a left atrial appendage to left pulmonary artery anastomosis. Each was studied 4 months later and compared to age-matched control animals (n=5). Spectra were obtained at 37°C, 15°C, during 2 hours of CA and 1 hour of normothermic reperfusion. Prior to CPB there were no significant differences between the two groups in ATP, CrP, or CrP/ATP (an index of free energy generated by metabolism). Upon institution of hypothermic CPB there was an increase in the CrP/ATP ratio in cyanotic animals with similar changes in the control animals as predicted from the temperature and pH effects on the creatine kinase equilibrium equation. During the period of arrest there was no difference between control and cyanotic animals in the changes of high energy phosphates. Control dogs had 50 - 70% recovery of ATP as did cyanotic dogs at 1 hr. of reperfusion but neither returned to baseline levels. The kinetics of depletion and repletion of high energy phosphates during ischemia and reperfusion are not significantly different between control and cyanotic dogs. It is postulated that the production of energy in the form of high energy phosphates is independent of the amount of oxygen present down to very low levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05012-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Acute Severe Post Ischemic Myocardial Depression Reversed by Triiodothyronine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fred W. Holland, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Paul S. Brown, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart Lung and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study was to determine the effects of tri-iodothyronine (T-3) on post-ischemic left ventricular performance and high energy phosphate content in a severe injury model. Isolated working rat hearts were studied with the Lagendorff apparatus as modified by Neely Rat hearts (n-63) received 20ml of hyperkalemic NIH #1 cardioplegia and were subjected to 20 min. of ischemia at 37°C. Treated hearts were reperfused with T-3 supplemented modified Krebs-Henseliett buffer. Control hearts did not receive T-3 supplementation. All treated hearts (n=44) performed work following ischemia. 26% (5/19) of the control hearts were not able to perform any left ventricular work following ischemia. Comparisons to pre-ischemic values demonstrated significant progressive hemodynamic recovery with increasing concentration of T-3 of 0.06, 0.15 and 0.00mg/ml LVSW recovery was 63, 72, 89, and 99% respectively ($p.<0.007$). There were corresponding recoveries of aortic flow, cardiac index, stroke volume and systolic blood pressure ($p<0.007$). Comparisons of post-ischemic high energy phosphate contents demonstrated no change with T-3 supplementation between treated and non-treated groups ($p>0.05$). Additionally, there were no significant changes in coronary sinus flow, ADP, AMP, and heart rate in any group at any time interval compared to pre-ischemic values. The administration of T-3 in a severe left ventricular injury model significantly augments ventricular recovery with no change in the decreased high energy phosphate stores.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 05013-01 SU

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Triiodothyronine Treatment of Low Cardiac Output Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fred W. Holland, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Eric N. Mendeloff, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, Md 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.6	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Low Cardiac output syndrome following cardiotomy and cardiopulmonary bypass is associated with high (10-50%) mortality. Previous studies by the Surgery Branch demonstrated low total and free triiodothyronine levels in 14 euthyroid patients having cardiac surgery. A study in isolated rat hearts showed high efficacy in treatment of low cardiac output in a severe injury model. The purpose of this study was to determine the effects of T-3 administration at the onset of reperfusion after 1 hour of global cardiac ischemia at 28°C. The data showed mild improvement in left and right ventricular fractional shortening as measured by sonomicrometers. These studies are to continue to establish the appropriate dose response relations.

LABORATORIES

NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI HL 00009-I6 LBG

PERIOD COVERED

October I, I989 - September 30, I990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Recognition and Synapse Formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marshall Nirenberg, Chief, LBG, NHLBI
Yongsók Kim, Visiting Fellow, LBG, NHLBI
Kohzo Nakayama, Visiting Fellow, LBG, NHLBI
Noriko Nakayama, Special Volunteer, LBG, NHLBI
Sadamitsu Asoh, Visiting Associate, LBG, NHLBI
Maral Mouradian, Guest Worker, LBG, NHLBI
Keith Webber, Guest Worker, LBG, NHLBI
Lan-Hsiang Wang, Staff Fellow, LBG, NHLBI
Adil Nazarali, Visiting Associate, LBG,NHLBI
Wha Seon Kwon, Predoctoral Student,LBG,NHLBI
Michael Mitas, Guest Worker, LGB, NHLBI

COOPERATING UNITS *(if any)*

Hemin Chin, Sr. Staff Fellow, LMB, NINDS

LAB/BRANCH

Laboratory of Biochemical Genetics

SECTION

Section of Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
I4	II	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The nucleotide sequences of cDNA and genomic DNA clones for NK-1, NK-2, NK-3 and NK-4 homeobox genes from Drosophila melanogaster were determined. NK-1 is expressed in small subsets of neurons and striated muscle cells in each thoracic and abdominal segment and in cells in the head. Genomic DNA was cloned from monkey and rat that corresponds to the Drosophila NK-1 homeobox DNA. Nucleotide sequence analysis showed that the amino acid sequences of the homeobox domains of monkey and rat are identical to that of the Drosophila NK-1 homeobox. NK-2 mRNA expression begins at the cellular blastoderm stage in the ventrolateral portion of the embryo as these cells become committed to the neuroectodermal pathway of development, which is the first step in the developmental program that leads to the formation of the nervous system. In 12 hr and older embryos NK-2 gene expression also was found in cells associated with the midgut. NK-3 and NK-4 are neighboring homeobox genes. NK-3 is expressed transiently in visceral mesoderm in 6-12 hr embryos in a segmentally repeated pattern. NK-4 mRNA also is expressed transiently starting as mesodermal cells appear during gastrulation (3 hr) and disappearing after 7.5 hr. Genomic DNA clones corresponding to 7 novel mouse homeobox genes were obtained and the nucleotide sequences of the homeobox regions were determined. Additional nucleotide sequence information was obtained for 2 of the 7 clones. Two novel species of Pou box-homeobox cDNA were cloned and the Pou box-homeobox regions were sequenced. Clones of mouse genomic DNA fragments with enhancer activity in N18TG2 mouse neuroblastoma cells were obtained by a selection method. CTF/NF-1 and LVc proteins extracted from N18TG2 nuclei bind to sites in cloned N1 DNA and function as enhancers. cDNA clones were obtained that correspond to species of NG108-15 poly A^+ RNA that increase in abundance when cells are treated with compounds that elevate cells cAMP. Four of the clones correspond to different regions of mitochondrial DNA, one clone corresponds to secretogranin I, and one corresponds to ribosomal protein S-10 mRNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00018-13 LBG

PERIOD COVERED

October 1, 1989 - September 30, 1990 (actually July 1, 1989 to July 1, 1990)

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Neuropeptide Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Steven L. Sabol, M.D., Ph.D., Medical Officer (Research), LBG, NHLBI
Jay Joshi, Ph.D., Senior Staff Fellow, LBG, NHLBI

COOPERATING UNITS *(if any)*

Daniel Kilpatrick, Ph.D., Worcester Foundation for Experimental Biology, Worcester, MA.
Jack Dixon, Ph.D., Department of Biochemistry, Purdue Univ., Lafayette, IN.

LAB/BRANCH

Laboratory of Biochemical Genetics

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project includes several studies on the genetic regulation of biosynthesis of protein precursors of neuropeptides in the mammalian nervous system.

One study concerns the regulation of the gene coding for neuropeptide Y (NPY), an important neurotransmitter in the central and peripheral nervous systems. We have found that increases in NPY mRNA levels and NPY gene transcription rates in PC12 rat pheochromocytoma cells are elicited by nerve growth factor (NGF) or by synergistic activation of the cyclic AMP and protein kinase C second-messenger systems. The action of NGF is profoundly inhibited by glucocorticoids, illustrating an important antagonism between NGF and glucocorticoids in neural development. We are attempting to identify the sequences near or within the rat NPY gene that are required for these responses and also for appropriate cell type-specific regulation.

Other studies concern the regulation of transcription of the gene coding for proenkephalin, the precursor of the enkephalin opioid peptides. The mechanisms of the positive regulation by cyclic AMP, glucocorticoids, and cell type are under investigation in C6 rat glioma cells and other cell lines. The proenkephalin gene was found to be expressed in QNR/D quail retina neuronal cells, a promising model of retinal amacrine neurons. The morphogen retinoic acid was found to increase proenkephalin gene expression in this line. The transactivator protein tax_1 of the human T-cell leukemia virus I (HTLV-I) was found to activate the proenkephalin gene promoter in cultured cell systems. This suggests that proenkephalin biosynthesis may be activated in some cells of patients afflicted with diseases caused by HTLV-I infection, such as tropical spastic paraparesis. The mechanism of this activation is under investigation.

These studies will hopefully shed light on the control of biosynthesis of peptides that are important in autonomic regulation, pain perception, and cognitive function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00151-20 LBG

PERIOD COVERED: October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Biology of Cyclic Nucleotides in E. coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Peterkofsky	Deputy Chief	LBG, NHLBI
Others:	N. Amin	Visiting Fellow	LBG, NHLBI
	S. Shah	Visiting Fellow	LBG, NHLBI
	H. Parikh	IRTA	LBG, NHLBI

COOPERATING UNITS *(if any)*

Frederick Cancer Center, Frederick, MD (I. Webber); University of California, San Diego, CA (J. Reizer); Nat. Inst. of Standards and Technology, Gaithersburg, MD (P. Reddy); Robert Wood Johnson Med. School, Piscataway, NJ (B. Ghosh), Centre de Biophysique Moleculaire, Orleans, France (F. Culard)

LAB/BRANCH: Laboratory of Biochemical Genetics

SECTION: Macromolecules Section

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. Crystal Structure of a cAMP-Independent Form of Catabolite Gene Activator Protein with Adenosine Substituted in One of Two cAMP-Binding Sites. Catabolite gene activator protein (CAP) in the presence of cAMP stimulates transcription from several operons in E. coli. A cAMP-independent variant, in which alanine-144 is replaced by threonine (CAP-91), is activated by adenosine, which does not activate the wild-type CAP. To test the effect of adenosine on the structure, a crystal of CAP91 grown as a complex with cAMP was soaked in a solution of adenosine and then X-ray diffraction data were accumulated. The data from the difference Fourier map suggested that adenosine becomes bound to one of the two subunits of CAP, while the other remains occupied by cAMP.

B. Effect of Site-specific Mutations in HPr on the Gram-positive Phosphoenolpyruvate:sugar Phosphotransferase System (PTS). The PTS is regulated in gram-positive bacteria by a protein kinase mediated phosphorylation of serine-46 of the phosphocarrier protein known as HPr. To define the mechanism of the regulation, site-specific mutations in serine-46 of HPr were constructed and then tested for PTS activity. The results indicate that imposition of a negative charge on serine-46 of HPr markedly inhibits its activity as a phosphocarrier.

C. Hyperexpression and Purification of Escherichia coli Adenylate Cyclase Using a Vector Designed for Expression of Lethal Gene Products. Since high levels of cAMP are toxic to E. coli, difficulties have been experienced in constructing strains that overproduce the enzyme adenylate cyclase, responsible for the synthesis of cAMP. A plasmid vector suitable for the expression of lethal genes was therefore constructed. Using a derivative of this vector, containing the gene for adenylate cyclase, it was possible to increase the cellular level of the enzyme about 7000 fold, corresponding to 30% of the total protein. A relatively simple procedure was devised to purify adenylate cyclase from extracts after hyperexpression to yield a nearly homogenous protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00153-03 LBG

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Differentiation of Excitable Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Mathew P. Daniels Research Biologist LBG, NHLBI

Others: None

COOPERATING UNITS *(if any)*

B. E. Flucher	LN-NINDS	J. Powell
M. Terasaki	"	Smith College,
H. Chin	LMB-NINDS	Biolog. Sciences Div.
S. Froehner	Dartmouth Med. School, Dept. of Biochemistry	

LAB/BRANCH

Laboratory of Biochemical Genetics

SECTION

Section of Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We do basic research on the cellular and molecular mechanisms involved in the differentiation of excitable membranes in: 1) the postsynaptic membrane of the skeletal neuromuscular junction 2) acetylcholine receptor aggregates, which form on muscle fibers in culture, in response to embryonic brain extract. These receptor aggregates are a model for the developing postsynaptic membrane 3) the membranes of the excitation-contraction coupling system of skeletal muscle (the transverse tubules, sarcoplasmic reticulum and the triad junctions they form).

Immunofluorescence and immunogold labeling at the electron microscope level shows that the peripheral membrane protein, ankyrin is associated with the triad junction in rat skeletal muscle, where it may be involved in organizing membrane channels or ion pumps, or in linking the triads to the muscle cytoskeleton.

In 18 day embryos of dysgenic (mdg/mdg) mice, which die at birth due to failure of excitation-contraction coupling (caused by a mutation in the gene for the voltage-sensitive Ca++ channel alpha-1 subunit) the expression of the alpha-1 subunit is sharply reduced or absent. However, spatial organization of the sarcoplasmic reticulum, transverse tubules and myofibrils (as seen by immunofluorescence) is normal, although these components are less prominently displayed than in normal embryos. It appears that the alpha-1 subunit is not required for the assembly of the excitation-contraction coupling system, but its absence may indirectly inhibit maturation.

In rat myotubes grown in culture, transverse tubule membranes form an extensive system which is distinct in molecular composition from the plasma membrane, despite the direct continuity of the two systems. The precursors of the transverse tubules appear to be intracellular (not connected to the plasma membrane) vesicles or tubules that first appear in myoblasts. Intracellular tubular membranes with markers for both transverse tubules and sarcoplasmic reticulum are associated with the Z lines of immature myofibrils in newly formed myotubes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00202-19 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Kinetics, Regulation, and Mechanism of Biochemical Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. Boon Chock, Chief, Section on Metabolic Regulation, LB, NHLBI

Others: James C. Cook, Ph.D., Staff Fellow, LB, NHLBI; Moon Bin Yim, Ph.D., Staff Fellow, LB, NHLBI; Luz Hermida, Special Volunteer, LB, NHLBI; Xiaomao Wu, Ph.D., Visiting Fellow, LB, NHLBI; Ephrem Tekle, Ph.D., NRC Fellow, LB, NHLBI; Siow-Kee Kong, Ph.D., Visiting Scientist, LB, NHLBI

COOPERATING UNITS *(if any)*

D. Yang, Georgetown University, Washington, DC; H. Gutfreund, Fogarty Scholar-in-Residence (Bristol University, Bristol, England); R.D. Astumian, National Institute of Standards and Technology, Gaithersburg, MD

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To study the physiological roles of the ubiquitination/deubiquitination cascade, we focused on the first enzyme in the ubiquitination pathway, the ubiquitin activating enzyme. During purification of this enzyme from rabbit reticulocytes, the enzyme copurified with the ubiquitin carrier proteins which suggests that they exist as a complex. Immunocytochemical data show significant compartmentalization of this enzyme in the cell nucleus from rat brain, heart, liver, and kidney and in HeLa cells. Immunochemical study revealed that the level of the activating enzyme is slightly lower in brain tissue from Alzheimer's patients relative to that of normal persons.

Oxygen-free radicals have been implicated in protein turnover, aging, and disease. To study the fundamental mechanism of these processes, we used EPR and spin trap methods to identify and monitor the formation and utilization of free radicals. This study revealed: (i) Mn(II) ions in bicarbonate/carbon dioxide buffer can catalyze the disproportionation of hydrogen peroxide and generate superoxide and hydroxyl radicals. Addition of an amino acid such as Leu yields a Leu-derived radical in place of the superoxide radical. Using various isotope-enriched Leu, this radical was identified as a hydronitroxide $-HOOCC(R)HNHO\bullet$. (ii) Cu,Zn-superoxide dismutase can catalyze the generation of •OH radicals in the presence of hydrogen peroxide. This finding implies that overexpression of the Cu,Zn-superoxide dismutase gene, such as in Down's syndrome, will result in the free •OH radicals generation *in vivo* and may be, in part, a cause of the illness.

It has been proposed that glycolytic enzymes form multienzyme complexes for direct transfer of metabolites. Reexamination of the evidence for direct transfer of NADH between its complexes with α-glycerol-3-phosphate dehydrogenase and with lactate dehydrogenase show the data are consistent with a free-diffusion mechanism.

We have developed an instrument which uses low amplitude, bipolar sinusoidal electric fields with variable pulse times as a means of introducing various macromolecules into cells. Our studies revealed that electroporation using a bipolar a.c. field provided the most efficient DNA transfection with the cells tested, and membrane permeabilization occurs symmetrically at the two hemispheres facing the electrodes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00204-23 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Structure: Enzyme Action and Control and Gene Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ann Ginsburg, Ph.D., Chief, Section on Protein Chemistry, LB, NHLBI

Others: Myun K. Han, Ph.D., Staff Fellow (5/88-), LB, NHLBI

Michal Zolkiewski, Ph.D., Visiting Fellow (3/90-), LB, NHLBI

Francis P. Cyran, Chemist, GS-9 (12/4/88-10/90), LB, NHLBI

Steven C. Van Noord, Summer Program (5/23/89-8/20/89), LB, NHLBI

Charles A. Counsil, Chemist GS-7 (7/17/89-7/12/90), LB, NHLBI

Mary Rose Burnham, Chemist, GS-7 (6/17/90-), LB, NHLBI

COOPERATING UNITS *(if any)*

J.B. Hunt, NSF (Chem. Div.); A. Shrake, Bur. Biologics; H.K. Schachman, Univ. of California, Berkeley; D. Eisenberg, Univ. of California, Los Angeles; Susan M. Green, Georgetown Univ.; Preston Hensley, Smith Kline & Beecham; J.R. Knutson, NHLBI

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Protein Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	4.75	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basic research on protein structure, function, and regulation has produced the following results:

(1) Heat-induced reversible partial unfolding of dodecameric Mn•glutamine synthetase from *Escherichia coli* at pH 7 involves transitions of two major thermodynamic domains, as evidenced by spectral and calorimetric measurements. Active-site ligands stabilize these domains. Interactions between domains occur and the cooperative unit is the dodecamer (rather than each subunit) in the partial unfolding transitions; ΔH = 185 kcal/(mol dodecamer).

(2) Zinc ion interactions with (a) isolated regulatory dimers of *E. coli* aspartate transcarbamoylase (ATCase) and ATCase, (b) transcriptional factor IIIA (TFIIIA) from immature oocytes of *Xenopus laevis*, and (c) yeast arginase have been studied by utilizing high-affinity, sensitive metallochromic indicators. Zinc ion binding stabilizes tertiary structures in (a-c) and also oligomer quaternary structures in (a) and (c); in (b) different affinity classes of zinc ion binding sites in the 9 Zn-fingers of TFIIIA were found and these could have a regulatory role in gene recognition.

(3) Fluorescence and spectral studies of zinc-dependent structures of TFIIIA are in progress. A specific labeling of Cys 287 of TFIIIA with a fluorescent probe was achieved by reacting 7*S* particle (in which all zinc clusters and all but one Cys are buried in the TFIIIA•5*S* RNA complex) with a thiol reactive reagent. TFIIIA interactions with labeled DNA fragments of the 5*S* RNA gene and purified TFIIIC now can be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00205-35 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis and Biochemical Roles of Selenoenzymes and Seleno-tRNAs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thressa C. Stadtman, Chief, Section on Intermediary Metabolism and Bioenergetics, Laboratory of Biochemistry, NHLBI

Others: Joe Nathan Davis Laboratory Research Assistant LB, NHLBI

COOPERATING UNITS *(if any)*

Dr. August Böck, University of München, München, West Germany; Dr. Dolph Hatfield, National Cancer Institute, NIH, Bethesda, MD; Dr. Richard S. Glass, University of Arizona, Tucson; Dr. Wei-Mei Ching, Naval Medical Research Institute, Bethesda, MD

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several lines of indirect evidence support the conclusion that a TGA termination codon in the open reading frame of the gene for a formate dehydrogenase, fdhF, in *Escherichia coli* is used to direct the incorporation of selenocysteine in the gene product. However, direct proof that the TGA codon in the gene sequence corresponds to the location of the selenocysteine residue in the polypeptide was lacking. A fusion gene product containing a portion of the formate dehydrogenase surrounding the putative selenocysteine locus fused to ß-galactosidase was isolated by affinity chromatography and digested with endoproteinase Asp-N. A resulting aspartate N-terminal peptide that contained [75Se]selenocysteine was purified and subjected to Edman automated amino acid sequence analysis. 75Se released from the peptide in the 7th cycle corresponded to the position of selenocysteine predicted from the gene sequence. Exact sequence homology of the first 19 residues of the peptide with that predicted from the gene sequence confirmed that UGA directs selenocysteine incorporation in this *E. coli* protein.

Based on the report of Arkowitz and Abeles [*JACS*, 1990] that the glycine reductase selenoprotein A is converted to its Se-carboxymethyl derivative during the reductive deamination of glycine, we have shown that the energy conserving step of the overall process involves the reductive cleavage of the selenoether derivative. Se[14C]carboxymethyl protein A was prepared directly by reaction with [14C]bromoacetate. When incubated with protein C (another enzyme component of the reductase system) in the presence of an added dithiol, the labeled carboxymethyl group was converted to an acetyl thiol ester derivative of protein C. Addition of phosphate converts the acetyl thiol ester to acetyl phosphate. The details of this novel reaction are investigated with pure selenoprotein A and pure protein C as reagents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00206-31 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Stereochemical Studies of Enzymatic Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Lin Tsai Research Chemist LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) For the identification of selenium-modified bases in tRNAs, 5-carboethoxy- and 5-carboethoxymethyl-2-selenouracils were synthesized and characterized.

(2) In order to gain insight into the biosynthetic steps for seleno-tRNAs, chemical activation of the sulfur function in 2-thiouracil derivatives was investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00211-17 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oxygen Toxicity

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Earl R. Stadtman, Ph.D., Chief, Laboratory of Biochemistry, NHLBI

Others: B.S. Berlett, Enzymes Section, LB, NHLBI

P.B. Chock, Ph.D., Head, Metabolic Regulation Section, LB, NHLBI

M.B. Yim, Ph.D., Metabolic Regulation Section, LB, NHLBI

P.E. Starke-Reed, Ph.D., Enzymes Section, LB, NHLBI

C.N. Oliver, Ph.D., Enzymes Section, LB, NHLBI

Osama Omar, Summer Research Fellowship Prgm., Enzymes Section, LB, NHLBI

COOPERATING UNITS *(if any)*

John Carney, University of Kentucky; Robert Floyd, Oklahoma Medical Research Foundation

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.35	3.2	1.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Previous studies in this laboratory have shown that a number of physiological and nonphysiological iron-dependent mixed-function oxidation (MFO) systems catalyze the generation of highly reactive oxygen species (viz, free-radicals, ferryl ion) which can damage proteins. Further investigations have shown that : (a) MFO-catalyzed oxidative damage to proteins is partly responsible for the increase in levels of catalytically inactive or less active forms of enzymes that occur during aging and oxidative stress; (b) the copper-zinc superoxide dismutase which is believed to protect cells from superoxide anion radical damage can in fact under some physiological conditions (i.e., in the presence of bicarbonate ion and hydrogen peroxide) catalyze the formation of the even more damaging hydroxyl radical ; (c) protein oxidation and concomitant loss of glutamine synthetase activity occurs during reperfusion following ischemia of the gerbil brain; (d) the ischemia-reperfusion-mediated protein damage can be attenuated by the addition of the free radical spin-trap N-tert-butyl-α-phenylnitrone (PBN) to the reperfusion medium; (e) exposure of some proteins to ozone leads to rapid loss of tyrosine, histidine and methionine residues, whereas these same residues in other proteins are resistant to attack by ozone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00224-13 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Calcium-Regulated Phosphorylation-Dephosphorylation and Enzyme Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles Y. Huang, Ph.D.	Metabolic Regulation Section	LB, NHLBI
Other:	Francesca Santini, Ph.D.	Metabolic Regulation Section	LB, NHLBI

COOPERATING UNITS *(if any)*

Isabel Climent, Department of Molecular Biology, University of Stockholm, Stockholm, Sweden

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) The physical and catalytic properties of a novel magnesium-dependent, calcium-inhibited protein phosphatase has been further characterized. Although 3 bands of 39,000, 25,000, and 16,000 molecular weight, were observed on sodium dodecyl sulfate gel electrophoresis, the two smaller bands were shown to be proteolyzed fragments of the larger band by experiments involving limited proteolysis and immunoblotting. The phosphatase was also shown not to attack phosphoseryl residues on either α or ß subunit of phosphorylase kinase and not inhibited by phosphatase inhibitor 2 or okadaic acid, thereby demonstrating that it is not a type 1 or type 2 phosphatase. Other properties of the phosphatase studied are: extinction coefficient at 280nm, pH optimum, inhibition constants for calcium in the presence and absence of a protein activator, effect of various divalent cations, several brain phosphoprotein as potential substrates, and further support for the enzyme being a calcium binding protein. A 120,000 molecular weight protein activator is found to be capable of 30 to 100-fold activation of the calcium-inhibited phosphatase.

(2) A method for analyzing kinetic parameters for two interacting components present at comparable levels has been developed and applied to the study of B1-B2 interaction and inhibition by various synthetic peptides in the *Escherichia coli* ribonucleotide reductase system.

(3) A method for differentiating inter- or intra-molecular autophosphorylation and autodephosphorylation has been proposed. The method is based on the change of apparent first-order rate constant obtained at different kinase or phosphatase concentrations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00225-13 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metal-Catalyzed Oxidation of Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Rodney L. Levine, M.D., Ph.D., Senior Investigator, LB, NHLBI

Others: Mah Shamim, Ph.D., Senior Staff Fellow, LB, NHLBI
Anders Karlström, Ph.D., Visiting Fellow, LB, NHLBI
Klaus Mittenbühler, Ph.D., Visiting Fellow, LB, NHLBI
Igor Gladstone, M.D., NIH Special Volunteer, LB, NHLBI
Hugh Mickel, M.D., NIH Special Volunteer, LB, NHLBI

COOPERATING UNITS *(if any)*

Department of Biochemistry, KabiGen AB, Stockholm, Sweden; Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA; Protein Expression Laboratory, OD, NIH; Department of Pediatrics, National Naval Medical Center, Bethesda, MD

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	3.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research focusses on metal-catalyzed oxidative modification of biopolymers, especially of proteins. The reaction is enabled by the binding of a metal such as ferrous iron to a cation binding site on the targeted protein. Hydrogen peroxide reacts at that site to generate an activated species of oxygen which then oxidizes amino acid residues at the binding site. This oxidation leads to an apparently irreversible, covalent modification of proteins which has been implicated in important physiologic and pathologic processes. These include the aging processes, arthritis, hypertension, intracellular protein turnover, oxygen toxicity, and reperfusion injury after ischemia. Determination of the actual roles of oxidative modification in these processes requires development of specific assays for modified proteins, identification of the structural and functional changes induced by modification, and understanding of factors which modulate the rate and specificity of oxidative modification *in vivo*. These are the current aims of this project.

In general, oxidatively-modified enzymes lose catalytic activity and become susceptible to proteolytic degradation. The cation binding site is weakened or destroyed and carbonyl groups are introduced into the side chains of the amino acid residues. These carbonyl groups are considered the hallmark of metal-catalyzed oxidative modification. Assays have been developed which permit detection and quantitation of these protein-bound carbonyl groups. Such assays are being applied to assess the extent of oxidative modification of proteins in human disease states. It also appears feasible to synthesize compounds which specifically oxidize an enzyme or a sequence of nucleic acid. Such compounds may have therapeutic value, particularly as agents against the human immunodeficiency virus. Current efforts are targeted towards developing drugs directed against the protease and against critical nucleic acid sequences of the virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00261-05 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CO Dehydrogenase and Acetoclastic Methanogenesis in *Methanosarcina barkeri*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: David A. Grahame Staff Fellow LB, NHLBI

COOPERATING UNITS *(if any)*

Steve Ragsdale, University of Wisconsin, Milwaukee, WI

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acetic acid is a major metabolic product of bacteria which anaerobically decompose complex organic materials. In certain natural microbial ecosystems and in those used by man for waste treatment the strictly anaerobic microorganism *Methanosarcina barkeri* is of prime importance in subsequent conversion of acetic acid to methane and carbon dioxide. The enzymes involved in acetic acid metabolism have been studied in this project since detailed information about this process is lacking. Steps involved in cleavage of the carbon–carbon bond of acetate have been studied by analysis of the enzyme carbon monoxide dehydrogenase (CODH). Subsequent methyl group transfer steps are involved prior to methane formation and previously this project has identified, purified, and produced antibodies against two methyl group transferase enzymes. One of these is present largely in cells grown on methanol, and the other is predominant in cells capable of acetate conversion to methane and carbon dioxide. Studies on these enzymes have been extended in order to compare their structural and catalytic properties in detail.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00263-05 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Signal Transduction Mechanism Involving Phosphoinositide

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Sue Goo Rhee, Ph.D.	Head, Section on Signal Transduction	LB, NHLBI
Others:	Uh Hyun Kim, M.D., Ph.D.,	Visiting Fellow	LB, NHLBI
	Jae Won Kim, Ph.D.	Visiting Fellow	LB, NHLBI
	Ha Kun Kim, Ph.D.	Visiting Fellow	LB, NHLBI
	Hyun Kim, M.D.	Visiting Fellow	LB, NHLBI
	Sang Soo Sim, Ph.D.	Special Volunteer	LB, NHLBI
	Hee Sook Kim, Ph.D.	Special Volunteer	LB, NHLBI
	Jae Woong Kim, Ph.D.	Special Volunteer	LB, NHLBI
	Do Joon Park, M.D.	Special Volunteer	LB, NHLBI

COOPERATING UNITS *(if any)*

Graham Carpenter, Vanderbilt University; Joseph Schlessinger, Rohr Research Laboratory; H. Shelton Earp, University of North Carolina at Chapel Hill; Gordon Guroff, NICHHD

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Signal Transduction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.8	8.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

(1) The mechanism by which PDGF and EGF activate phospholipase C was studied. Stimulation with the growth factors promotes the association of PLC-γ with the growth factor receptors and increase of tyrosine and serine phosphorylation of PLC-γ. We identified 4 tyrosine phosphorylation sites in PLC-γ. The tyrosine phosphorylation did not affect the catalytic activity of PLC-γ *in vitro*. We proposed, therefore, that the phosphorylation of PLC-γ by EGF receptor kinase alters its interaction with putative inhibitory protein and leads to its activation. **(2)** Treatment of NIH 3T3 cells with EGF or PDGF induced a translocation of PLC-γ from cytosol to membrane. **(3)** Although the nerve growth factor (NGF) receptor is not a protein kinase, NGF induces an increase in tyrosine and serine phosphorylation. This result suggests the possibility that the NGF-dependent increase in phosphoinositide hydrolysis in PC12 cells is due to selective phosphorylation of PLC-γ by serine and tyrosine protein kinase coupled to the NGF receptor. **(4)** Activation of protein kinase C attenuates the receptor-coupled PLC activity and provides a negative feedback signal to limit the magnitude and duration of receptor signalling. We identified PLC-ß and EGF receptor as the target of protein kinase C. **(5)** A rapid method for purification of inositol (1,4,5)trisphosphate 3-kinase (IP3K) from rat brain was developed. Inclusion of calpain inhibitor prevented the progressive degradation of the 53-kDa protein to several catalytically active fragments. Using the nonproteolyzed 53-kDa enzyme, we showed that cAMP-dependent protein kinase and protein kinase C phosphorylate, and thereby modulate the activity of IP3K. **(6)** Monoclonal antibodies to IP3K were prepared. Using these antibodies, we isolated a cDNA clone that encodes IP3K from a rat brain cDNA expression library. This clone had an open reading frame that would direct the synthesis of a protein of 449 amino acids. The putative protein revealed a potential calmodulin-binding site and 6 regions with amino acid compositions common to proteins that are susceptible to calpain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00265-04 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Factors Affecting Expression of a Selenium-Containing Enzyme

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Milton J. Axley	Staff Fellow	LB, NHLBI
Others:	Thressa C. Stadtman	Section Chief	LB, NHLBI
	David A. Grahame	Staff Fellow	LB, NHLBI

COOPERATING UNITS *(if any)*

Dr. August Böck, University of München, München, West Germany

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mammals, birds, and several species of bacteria incorporate selenium as selenocysteine at specific sites of a few essential proteins. The bacterium *Escherichia coli* produces a selenocysteine-containing enzyme, formate dehydrogenase, when grown under anaerobic conditions. We have used this as an easily manipulable model system for analyzing the regulation of gene expression at the transcriptional and translational levels. In contrast to the results found for other anaerobic-specific genes, we have found that inhibition of gyrase activity (which increases the supercoiling of DNA) enhances the expression of formate dehydrogenase.

We have purified this formate dehydrogenase to near homogeneity. This has allowed studies on the properties of this selenoprotein. The chemical function of the selenocysteine moiety in the enzyme's reaction mechanism can be analyzed by comparison of this protein with a mutant protein in which the selenocysteine is replaced by cysteine. This mutant species has been purified recently. We have found this mutant enzyme binds its substrate with an affinity similar to the wild-type enzyme, but its catalytic activity is greatly reduced.

Elucidation of the biochemical mechanism of selenium utilization would allow a greater appreciation of the essential role of selenium in the diet. With an understanding of the mechanism of selenocysteine incorporation into protein, one could direct the mutagenesis of a protein such that selenocysteine replaces cysteine. Such protein engineering could significantly alter the catalytic properties of many enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00266-04 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning of Selenoprotein A Gene from *Clostridium sticklandii*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gregory E. Garcia Staff Fellow LB, NHLBI

Others: Thressa C. Stadtman Section Chief LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Selenoprotein A (SPA) of the glycine reductase complex from Clostridium purinolyticum was purified and nearly a third of the protein sequence determined. Oligonucleotide primers were synthesized to the deduced mRNA and gene sequences corresponding to the amino acid sequences of the N-terminus and an internal peptide. These primers were used to amplify a specific DNA fragment from genomic DNA by the polymerase chain reaction (PCR) technique. The PCR product was used to select a 3,600 bp DNA fragment from total genomic DNA digested to completion with restriction enzyme Hind III. The fragment was inserted into the Hind III site of vector pUC-13 and cloned into E. coli strain DH5. The entire SPA gene was identified within the insert by Sanger dideoxynucleotide sequencing with appropriate oligonucleotide primers. Both DNA strands of the gene were sequenced. It was found that the gene encodes a protein of 150 amino acids in length and predicts a UGA codon in the mRNA corresponding to the location of selenocysteine in the protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00267-04 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Ubiquitination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: James C. Cook, Ph.D., Staff Fellow, LB, NHLBI

Others: P. Boon Chock, Ph.D., Section Chief, LB, NHLBI
Luz Hermida, Special Volunteer, LB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed toward understanding the role of the Ubiquitin System in cellular metabolism.

Reagents for immunochemical studies of Ubiquitin and Ubiquitin Activating Enzyme were developed and used for quantitation of these two proteins in Normal and Alzheimer's Diseased brains. Slightly lower levels of Ubiquitin Activating Enzyme were detected in Alzheimer's Diseased tissue.

Immunocytochemical studies were carried out with rat brain, heart, liver and kidney and with HeLa cells. Results suggest significant compartmentalization of Ubiquitin Activating Enzyme in the cell nucleus.

Two isoforms of Ubiquitin Activating Enzyme have been detected in several cell types. Characterization of these isoforms is in progress.

Copurification of Ubiquitin Activating Enzyme and Ubiquitin Carrier Protein from rabbit reticulocytes has been observed, and may be an indicator of a specific protein-protein complex. Such an interaction may be the basis of a regulatory mechanism. Confirming evidence for complex formation is being sought.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00268-04 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Oxidation of Proteins and Model Polymers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: J. Michael Poston, Ph.D., Research Chemist, Enzymes Section, LB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues XX ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Homopolymers of L-isomers of alanine, arginine, histidine, lysine, and proline have been oxidized in the presence of ferrous iron and a chelator, generally citrate. Oxidation of the polymers introduces carbonyls which may be detected by reaction with 2,4-dinitrophenylhydrazine or with *p*-aminobenzoic acid in the presence of sodium cyanoborohydride. Oxidation results in loss of acid precipitability of these homopolymers. When similar oxidations were conducted with insulin beta chain, similar oxidative changes are seen: increased carbonyl content and decreased precipitation with acid. Amino acid analysis of the insulin beta chain suggests that the attack on the peptide's residues must be random.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00272-02 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

EPR Study of Free Radicals in Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Moon Bin Yim, Ph.D.	Senior Staff Fellow	LB, NHLBI
Others:	P. Boon Chock	Section Chief	LB, NHLBI
	Earl R. Stadtman	Chief	LB, NHLBI
	Barbara Berlett	Biologist	LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The list of mammalian disease processes in which oxygen radicals and their cascaded reactive radicals are implicated continues to grow. In order to examine the structural identities of these species and the mechanism of the oxidative modification of biomolecules, we investigated the effects of superoxide dismutase (SOD) and transition metal ions on the formation of these radicals. Electron Paramagnetic Resonance (EPR) spectroscopy and a spin trapping method were employed for the studies.

The SOD catalyzes the reaction of superoxide dismutation and thus protects biomolecules from the superoxide toxicity. However, its own reaction product hydrogen peroxide is also toxic and is known to inactivate Cu,Zn-SOD itself. We have found in this study that during the inactivation of Cu,Zn-SOD by hydrogen peroxide, "free" •OH radicals are produced and escape from the active site channel. This finding implies that the overexpression of the Cu,Zn-SOD gene (SOD1), such as in Down's syndrome, will result in the formation of "free" •OH radicals *in vivo* and may be, in part, a cause of the syndrome.

Transition metal ions are generally required for the generation of oxygen radicals. We have studied the effects of Mn(II) ions in physiological concentrations of bicarbonate and carbon dioxide. During the disproportionation of hydrogen peroxide catalyzed by Mn(II), superoxide and hydroxyl radicals are detected. Addition of an amino acid in the solution resulted in production of an amino acid-derived radical that replaced the superoxide radical. By employing various isotope-enriched amino acids we have identified this radical as a hydronitroxide $-OOCC(R)HNHO\bullet$. The data are consistent with the formation of a transient "caged" •OH in the inner coordination sphere of Mn(II). Two reaction schemes are proposed to account for the experimental results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00273-01 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chaperone Assisted Protein Renaturation Using GroEL/ES from *E. coli*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Mark T. Fisher, Ph.D., Staff Fellow, Enzyme Section, LB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues XX ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The correct expression and assembly of multisubunit proteins (oligomers) and monomers containing multidomain structures depends on the cell's ability to prevent inappropriate aggregation and misfolding of newly synthesized polypeptides. In recent years, experimental evidence has accumulated which suggests that protein factors, termed molecular chaperones, aid in this process by preventing detrimental aggregation through transient binding events. The exact molecular details involved in both the recognition and release of bound polypeptide are unknown at present. These molecular chaperones have been identified as intrinsic heat shock proteins (levels of these proteins increase under conditions of heat stress) and are, thus far, found to be ubiquitous in all living organisms characterized to date. These chaperones are absolutely required by the cell and deletion of such factors is lethal. The development of appropriate assays using model protein systems is essential for assessing and elucidating the molecular role these molecular chaperones play during *in vivo* protein folding and assembly. To this end, a series experiments were designed to compare protein refolding between near-physiological and nonphysiological solution conditions using dissociated/unfolded *E. coli* glutamine synthetase (GS) oligomer and unfolded monomeric carbonic anhydrase. Under conditions of physiological ionic strength, pH, temperature, and appropriate cofactor concentrations (in this case, physiological metal ion concentrations), these proteins refold and/or reassociate with low yields and activity. The use of non-physiological solution conditions such as high ionic strengths, inclusion of small amounts of protein denaturants such as urea or guanidine HCl, large concentrations of metal ions, and low temperature were found to decrease the rates of inappropriate aggregation and resulted in higher yields of renatured and active proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00274-01 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In vitro incorporation of selenium into tRNAs of *Salmonella typhimurium*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Zsuzsanna Veres	Visiting Associate	LB, NHLBI
Others:	Thressa C. Stadtman	Section Chief	LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Broken cell preparations of *Salmonella typhimurium* rapidly incorporated radiolabeled selenium from radiolabeled selenite into tRNA by an ATP-dependent process. Selenium incorporation in the presence of radiolabeled selenite was enhanced by the selenocysteine precursor, O-acetyl-L-serine. This increase in incorporation was a function of O-acetyl-L-serine concentration. Neither O-acetyl-L-homoserine nor O-phospho-L-serine stimulated the incorporation of selenium into tRNA. The incorporation of radiolabeled selenium from radiolabeled selenite was decreased by the addition of L-selenocysteine but not by the D isomer. When homologous bulk tRNA was added to the broken cell preparations, an increased rate of radiolabeled selenium labeling was observed. The supernatant fraction of the broken cell preparation contained all of the enzymes required for this process. Reversed-phase HPLC analysis of labeled bulk tRNA digested to nucleosides showed the presence of a labeled compound that co-eluted with authentic 5-methylaminomethyl-2-selenouridine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00275-01 LB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis and characterization of seleno-tRNAs from *Methanococcus vannielii*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Politino	IRTA Fellow	LB, NHLBI
Others:	Thressa C. Stadtman	Section Chief	LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Selenium–containing nucleosides are natural components of several tRNA species in *Methanococcus vannielii*. In the present study, the incorporation of selenium into these macromolecules was investigated using sonic extracts of *M. vannielii*. Nucleoside analysis of the Selenium 75–labeled tRNAs from these *in vitro* reaction mixtures demonstrated that the selenium was present in Selenium 75–labeled nucleosides identical to the two naturally occurring 2–selenouridines produced *in vivo*. Incorporation of selenium into these nucleosides was ATP–dependent and was maximal after twenty minutes. Addition of O–acetylserine enhanced the activity two– to three–fold, implicating a role for selenocysteine in the reaction. Added L–selenocysteine could function as a selenium donor, but the D isomer and D,L–selenomethionine were inactive. RPC–5 chromatography of bulk tRNA isolated from *M. vannielii* grown on radioactive selenite separated five major species of seleno–tRNAs. The amino acid–accepting activity of these tRNAs was investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 01002-16 LBC

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Investigations by Nuclear Magnetic Resonance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Edward A. Sokoloski, Chemist, LBC:NHLBI
Other: Govind Kapida, Howard University, Washington, D.C.

COOPERATING UNITS *(if any)*
Govind Kapida Howard University, Washington, D.C.

LAB/BRANCH: Laboratory of Biophysical Chemistry

SECTION: Nuclear Magnetic Resonance

INSTITUTE AND LOCATION
NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.00	2.00	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nuclear Magnetic Resonance and Mass Spectroscopy are complementary instrumental methods for structural determination of organic and bio-organic chemical compounds. The determination of the structure of several indole alkaloids from the seeds of Picralima nitida has been completed. A new indole alkaloid, picratidine, was found. Control studies for a project to examine antibody binding by deuterium NMR are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01003-18 LB C

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure of Natural Products Using Instrumental Methods

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.M. Fales, Ph.D. Chief Laboratory of Chemistry
Other: R. Mason, Ph.D. Staff Fellow

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Biophysical Chemistry

SECTION Chemical Structure Section

INSTITUTE AND LOCATION NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A compound from the action of high molecular weight B-cell growth factor on cell walls has been studied to determine its structure. The natural killer cell factor, presumably a peptide, has also been analyzed by mass spectrometry and nmr. Pheromones from the Guam tree snake and an antioxidant from nasal effluvia have been identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01005-19 LB C

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Solid State and Computer Studies of Physiologically-Important Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.V. Silverton, LBC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Biophysical Chemistry

SECTION Chemical Structure

INSTITUTE AND LOCATION

NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Solid-state and **computational** research into **structure, conformation** and **configuration** of **biologically** important chemicals. Compounds investigated were relevant to **AIDS therapy, enzyme action, synthesis** and **optical purity.**

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01006-19 LB C

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Natural Materials and Metabolic Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert J. Highet, LBC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Biophysical Chemistry

SECTION Structural Nuclear Magnetic Resonance Section

INSTITUTE AND LOCATION
NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nuclear Magnetic Resonance studies have established the structure of a product of the trichloromethyl radical on myoglobin, of two mold metabolites and of products of the interaction of betamethylaminoalanine and bicarbonate ion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 01027-08 LBC

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Three-dimensional structures of biological macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Ferretti, J. A., LBC: NHLBI
Others: Han, K.-H., Senior Staff Fellow, LBC
Yang, J.-S., Visiting Fellow, LBC

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Biophysical Chemistry

SECTION Nuclear Magnetic Resonance

INSTITUTE AND LOCATION

NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Solid State and solution nuclear magnetic resonance spectroscopy has been used to study the structure of peptides in various noncrystalline states which may be similar to the physiological environment. The peptides we have studied include a series of peptides which is a part of the integral membrane protein, gp41, found at the surface of human immunodeficiency virus. As a result of these conformational studies we have identified transmembrane regions as well as other regions which interact exclusively with the phosphate polar headgroups of the membrane bilayer. We have also studied the solution structure of two peptides associated with a region of the gp41. One peptide is highly antigenic and distinguishes between the sera of healthy HIV positive patients from those with AIDS. The corresponding peptide where alanine is replaced by threonine in position seven does not show such a distinction. We have also developed new NMR pulse methods for studying these systems and we are continuing to evaluate the errors in the corresponding measurements.

We have developed and implemented new NMR pulse methods for studying these systems and we are continuing to evaluate the errors in the measurements.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01029-03 LB C

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioorganic Chemistry of Natural Amines and Other Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Tappey Jones, LBC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Biophysical Chemistry

SECTION Chemical Structure Section

INSTITUTE AND LOCATION

NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Structural elucidation studies have been successfully conducted on the alkaloidal venom of a South American ant species, **Megalomyrmex leoninus,** and on the probable sex pheromones of the Brown Tree Snake. New synthetic routes to a lipophilic C-terminal cysteine derivative, a set of unique tricyclic azaacetal venom alkaloids, the probable sex pheromone of the Brown Tree Snake, and a unique spiropyrrolizidine frog neurotoxin have been undertaken or completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01030-02 LB C

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigations of mass spectral techniques and processes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: H.M. Fales, Ph.D. Chief, Laboratory of Chemistry

OTHER: R.T. Mason, Ph.D. Staff Fellow

Louis Pannell, NIDDK

COOPERATING UNITS *(if any)*

Louis Pannell, Ph.D. NIDDK

LAB/BRANCH

Laboratory of Biophysical Chemistry

SECTION

Chemical Structure Section

INSTITUTE AND LOCATION

NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ion trap mass spectrometer has been investigated in detail to determine the conditions under which self-CI occurs. A reflectron has been adapted to the PDMS spectrometer, reducing noise and increasing resolution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 01031-01 LBC

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Preparative High-Speed Countercurrent Chromatograph with Three Multilayer Coils.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Yoichiro Ito Senior Investigator LBC:NHLBI

Others. Eiichi Kitazume Visiting Fellow LBC:NHLBI
Jimmie L. Slemp Machinist BEIB: NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Biophysical Chemistry

SECTION

INSTITUTE AND LOCATION
NIH:NHLBI Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A high-speed CCC centrifuge was designed and constructed for performing preparative-scale separations. The apparatus holds three multilayer coils (2.6mm ID) which are connected in series to make up a total capacity of about 1600ml. The maximum speed is 1000 rpm. Preparative capability of the apparatus was demonstrated on the separation of a 4g quantity of DNP amino acids with a two-phase solvent system composed of CHCl3/CH3COOH/0.1NHCl (2:2:1). Four components were resolved in 7.5h at partition efficiencies ranging from 1000 to 1800 theoretical plates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 01413-28 LBC
(formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Biophysical Methods for Studying Bio-molecular Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I: R.L. Berger, Chief, Biophysical Instrumentation Section
Others: J. Forehlich NIA LMB
H. Cascio, BEIB
Ira Levin, NIDDK

COOPERATING UNITS *(if any)*

Commonwealth Technology, Alexandria, Va
NASA Houston, NIA, BEIB, NIDDK

LAB/BRANCH Laboratory of Biophysical Chemistry (Formerly LTD)

SECTION

INSTITUTE AND LOCATION

NIH:NHLBI Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The utilization of adenosine triphosphate as the prime energy source of many muscle and ion transport reactions is of considerable interest to the understanding of cellular processes. A major problem in studying the pre-steady state kinetics of these reactions has been the lack of ability to follow the disappearance of ATP and the appearance of the reaction products ADP and PO4. We have recently utilized near infra-red spectroscopy to detect each of these compounds and have been able to make calibration curves for each in the 1 to 25 micromolar region. Many attributes of this system must be carefully controlled to allow us to have really good reproducibility and sensitivity i.e., approaching that of radiolabelled compounds which utilize P32 labelled ATP. Considerable progress has been made on the problem this year and we now know the measurements can be made. Laser Raman spectroscopy has been carried out on the PO4 mixture at pH11, 9, 6, and 1 as well as pH 7.4 where a mixture of the H2P04, HP04 occurs but where we get the best spectral signature of ATP, ADP and PO4. Much further work will be needed to elucidate the source of the NIR spectra and determine the conditions that affect the spectra. Work on the development of instrumentation for blood substitute continues but a number of technical problems has slowed down the completion of these instruments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01414-18 LBC
(formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Biocalorimeters for Solution and Cell Biochemical Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. R.L. Berger, Chief, Biophysical Instrumentation Section, LBC
Other: C.P. Mudd, Biomedical Engineer, BEIB:DRS
N. Gershfeld, Lab Physical Biology, NIAMS
W. Friauf, Chief, Section on E.E., BEIB:DRS

COOPERATING UNITS *(if any)*

Pennsylvania State University
Commonwealth Technology, Inc. Alexandria, VA. & U. of Milan, Italy
BEIB, NIAMS

LAB/BRANCH

Laboratory of Biophysical Chemistry (Formerly LTD)

SECTION

INSTITUTE AND LOCATION

NHLBI:NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of the reaction of NAD-NADASE have been extended on the all tantalum stopped-flow microcalorimeter to 10°C, 25°C, and 37°C confirming the endothermic nature of the reaction by demonstrating the 5-fold increase in activity from 25°C to 10°C with the almost complete loss in activity at 37°C.

A new heat capacity measuring microcalorimeter has been developed which is capable of measuring charges of 1 part in one hundred thousand of total heat capacity of phospholipids. A reaction heat of 60 calories/mole was found as they went from multilamellar vesicles to unilamellar vesicles. The total change in heat capacity was 2 to 3% depending on the system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01462-04 LBC
(formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cross-Axis Synchronous Flow-Through Coil Planet Centrifuge

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Yoichiro Ito Senior Investigator LBC:NHLBI

Others: Eiichi Kitazume Visiting Fellow LBC:NHLBI
Molina Bhatnagar Research Aid LBC:NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biophysical Chemistry (Formerely LTD)

SECTION

INSTITUTE AND LOCATION

NIH:NHLBI Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The fourth prototype of the X-axis CPC (type XLL) was constructed. The apparatus holds a pair of column holders at L/R = 2 where R (7.6cm) is the distance between the holder axis and the central axis of the centrifuge and L (15cm), the lateral disposition of the column holder along the holder axis, this is compared with L/R = 1 in the third prototype (type XL) reported earlier.

Using short coils of 2.6mm ID PTFE (polytetrafluoroethylene) tubing with 7.6cm and 24cm helical diameters, retention of the stationary phase was measured in 10 pairs of two-phase solvent systems under various experimental conditions by combining three factors, i.e., the planetary motion (P(I) and P(II)), the head-tail elution mode (H:head to tail;T:tail to head), and the inward-outward elution mode (I:inward;O:outward). The results indicated that a satisfactory retention (over 50%) of the stationary phase is obtained in both diameter coils by choosing the optimum combinations of those three factors. Among the 8 possible combinations, P(I)-T-I and P(II)-H-I produced the highest retention of the lower phase and P(II)-T-O and P(I)-H-O produced the highest retention of the upper phase.

Using a simple statistical analysis, the effects of each factor on the stationary phase retention were isolated. The unique hydrodynamic mechanism involved in the present X-axis CPC system was discussed with the aid of the force distribution diagrams obtained from a mathematical analysis of acceleration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01463-04 LBC (formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Analytical Countercurrent Chromatographs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Yoichiro Ito Senior Investigator LBC:NHLBI
Hisao Oka Senior Researcher Aichi Prefectural Institute of Public Health
Nagoya, Japan

COOPERATING UNITS *(if any)*

Aichi Prefectural Institute of Public Health, Nagoya, Japan

LAB/BRANCH Laboratory of Biophysical Chemistry (Formerly LTD)

SECTION

INSTITUTE AND LOCATION

NIH:NHLBI

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Performance of two countercurrent chromatographic models, high-speed countercurrent chromatograph (HSCCC-4000) and analytical toroidal coil centrifuge (TCC), is evaluated in terms of theoretical plate number, resolution factor and separation time to assess their analytical capability. A series of experiments was conducted to investigate the effects of internal diameter and length of the coiled column and flow rate of the mobile phase on the separation of indole auxins in two-phase solvent systems composed of n-hexane/ethyl acetate/methanol/water at different volume ratios. The three components of the indole auxins were completely resolved in 16 min with the HSCCC system equipped with a multilayer coil of a 0.55 mm ID PTFE tube with theoretical plates ranging from 829 to 1,290. Similar separation was achieved in 24 min with the TCC system equipped with a 0.3mm ID PTFE tube with theoretical plates ranging from 969 to 1,811. It is concluded that both systems have comparable analytical capability at the present stage of development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01468-02 LBC
(formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Improved High-Speed Countercurrent Chromatograph with Multiple Column Holders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Yoichiro Ito Senior Investigator LBC:NHLBI

Others: Hisao Oka Visiting Scientist
Eiichi Kitazume Visiting Scientist
Molina Bhatnagar Research Aid
Y.-W. Lee Senior Scientist Research Triangle Institute, NC.

COOPERATING UNITS *(if any)*

Research Triangle Institute, North Carolina

LAB/BRANCH Laboratory Biophysical Chemistry (Formerly LTD)

SECTION

INSTITUTE AND LOCATION

NIH:NHLBI

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

For performing semianalytical separations using the present high-speed countercurrent chromatograph, a set of three identical multilayer coil separation columns was newly fabricated. Each column was prepared from a 100m length of 1.07 mm ID PTFE (polytetrafluoroethylene) tubing by winding it directly onto the holder hub (7.6cm ID) forming multiple coiled layers. The three multilayer coils were connected in series to make up a total capacity of about 270 ml. The performance of the apparatus was evaluated in separations of several synthetic sample mixtures including DNP (dinitrophenyl) amino acids (standard testing sample for comparative studies), indole auxins, tetracycline derivatives, and rare earth elements. The method was applied to separations of natural products such as bacitracin components, favonoids from a crude ethanol extract of Hippophae rhamnoides, and triterpenoic acids from Boswellia carterii.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04601-03 CE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of Cellular Energy Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. S. Balaban	Chief	LCE, NHLBI
Others:	T. Ceckler	Guest Worker	IRTA
	T. Fralix	Guest Worker	IRTA
	V. Kuprianov	Guest Worker	USSR, Cardiology Research Center
	F. Heineman	Medical Staff Fellow	LCE, NHLBI
	D. Kim	Guest Worker	NRC Fellow

COOPERATING UNITS *(if any)*

Howard Hughes Medical Institute, USSR Cardiology Research Center, G.E. Corporate Research Center

LAB/BRANCH

Laboratory of Cardiac Energetics

SECTION

Energy Metabolism

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	4.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of these studies is to establish a better understanding of energy metabolism in intact tissues. Toward this goal, this laboratory concentrates on the use of non-invasive and techniques to evaluate the biochemical function of the heart. These techniques include optical and nuclear magnetic resonance spectroscopy to monitor various aspects of tissue function. In the past year we have concentrated on establishing the cytosolic feedback mechanism operating between oxidative phosphorylation and mechanical work in the intact heart. The following major findings were made: 1) Using optical spectroscopy, we have shown that the oxygen tension of the heart, under control of blood flow autoregulation, is below the maximum concentration for oxidative phosphorylation. This data demonstrates that the heart actively maintains the oxygen tension below the saturation point of this process, indicating that tissue oxygen tension could play an active role in the limitation of energy metabolism. 2) Using a Ca++ sensitive dye in the intact heart, we have demonstrated that the mean intracellular Ca++ (Ca++i) concentration increases in proportion to the increase in cardiac afterload work and oxygen consumption. In paired experiments, the mitochondrial NADH concentration increases in proportion to Ca++i. This is consistent with the notion that Ca++i could regulate the rate of oxidative phosphorylation by modulating the NADH redox state via mitochondrial dehydrogenases. 3) The effects of ketone infusions on the metabolism of the heart in vivo was established demonstrating that the mitochondrial NADH redox state is a viable control point in the regulation of oxidative phosphorylation in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04602-03 CE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Non-invasive Techniques for Monitoring Cellular Function and Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.S. Balaban, Chief, LCE, NHLBI

Others: T. Ceckler, Guest Worker, IRTA
K. Karino, Guest Worker, NEI, NHLBI
S.D. Wolff, Res. Scholar, HHMI, NHLBI
T.A. Fralix, Guest Worker, IRTA
F. Heineman, Sen. Staff Fellow, LCE, NHLBI
S. Simon, Professor, Duke University
S. Chesnick, Special Expert, LCE, NHLBI
P. Kador, Senior Scientist, NEI, LCE

COOPERATING UNITS *(if any)*

Howard Hughes Medical Institute, Duke University
National Eye Institute

LAB/BRANCH

Laboratory of Cardiac Energetics

SECTION

Non-invasive Technology

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	4.0	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These investigations are devoted to the development of noninvasive methods of accessing tissue structure and function. Two general techniques are being developed: nuclear magnetic resonance (NMR) and optical spectroscopy/imaging. Over the last year we have made the following developments in NMR technology: (1) We have further developed the theory and application of magnetization transfer contrast (MTC) to the study of biological tissues. This approach permits the imaging of the interaction rate between water and macromolecules in the body resulting in a unique form of high resolution image contrast and tissue characterization. The quantitative aspects of this approach, defining its frequency and power dependence, were established outlining the methods of obtaining quantitative rate constant maps in vivo. (2) 3-H NMR studies have revealed that the interaction of water with macromolecules, the dominant source of contrast in tissues, is due to spin diffusion between the water and the macromolecules. (3) The molecular basis of the dipolar interaction of water with macromolecules was evaluated which demonstrated a unique role for hydroxyl groups in lipid bilayers. (4) The first human images using MTC were collected by adding a second radiofrequency channel to a standard clinical scanner. These images were 3-dimensional images of the knee revealing excellent soft tissue contrast. (5) A quantitative relationship between 3-fluorosorbitol production from 3-fluorodeoxyglucose and aldose reductase activity was established in the isolated lens of the eye, suggesting that this approach is a viable method of monitoring aldose reductase activity in vivo. Using optical spectroscopy the following advancements were made: (1) The effects of inner filters on the optical behavior of calcium indicating dyes and NAD(P)H fluorescence in the intact heart were defined and methods developed to correct for these serious artifacts. (2) Methods were further developed for the monitoring of tissue oxygenation in vivo using optical spectroscopy in the 500 to 650 nm range.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 00401-24 LCB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thermodynamic and kinetic studies of cytochrome c oxidase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard W. Hendler — Section Head — LCB, NHLBI

Others: Pardha Saradhi — Visiting Fellow — LCB, NHLBI

COOPERATING UNITS *(if any)*

Walter Friauf, John Cole, BEIB, DRS; Arthur Schultz, Hal Fredrickson, CSL, DCRT; Ira Levin, Paul Harmon, LCP, NIDDK

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Membrane Enzymology

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous work from our laboratory has revealed a highly interactive and complicated redox potentiometric behavior involving all of the redox centers in mammalian cytochrome *aa3*. Although all of the redox centers are on 2 subunits, the enzyme has 13. Perhaps the additional peptides are responsible for this complexity. To test this idea, we examined a natural 2 subunit cytochrome *aa3* isolated from *Paracoccus denitrificans*. It was found that all of the complicated behavior was present with the structurally simpler enzyme. Therefore, the observed redox pattern must be related to the necessary energy transduction function of the enzyme. A new system for automated electrodic potentiometry was developed using the IBM PS2/80 microcomputer with all of the software written in the language of "C". Steps were taken to design and build a new kind of rapid scan spectrometer. This instrument will be able to take a continuous series of optical spectra every 10 μsec. These spectra will be examined by SVD to define the kinetic sequence of intermediates during cytochrome *aa3* turnover. A new collaborative project was initiated to combine potentiometry and absorption spectroscopy with Resonance Raman spectroscopy in order to learn more about events taking place at redox centers during oxidation and reduction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00418-10 LCB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Energetic and stoichiometric relationships involving respiration $\Delta\mu$ H+ and ATP

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard W. Hendler	Section Chief, Membrane Enzymology,	LCB, NHLBI
Others:	Pardha Saradhi	Visiting Fellow	LCB, NHLBI
	Baltazar Reynafarje	Expert	LCB, NHLBI
	Anna Zolkiewska	Visiting Fellow	LCB, NHLBI

COOPERATING UNITS *(if any)*

Richard I. Shrager, Mathematician, LAS, DCRT

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Membrane Enzymology

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of a new computer-based system for obtaining realtime measurements of $\Delta\Psi$, and ΔpH, $\Delta\mu$H+ and H+-fluxes has been completed. This system monitors [TPP+], makes corrections for TPP+-binding, computes $\Delta\Psi$, measures fluorescence of internally trapped pyranine and converts this to internal pH, measures external pH, reports ΔpH, measures [O2], and computes proton fluxes. This system was used to compare the energy transducing abilities of 13-subunit mammalian cytochrome *aa3* with that of the 2-subunit enzyme from *Paracoccus denitrificans*, using proteoliposomes. The bacterial enzyme was able to form a $\Delta\Psi$ of 217 mV compared to 184 mV for the mammalian enzyme. Both systems form a ΔpH of ~0.15 units, or 9 mV. The small ΔpH has a greater role, however, in respiratory control than the $\Delta\Psi$. The H+/O pumping stoichiometry is quite comparable for the 2 systems. Therefore, only 2 peptide subunits are necessary for energy transduction by cytochrome *aa3*. It was determined that because of the very low internal volume of cholate-dialysis-prepared liposomes and the high buffer capacity of the liposomal membrane, this system cannot be used for proton flux measurements between the outer and inner aqueous compartments. Preliminary experiments to measure ATP synthesis using liposomes also uncovered insurmountable problems that prevent the use of ATPase liposomes to study ΔpH and $\Delta\Psi$-driven ATP synthesis. For these reasons, attention is being shifted from liposomes to submitochondrial particles for a continuation of these studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00419-10 LCB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-function relationships in eukaryotic cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Blair Bowers, Research Biologist, LCB, NHLBI

Others: Thomas Olszewski, Biologist, LCB, NHLBI
Marcel Nwulia, Student Assistant, LCB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The small soil amoeba, Acanthamoeba castellanii, is being used as a model system to study the flow of membrane through successive cellular compartments during endocytosis. We have developed greatly improved preparative procedures for ultrastructural studies of the membrane systems in this amoeba. Rapid freezing in -190° propane, followed by freeze substitution, adequately preserves for the first time a complex system of vesicles and tubules that function in membrane and content transport. Rapid freezing also stabilizes the forming fluid-uptake vesicles and shows that fluid uptake vesicles are non-coated and have the same mechanism of release into the cytoplasm as phagosomes. The neck of the endosome elongates and is constricted by microfilaments, releasing the endosome to move in the cytoplasmic flow. In order to follow the path of membrane that enters the cell during endocytosis, we have used a gold-labeled monoclonal antibody that is specific for membrane proteins. Within 1-2 minutes after the cell surface was labeled with the specific gold probe, the label was found in small vesicles and tubules. Within 10 minutes the label appeared in large (diameter >1 μm) vacuoles. The large vacuoles contain acid hydrolases and are a degradative compartment. The gold label was also present at the earliest time intervals in intermediate size vesicles that contained membrane remnants, suggesting that a considerable fraction of membrane is degraded immediately after internalization. Thus the small vesicle compartment may be a sorting compartment analogous to the early endosomes in mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00501-17 LCB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Actin Polymerization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Edward D. Korn, Chief, NHLBI, LCB

Others: Michael Bubb, Medical Staff Fellow, LCB, NHLBI; Arun K. Attri, Visiting Associate, LCB, NHLBI

COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Branch, DRS
Laboratory of Genetics, State University of Ghent, Belgium

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis by analytical ultracentrifugation indicates that actin oligomers (approximately tetramers) form from monomeric ATP-actin below its critical concentration. Slightly above its critical concentration in the absence of free Mg-ATP, about 50% of the actin may be converted to small oligomers, approximately dimers, which still contain actin-bound ATP. At steady state in the presence of F-actin, most of the unpolymerized actin appears to be small oligomers, approximately dimers to tetramers, as determined in the analytical ultracentrifuge after clearance of F-actin. These results are consistent with Oosawa's theory for the polymerization of helical polymers.

Actobindin, an 88-amino acid protein from *Acanthamoeba* that is a potent inhibitor of actin polymerization, has been shown to form a ternary complex with 2 actin monomers. The first actin binds with a K_D of about 2-5 μM and the second actin binds either with the same K_D but with negative cooperativity or with a significantly lower K_D.

GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00506-15 LCB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Acanthamoeba myosins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Edward D. Korn, Chief, LCB, NHLBI

Others: Hanna Brzeska, Visiting Associate, LCB, NHLBI; Thomas J. Lynch, Staff Fellow, LCB, NHLBI; Ray Scharff, Chemist, LCB, NHLBI; Chhanda Ganguly, Staff Fellow, LCB, NHLBI; Venugopal Sathyamoorthy, Staff Fellow, LCB, NHLBI; Ivan Baines, IRTA Fellow, LCB, NHLBI; Dorota Kulesza-Lipka, Visiting Fellow, LCB, NHLBI

COOPERATING UNITS *(if any)*

Neurosciences Branch, NIMH

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.7	5.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The essential elements for substrate specificity for myosin I heavy chain kinase has been studied with the use of synthetic peptides as substrates. One basic amino acid has been found essential, and two preferable, on the N-terminal side of the phosphorylation site and a tyrosine reside is required 2 positions away on the C-terminal side. The activity of the kinase is about 50-fold enhanced by autophosphorylation of up to 8 sites and this autophosphorylation is about 20-fold stimulated by the presence of acidic phospholipids.

Previous fluorescence immunolocalization studies of myosin I have been extended by immunoelectron microscopy. Myosin I has been found to be located at the plasma membrane, as previously inferred, and also at the membrane of the contractile vacuole, a new observation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00514-07 LCB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The structure and function of nonmuscle myosins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John A. Hammer III, Research Biologist — LCB, NHLBI

Others: Goeh Jung, Visiting Associate — LCB, NHLBI
Raul Urrutia, Visiting Fellow — LCB, NHLBI
Gu Jun, Visiting Fellow — LCB, NHLBI
Edward D. Korn, Chief — LCB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in the structure, regulation and in vivo function of myosins in nonmuscle cells. Our approach is to use anatomical, biochemical, cell biological and genetic methods to study the two distinct forms of nonmuscle myosin, myosin I and myosin II. We have cloned several different genes encoding the heavy chain subunits of both myosin II (a nonmuscle myosin possessing conventional structure) and myosin I (a low molecular weight, monomeric, nonfilamentous nonmuscle myosin). Current efforts are directed at: (1) determining the in vivo function of myosin I by examining the phenotype of myosin I-deficient cells generated by genetic means, (2) structure/function analysis of the unconventional C-terminal domain of myosin I (both protozoan myosin I and a vertebrate form of myosin I, the intestinal brush border 110 kDa protein), (3) full characterization of a third type of nonmuscle myosin distinct from myosin I and myosin II, and (4) testing models of myosin II filament formation and enzymatic regulation using site-directed mutagenesis and expression in E. coli. These basic studies shed light on the molecular basis of actomyosin-linked cellular motility, which in turn may increase our understanding of many cellular processes crucial to clinical medicine, such as white blood cell chemotaxis, cancer cell migration, angiogenesis, and wound healing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00516-04 LCB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

70-kDa Heat shock proteins and the homologous uncoating ATPase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Evan Eisenberg, Section Head, NHLBI, LCB
Lois E. Greene, Research Chemist, NHLBI, LCB

Others: Winifred Barouch, IRTA Fellow; Yumiko Emoto, Visiting Fellow; Bao-chong Gao, Visiting Associate; Myrna Mandel, Staff Fellow (NHLBI, LCB)

COOPERATING UNITS *(if any)*

Elizabeth A. Craig, University of Wisconsin, Dept. of Physiological Chemistry

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Physiology

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall focus of our laboratory is the study of the 70-kDa heat shock proteins and their role in both normal cellular processes and heat shock. First, we are investigating one of the only defined functions of a 70-kDa heat shock protein--the ability of the 70-kDa uncoating (UC) ATPase isolated from bovine brain to remove clathrin from clathrin coated vesicles in an ATP dependent reaction. Our results show that when the UC ATPase with bound ATP is mixed with coated vesicles, there is an initial burst of uncoating followed by slow steady-state uncoating. Based on these data we have proposed a model where the UC ATPase rapidly removes a stoichiometric amount of clathrin, while ATP is hydrolyzed at the active site. Slow release of ADP and Pi from the resulting enzyme•clathrin•ADP•Pi complex then limits the rate at which further uncoating can occur. This model predicts that, during the steady-state uncoating reaction, a ternary complex composed of the UC ATPase, clathrin, and nucleotide will be present. In support of this model, using FPLC, we have been able to isolate a long lived enzyme•clathrin•ADP•Pi complex following the initial burst of uncoating. We have also been able to show that a similar complex forms when free clathrin is mixed with the UC ATPase. The amount of complex which forms depends on whether equilibrium or steady-state conditions prevail. In addition to these binding studies, we have cloned the bovine brain UC ATPase and plan to express it in a yeast strain which lacks the endogenous genes which produce proteins which we have shown have similar activity to the bovine brain UC ATPase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01404-22 LCB
(Formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membrane Lungs for Long Term Respiratory, Cardiac and Cardiopulmonary Assist

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kolobow, Theodor (Medical Officer)	LCB, NHLBI
	Vitale, Giovanni (Visiting Fellow)	LCB, NHLBI
	Mandava, Srinivas (IRTA Fellow)	LCB, NHLBI
	Muller, Eckhardt (Special Volunteer)	LCB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Pulmonary and Cardiac Assist Devices

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have improved on our system to provide demand cardiopulmonary assistance through peripheral cannulation alone, using an extracorporeal membrane lung perfusion system. The key to its success lies in the decompression of the left ventricle using a specially designed helical coil to render the tricuspid and pulmonary artery valves partially/totally incompetent; the gentle ventilation of the lungs with 5% of CO2 in room air; the use of pulsatile blood flow; and the use of special blood catheters to allow high flow extracorporeal bypass through peripheral cannulation alone. We have greatly expanded our own understanding of the nature of acute lung failure secondary to the use of high tidal volumes, at high PIP. Depending on the respiratory rate, this evolving acute respiratory failure can rapidly transform a mild acute respiratory failure, into a sever ARDS, with multiorgan system failure that includes primary lung failure, and failure of the CNS, hepatic renal and the cardiocirculatory systems. The cardiocirculatory system failure does not lend to recovery by means now practiced in the clinical intensive care unit, or in the surgical research laboratory. To reduce our reliance on mechanical ventilators, we have developed a system to provide ventilation to an important anatomical structure that does not in and of itself directly participate in gas exchange, the trachea. We pass at a high flow humidified warm gas through a 1 mm catheter directly to the level of the carina. The trachea becomes thus well ventilated, and the effectiveness of spontaneous ventilation or the effectiveness of mechanical ventilation is thereby greatly enhanced. We have shown that this technique (ITV) can be successfully employed to sustain spontaneous ventilation (no mechanical ventilation) with as little as 6-12% of remaining normal lungs. Similarly, this technique can greatly improve on mechanical ventilation by allowing better alveolar ventilation at lower tidal volumes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01407-27 LCB
(Formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Fluorescence Spectroscopic Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Raymond F. Chen, Sr. Investigator — LCB, NHLBI
Jay R. Knutson, Sr. Investigator — LCB, NHLBI
Chen-Lu Tsou, Fogarty Scholar
Herman Ziffer, Sr. Investigator — LCP, NIADDK

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Laser excited ultraviolet fluorescence of tryptophan peptides was studied as a model for protein fluorescence, which arises from tryptophan residues. From the heterogeneity of the fluorescence parameters, it could be deduced that these peptides assumed different conformations in solution. Some conformations were non-fluorescent due to static self-quenching, a phenomenon which was demonstrated for the first time. Such quenching probably occurs in proteins, explaining certain puzzling features of their fluorescence.

The detailed physical processes occurring after photoexcitation of indole derivatives such as tryptophan was studied by fluorescence and n.m.r. analysis of products formed by irradiation with ultraviolet irradiation in heavy water (D20). Proton exchange occurred with some compounds but not others. Proton transfer thus is not necessary for quenching of indolyl fluorescence, but may occur secondarily after electron delocalization.

Energy transfer between protein tryptophan residues and attached dyes was studied using the technique of global analysis of fluorescence decay. Combined with spectral data, this method was able to determine the distance between energy donor and acceptor without prior knowledge of the degree of binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01452-07 LCB
(Formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Time Resolved Fluorescence Spectroscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jay R. Knutson, Research Biophysicist LCB, NHLBI

Others: Raymond F. Chen, Senior Investigator, LCB, NHLBI; Denise K. Porter, Biological Laboratory Technician, LCB, NHLBI

COOPERATING UNITS *(if any)* P. Hensley (Smith, Kline & Beecham), M. Han, F. Cyran, M. Fisher (H:LB), M. Bubb, A. Attri, E. Korn (H:LCB), D. Sackett, J. Wolff (K:CE), M. Clague R. Blumenthal (C:DCBD), Y. Raviv, A. Russo (C:DCT:RO), L. Davenport (Brooklyn College), S. Green (Georgetown/JHU), L. Brand (JHU), F. Gleason (Univ. Minn)

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.1	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have constructed (and continue to develop) a laser-based facility for time-resolved fluorescence spectroscopy of biomolecules. This facility provides rapid collection and analysis of luminescence data related to macromolecular size, flexibility, folding and structural fluctuations. This gives us insight into the structure and movements of proteins and membranes. The method is also sensitive enough to study nanogram quantities (100 fold more sensitive than nmr).

Our main "time-correlated" fluorometer was utilized to study the folding and dynamics of many different proteins: interleukin-1-β, a potent immunomodulator that is drawing interest in AIDS and cancer therapies; v1 & v2, cloned fragments of the T-cell receptor "targeted" by HIV particles; TF3A, a DNA-binding protein whose zinc "fingers" control transcription; 'HApep', a protein fragment that the influenza virus uses to anchor to and fuse with our cell membranes; thioredoxin, an important cofactor for genetic processing; actin, actobindin and tubulin, structural elements in the "self-assembling scaffolding" of cells called cytoskeleton; arginase, a metabolic enzyme complex with manganese-controlled "switching", and several other proteins and membranes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01470-02 LCB (Formerly LTD)

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Surrogate host for testing genetically altered cell grafts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.L. Bowman	Chief, LTD	LTD, NHLBI
Others:	F. Anderson	Chief, LMH	LMH, NHLBI
	M. Flugelman	Special Volunteer	Cardiology Branch, NHLBI
	D. Dichek	Medical Staff Fellow	LMH, NHLBI
	Nga Nguyen	Biologist	LMH, NHLBI
	M. Gallelli	Summer Employee	LMH, NHLBI

COOPERATING UNITS *(if any)*

none

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to test whether gene-engineered endothelial cells would adhere and thrive and express a recombinant marker when introduced into a segment of arterial graft, it is necessary to provide a mock circulatory system or surgically implant the graft into a living host animal.

The present work provides a system whereby tissue culture fluid is presented to the graft segment containing the altered cells at pulse pressures and flow values mimicking host that would be encounted by a graft implanted in a living animal.

The mock circulation is designed to maintain a biochemical milieu consistent with optimal tissue growth and survival while challenging the adhesive and secretory activity through the mechanical forces tending to dislodge the grafted cells.

Changes in the procedure such as the material of the graft and the provision of ports for insertion of a balloon catheter to insert a stent previously seeded with cells genetically modified to elaborate tissue plasminogen activator have been accomplished. Preliminary results demonstrated satisfactory survival and function of cells on the stents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00622-13 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cyclic Nucleotide Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mech.	CM, NHLBI
Others:	Su-Chen Tsai, Ph.D.	Res. Chemist	CM, NHLBI
	S. Russ Price, Ph.D.	Staff Fellow	CM, NHLBI
	Chii-Ming Lee, M.D.	Visiting Fellow	CM, NHLBI
	Lee McDonald, Ph.D.	Guest Researcher	CM, NHLBI
	Sally Stanley	Chemist	CM, NHLBI
	Catherine Welsh, M.D.	Staff Fellow	CM, NHLBI

COOPERATING UNITS *(if any)*

Randall K. Holmes, Department of Microbiology, USUHS.

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	3.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Guanine nucleotide-binding proteins are critical elements in many signal transducing and metabolic pathways in animal cells. One of these proteins, ADP-ribosylation factor, was identified based on its ability to enhance, in the presence of GTP, the ADP-ribosyltransferase activity of cholera toxin, a reaction believed to be important in the pathogenesis of cholera. These studies were consistent with the conclusion that ARF serves as an allosteric activator of the toxin. To study in more detail the effects of ADP-ribosylation factor, a recombinant protein was synthesized in E. coli. The recombinant ARF exhibited high affinity guanine nucleotide-binding and stimulated the ADP-ribosyltransferase activities of cholera toxin and E. coli heat-labile enterotoxins LT-I, LT-IIa and LT-IIb. The E. coli heat-labile enterotoxins were previously shown to catalyze enzymatic reactions similar to those of cholera toxin and to have similar effects on target tissues. Cholera toxin and the E. coli toxins exhibit considerable conservation in structure, particularly in the catalytic subunit. These studies are consistent with the conclusion that both the catalytic activity and the allosteric sites are conserved in the toxins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00627-12 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

GTP-Binding Proteins and Adenylyl Cyclase

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Su-Chen Tsai, Ph.D.	Research Chemist	CM, NHLBI
Others:	Mikako Tsuchiya, M.D., Ph.D.	Visiting Fellow	CM, NHLBI
	Randy Haun, Ph.D.	Staff Fellow	CM, NHLBI
	Ronald Adamik	Biologist	CM, NHLBI
	Patrick P. Chang	Chemist	CM, NHLBI
	Mary Walker, Ph.D.	PRAT Fellow	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mech.	CM, NHLBI
	Martha Vaughan, M.D.	Chief	CM, NHLBI

COOPERATING UNITS *(if any)*

H.-C. Chen, Endocrinology and Reproduction Research Branch, NICHD

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Metabolic Regulation

INSTITUTE AND LOCATION

NHBLI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	2.3	1.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Cholera toxin, the secretory product of Vibrio cholerae responsible in part for the devastating diarrheal syndrome characteristic of cholera, activates adenylyl cyclase by catalyzing the ADP-ribosylation of Gsα, the stimulatory guanine nucleotide-binding protein of the cyclase system. This toxin-catalyzed reaction is stimulated, in the presence of GTP, by ∿20 kDa guanine nucleotide-binding proteins, termed ADP-ribosylation factors or ARFs. Two forms of ARF, sARF I and sARF II, were isolated from bovine brain cytosol. Rabbit polyclonal antibodies against bovine sARF II reacted with soluble and membrane ARFs but did not react with other guanine nucleotide-binding proteins such as the 20 kDa protein ras and the heterotrimeric transducing G proteins (e.g., Gtα, Gsα, Giα, and Goα). The anti-ARF antibodies recognized ∿20 kDa ARF-like proteins in a variety of species and organ systems. The highest levels of immunoreactivity were observed in brain and other neural tissues. In these tissues an ARF doublet was observed, with the upper band (sARF II) being the predominant form. In other tissues, an immunoreactive band corresponding to the lower molecular weight species (sARF I) was present at higher concentration.

Levels of ARF, when quantified by immunoreactivity, correlated with those determined by a functional assay, stimulation of cholera toxin-catalyzed ADP-ribosylation. During rat brain development, when quantified by both immuno-reactivity and function, sARF II was lowest at birth, showed some increase at 10 days, and was maximal at 27-60 days; sARF I was unchanged. In spleen and heart, sARF I predominated over sARF II; in spleen, it increased with age while in heart, it decreased. Based on these studies, it appears that ARF proteins share epitopes and are expressed at different levels during development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00634-10 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of cGMP-stimulated Cyclic Nucleotide Phosphodiesterase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Takayuki Tanaka, M.D., Ph.D.	Visiting Fellow	CM, NHLBI
Others:	Steven Hockman	Biologist	CM, NHLBI
	Vincent C. Manganiello, M.D. Ph.D.	Head, Section on Biochemical Physiology	CM, NHLBI

COOPERATING UNITS *(if any)*

Dr. M. Moos, FDA, Bureau Biologics

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Biochemical Physiology

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
1.7	1.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A particulate cGMP-stimulated PDE was purified from bovine brain cerebral cortex. Photolabelling of cGMP-stimulated PDEs from bovine brain or calf liver with [32P]cGMP, followed by proteolytic digestion with V8 protease and peptide mapping (Cleveland et al., J. Biol. Chem. 252: 1102, 1977), indicated that 32P was predominantly associated with peptide(s) of ~12-14 kDa, suggesting that cGMP binding sites might be located in conserved domains of different cGMP-stimulated PDEs. Partial amino acid sequences were determined for the photolabelled fragment and other peptides. On Western immunoblots, antibodies against a synthetic peptide with a sequence matching part of that of the photolabelled fragment cross-reacted with intact PDE and the photolabelled fragment. Deduced amino acid sequence from a partial cDNA clone (isolated from a Lambda Zap II bovine brain library screened with an oligonucleotide probe based on the amino acid sequence of another peptide), as well as the partial sequence of the photolabelled fragment, exhibited considerable identity with the putative cGMP-binding domain of a cardiac cGMP-stimulated PDE (Charbonneau et al., Proc. Natl. Acad. Sci. 87: 288, 1990). These results suggest that cGMP-stimulated PDEs will exhibit considerable homology in at least the cGMP-binding region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00636-09 CM

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of cGMP-inhibited low Km cAMP Phosphodiesterase in Rat Adipocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Carolyn J. Smith, Ph.D.	Sr. Staff Fellow	CM, NHLBI
Others:	Valeria Vasta, Ph.D.	Special Volunteer	
	Vincent Manganiello, M.D., Ph.D.	Head, Sec. on Biochem. Physiology	CM, NHLBI

COOPERATING UNITS *(if any)*
Eva Degerman, Ana Rascon, and Per Belfrage, Department of Physiological Chemistry, University of Lund, Lund, Sweden.

LAB/BRANCH
Laboratory of Cellular Metabolism, NIH, NHLBI, Bethesda, MD

SECTION
Biochemical Physiology

INSTITUTE AND LOCATION
NHLBI, National Institutes of Health, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

In 32PO4-labeled rat adipocytes, isoproterenol (ISO), adenosine deaminase (ADA), or insulin rapidly increased phosphorylation of a particulate 135 kDa protein which was identified as native cGMP-inhibited "low Km" cAMP phosphodiesterase (cGI PDE) and was selectively immunoprecipitated from solubilized particulate fractions with anti-cGI PDE IgG. The time course and concentration dependencies for phosphorylation and activation of cGI PDE were similar for ADA, ISO and insulin; maximal agonist-dependent labeling was similar for all three agents. A particulate 135 kDa phosphoprotein was also isolated from 32PO4-labeled 3T3-L1 differentiated adipocytes incubated with ISO or insulin. In rat adipocytes incubated with ADA, phenylisopropyladenosine (PIA) (an ADA-resistant adenosine analog) prevented or reversed both phosphorylation and activation of cGI PDE, and inhibited the stimulatory effects of ADA on cAMP-dependent protein kinase (A-kinase) and lipolysis. Incubation of rat adipocytes with insulin in the presence of ADA or ISO transiently evoked ∿40-300% greater phosphorylation and activation of cGI PDE than the added effects of insulin and lipolytic agent alone; activation of cGI PDE preceded insulin-dependent decreases in A-kinase and lipolysis. These and other results suggest that ADA and β-agonist phosphorylate and activate cGI PDE via A-kinase; that the antilipolytic effect of insulin is associated with phosphorylation/activation of cGI PDE via an unidentified intracellular serine kinase, and does not require adenosine-mediated inhibition of adenylate cyclase; and that regulation by Gi (the guanine nucleotide binding protein which inhibits adenylate cyclase) is important for the cAMP-dependent activation of hormone-sensitive PDE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00638-08 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genes for GTP-binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James Murtagh, M.D.	Md. Staff Fellow	CM, NHLBI
Others:	Lucia Monaco, Ph.D.	Visiting Fellow	CM, NHLBI
	Su-Chen Tsai, Ph.D.	Res. Chemist	CM, NHLBI
	Kenneth Newman, M.D.	Md. Staff Fellow	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. on Mol. Mechanisms	CM, NHLBI
	Martha Vaughan, M.D.	Chief	CM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews

XX ☐ (b) Human tissues

☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

ADP-ribosylation factors (ARFs) are ∿20 kDa proteins that act as GTP-dependent allosteric activators of cholera toxin. Using inosine-containing degenerate oligonucleotide primers directed to conserved GTP-binding domains in ARFs, the polymerase chain reaction (PCR) was used to amplify simultaneously from human DNA portions of three ARF genes that include codons for 102 amino acids and intervening sequences. Amplification products, which differed in molecular weight because of differences in intron sizes, were separated by agarose gel electrophoresis. One amplified DNA contained no introns, and had a sequence different from but related to those of known ARFs. Based on this apparently unique sequence, selective oligonucleotide probes were prepared and used to isolate clone ΨARF 4, a putative ARF pseudogene, from a human genomic library in λ phage EMBL3. Reverse transcription-PCR, using primers in the 5'- and 3' untranslated regions, was then used to clone from human poly(A)+ RNA a cDNA containing the entire coding sequence corresponding to the expressed homolog of ΨARF 4, and referred to as human ARF 4. It appears that ΨARF 4 arose during human evolution by integration of processed ARF 4 mRNA into the genome. Human ARF 4 differs significantly from previously identified mammalian ARFs 1, 2, and 3. Hybridization of ARF 4-specific oligonucleotide probes with human, bovine, and rat poly(A)+ RNA revealed a single 1.8 kb mRNA, which was clearly distinguishable from the ARF 1 1.9 kb mRNA. The polymerase chain reaction provides a powerful tool for investigating diversity in this and other multigene families, especially with primers targeted at domains believed to have functional significance. Current investigations focus on the diversity of the ARF multigene family, the relationship between ARFs and other multigene families coding for guanine-nucleotide binding proteins, and evolutionary relations of ARF genes across species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00643-04 CM

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Structure-Function Relationships of Goα Mutants Expressed in E. coli

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joel Avigan, Ph.D.	Research Chemist	CM, NHLBI
Others:	James J. Murtagh, M.D.	Md. Staff Fellow	CM, NHLBI
	Linda Stevens	Chemist	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. on Mol. Mechanisms	CM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cellular Metabolism

SECTION
Molecular Mechanisms

INSTITUTE AND LOCATION
NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Go is a guanine nucleotide-binding protein that is involved in the regulation of signal transduction in animal cells. It is composed of α, β, and γ subunits, with the α subunits possessing a guanine nucleotide-binding site and being the target for ADP-ribosylation catalyzed by pertussis toxin; toxin-catalyzed ADP-ribosylation uncouples the Goα protein from receptor and inhibits signaling. The signaling process depends on interaction of Goα with other components of the system, including hormone receptors, βγ subunits, guanine nucleotides, and effector enzymes. To study the role of the various domains of Goα, oligo-nucleotide-directed in vitro mutagenesis was used; mutants of Goα were expressed in E. coli, and partially purified. The recombinant Goα mutants were analyzed with regard to immunoreactivity with anti-Goα polyclonal antibodies and their ability to serve as substrates in the ADP-ribosylation reaction. Previously it was shown that mutants in which cysteine-351 was replaced with glycine, or in which two carboxy-terminal amino acid residues were deleted, were not substrates in the toxin-catalyzed ADP-ribosylation reaction. Deletion of the terminal tyrosine did not block ADP-ribosylation; replacement of leucine-357 with glycine or alanine produced mutants which were poor substrates for toxin-catalyzed ADP-ribosylation. These studies indicate that pertussis toxin recognizes the carboxy terminus of the α-chain; small differences in structure alter the ability of the mutant α chains to serve as ADP-ribose acceptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00645-03 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tissue-Specific Expression of Guanine Nucleotide-binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Inez M. Serventi, Ph.D.	Sr. Staff Fellow	CM, NHLBI
Others:	Mikako Tsuchiya, M.D., Ph.D.	Visiting Fellow	CM, NHLBI
	James J. Murtagh, M.D.	Md. Staff Fellow	CM, NHLBI
	Kenneth Newman, M.D.	Md. Staff Fellow	CM, NHLBI
	Eleanor Cavanaugh	Chemist	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mech.	CM, NHLBI
	Martha Vaughan, M.D.	Chief	CM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Go is a heterotrimeric GTP binding protein found predominantly in neural and neuroendocrine tissues and to a lesser extent in heart, fat cells and possibly lung. It appears to transmit signals from a family of receptors to intracellular effectors. In vitro it couples with rhodopsin and α2-adrenergic, muscarinic, GABAB, opioid and f-MetLeuPhe receptors and appears to regulate ion channels. The α subunit of Go is a 39 kD polypeptide which binds and hydrolyzes GTP and is ADP-ribosylated by pertussis toxin. Several cDNA clones from a variety of species and tissues were isolated and sequenced. All encoded a protein of 354 amino acids exhibiting a high degree of amino acid homology (98%) among species (Goα1). Immunologic data, however, were consistent with the hypothesis that there exists another closely related form of Goα. Hybridization of mammalian brain and retinal poly (A)+ RNAs using a bovine Goα1 cDNA probe showed four major forms of Goα mRNA ranging in size from 6.2 to 2 kB. Previous studies showed that the 4, 3 and 2 kB forms of Goα mRNA arise via an alternative splicing mechanism resulting in Goα1 transcripts which apparently differed only in their 3' untranslated regions (UTRs). Recently, another group isolated and sequenced a new form of Goα cDNA (Goα2) from a hamster cDNA library which is completely identical in nucleotide and amino acid sequences to hamster Goα1 through amino acid 241; it differs in the terminal 3'-coding and -untranslated regions. Two 36-base oligonucleotide probes complementary to analogous regions of the 3' coding regions of Goα1 and Goα2 and species-specific 32-base oligonucleotide probes complementary to the 5' UTR of Goα1 were hybridized to bovine brain and human neuroblastoma poly (A)+ RNA. Results of these studies indicate that the 4, 3 and 2 kB forms are Goα1-specific mRNAs whereas the 6.2 kB form is the human and bovine equivalent of Goα2 mRNA. The mechanism by which this new form of Goα mRNA arises and how it relates to the Goα1 gene is the subject of our future efforts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00646-03 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Small GTP-binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mikako Tsuchiya, M.D., Ph.D.	Visiting Fellow	CM, NHLBI
Others:	Su-Chen Tsai, Ph.D.	Res. Chemist	CM, NHLBI
	David Bobak, M.D.	Staff Fellow	CM, NHLBI
	Randy Haun, Ph.D.	Staff Fellow	CM, NHLBI
	S. Russ Price, Ph.D.	Staff Fellow	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mech.	CM, NHLBI
	Martha Vaughan, M.D.	Chief	CM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒X(c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Some hormones, neurotransmitters and bacterial toxins affect cellular metabolism by altering regulatory pathways involved in the generation of second messengers. In some instances, binding of ligand to a receptor site on the exterior of the cell surface triggers an effect on an intracellular target. Coupling of receptor to effector is mediated through guanine nucleotide-binding (G) proteins. The activities of some of these G proteins are altered by certain bacterial toxins (e.g., pertussis toxin, cholera toxin) through a covalent modification known as ADP-ribosylation. Cholera toxin-catalyzed ADP-ribosylation results in activation of the G protein and persistent stimulation of the effector. The toxin-catalyzed reaction is in turn enhanced by 20 kDa guanine nucleotide-binding proteins, termed ADP-ribosylation factors or ARFs. In prior studies, cDNA clones for three different ARFs were isolated from human and bovine libraries; based on hybridization patterns of mammalian poly(A)+ RNA with a bovine ARF 2 probe, it was postulated that other ARF genes existed. Using differential hybridization with specific cDNA and oligonucleotide probes, cDNA clones that encoded new ARF forms were isolated from a cyclic AMP-differentiated HL-60 Lambda ZAP library. Thus far, six different putative ARF proteins have been described; they would appear to fall into three classes based on size (175, 180, or 181 amino acids), amino acid identity, and regions of amino acid sequence homology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 H1 00647-02 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Expression of Go and ADP-ribosylation factor in the baculovirus cloning system

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barbara Kunz, Ph.D.	Visiting Associate	CM, NHLBI
Others:	Su-Chen Tsai, Ph.D.	Res. Chemist	CM, NHLBI
	Patrick Chang	Chemist	CM, NHLBI
	Catherine Welsh, M.D.	Staff Fellow	CM, NHLBI
	Sally Stanley	Chemist	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mech.	CM, NHLBI
	Martha Vaughan, M.D.	Chief	CM, NHLBI

COOPERATING UNITS *(if any)*

Hao-Chia Chen, Section on Molecular Structure and Protein Chemistry, Endocrin. and Repro. Res. Branch, NICHD

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.6	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An ADP-ribosylation factor (ARF), a 20 kDa guanine nucleotide-binding protein that enhances cholera toxin ADP-ribosyltransferase activity, and the α subunit of Go (Goα), a guanine nucleotide-binding protein that is believed to regulate ion channels, have been expressed in an insect cell line (Spodoptera frugiperda, Sf-9) using modified baculovirus vector pVL941, containing inserts encoding Goα (pVL941-GO9) and ARF2 (pVL941-ARF2).

As described previously, lysates of pVL941-Go9-infected cells contained two proteins reactive with an anti-serum specific for Goα. One co-migrated on SDS-PAGE with Goα purified from bovine brain (∿39 kDa), was present in both soluble and particulate fractions of Sf-9 cells and was ADP-ribosylated with [32P]NAD and pertussis toxin (PT) in the absence of exogenous βγ subunits. As shown in the present report, this protein incorporated [3H]myristic acid. The second protein (∿43 kDa) was present only in the 50,000 x g supernatant; it was not ADP-ribosylated by pertussis toxin or [3H]myristoylated.

Sf-9 cells infected with a modified baculovirus, pVL941-ARF2, contained proteins of ∿20 and ∿21 kDa which reacted with anti-ARF polyclonal antibodies on an immunoblot. The 20 kDa protein incorporated [3H]myristic but not [3H]palmitic acid. Acid methanolysis and subsequent separation of the products on a reversed-phase HPLC-column confirmed that the expressed 20 kDa ARF was myristoylated. The sequence of the amino-terminal 15 amino acids of the unmyristoylated protein corresponded to the sequence derived from the cDNA used in construction of the pVL941-ARF2 vector. Myristoylated and unmyristoylated ARF were found in the 150,000 x g supernatant and membrane fractions of infected insect cells. Myristoylation of ARF was therefore neither necessary nor sufficient for targeting of this protein to the membrane. Myristoylated and unmyristoylated ARF enhanced cholera toxin-catalyzed ADP-ribosylation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00649-02 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Functional Domains in Guanine Nucleotide-Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Michael Bliziotes, M.D.	Sr. Staff Fellow	CM, NHLBI
Others:	Linda Stevens	Chemist	CM, NHLBI
	Patrick Chang	Chemist	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mechanisms	CM, NHLBI

COOPERATING UNITS *(if any)*

H.-C. Chen, Endocrinology and Reproduction Research Branch, NICHD

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.55	1.1	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Guanine nucleotide-binding (G) proteins are important in a number of membrane signal-transducing systems, including the hormone-sensitive adenylyl cyclase and the retinal light-sensitive cyclic GMP phosphodiesterase complex. In retinal rods, transducin (or Gt), a G protein, couples the photoreceptor rhodopsin to the cyclic GMP phosphodiesterase. Like all G proteins, transducin is a heterotrimer composed of α, β, and γ sununits. The α subunit binds guanine nucleotides and possesses intrinsic GTPase activity. The βγ subunits, which are isolated as a complex, facilitate the interactions of α with receptor. The amino-terminus of transducin α (Gtα) may be involved in its binding to the βγ subunits. To identify domains responsible for functional interactions between the α and βγ subunits, monoclonal antibodies against Gt were prepared. One monoclonal, MSN1, inhibited photolyzed rhodopsin-stimulated GTP hydrolysis catalyzed by Gtα in the presence of Gtβγ, as well as βγ-stimulated pertussis toxin-catalyzed ADP-ribosylation of Gtα. Proteolytic fragments of Gtα generated with V8 protease and trypsin, and lacking the amino-terminus, did not react with MSN1. MSN1 failed to cross-react with Gsα, Giα, or Goα. Both βγ, which is believed to interact with the amino-terminus of α, and light-activated rhodopsin, which is thought to interact with the carboxyl-terminus, inhibited the reaction of α with MSN1 on immunoblots; dark-adapted rhodopsin had no effect. Synergistic inhibition of MSN1 binding was observed in the presence of both βγ and photolyzed rhodopsin, probably due to βγ-enhanced binding of α to receptor. These results are compatible with the hypothesis that binding of light-activated rhodopsin at the carboxyl-terminus of α can influence conformation of the amino-terminus or that the amino- and carboxyl-termini are in close proximity in the native protein, permitting bound rhodopsin to interfere with MSN1 interaction with an epitope near the amino-terminus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00651-02 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

GTP-Binding Protein Substrates for Cl. botulinium C3 ADP-ribosyltransferase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim C. Williamson, Ph.D.	Staff Fellow	CM, NHLBI
Others:	Sally Stanley	Chemist	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. on Mol. Mechanisms	CM, NHLBI
	Martha Vaughan, M.D.	Chief	CM, NHLBI

COOPERATING UNITS *(if any)*

Leonard A. Smith, Ph.D., Head, Laboratory of Molecular Biology, Pathology, USAMRIID

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Although a large number of ∿20-26 kDa guanine nucleotide-binding proteins with sequence homology to the protooncogene ras have been identified by cDNA cloning, only some of the corresponding proteins have been purified and characterized. Recently it was found that members of this ras superfamily, rhoA, B, and C and rac1 and 2 are substrates for Clostridium botulinum C3 ADP-ribosyltransferase (C3). The functions of rho and rac have not been defined, although injection of C3 may lead to effects similar to those observed with activated ras. Two C3 substrates were purified from bovine brain cytosol and identified by microsequencing. The partial amino acid sequences were identical to the deduced amino acid sequences of rhoA and rhoB, respectively and hence they are referred to as rhoA* and rhoB*. rhoB* is the first identified soluble form of rhoB; it exhibits properties similar to those reported for membrane-associated rhoB. rhoA* has characteristics clearly different from other rhoA-like proteins. rhoA* migrates as a 77-80 kDa protein on gel filtration; however on SDS-PAGE, the ADP-ribosylated protein has a mobility consistent with a protein of 21.5 kDa. C3-catalyzed ADP-ribosylation of rhoA* was dependent on guanine nucleotides even in 1 mM Mg^{2+}. Half-maximal stimulation by GTP, GTPγS, Gpp(NH)p, and GDP was observed at 16, 20, 220, and 380 nM, respectively. GDPβS, GMP and adenine nucleotides were ineffective. Additionally, in the presence of guanine nucleotide, the rate and extent of ADP-ribosylation were enhanced by dimryistylphophatidylcholine (DMPC) and cholate. These findings are consistent with the possibility the rhoA* is isolated as part of a complex in which it exhibits sensitivity to guanine nucleotides. The complex could be self-associated rhoA* or rhoA* tightly associated with a cytosolic protein in a manner that makes ADP-ribosylation by C3 sensitive to guanine nucleotides and phospholipids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00652-01 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Characterization of cAMP Phosphodiesterase in Human Platelets and Myocardium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Elizabetta Meacci, Ph.D.	Visiting Fellow	CM, NHLBI
Others:	Carolyn J. Smith, Ph.D.	Senior Staff Fellow	CM, NHLBI
	Valeria Vasta, Ph.D.	Guest Worker	CM, NHLBI
	Steven Hockman	Biologist	CM, NHLBI
	Vincent C. Manganiello, M.D. Ph.D.	Head, Section on Biochemical Physiology	CM, NHLBI

COOPERATING UNITS *(if any)*

Dr. M. Moos, FDA, Bureau Biologics, FDA; Matthew Movsesian, Div. Cardiol., Univ. of Utah Med. Ctr., Salt Lake City, Utah; Eva Degerman and Per Belfrage, Dept. of Med. and Physiol. Chem., Univ. of Lund, Lund, Sweden

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Biochemical Physiology

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.9	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have purified cGMP-inhibited cAMP phosphodiesterases (cGI PDEs) from rat and bovine adipose tissues and more recently from human platelets by chromatography on DEAE-Sephacel and an affinity column constructed by coupling the isothiocyanate derivative of the specific inhibitor cilostamide to aminoethyl agarose (CIT-agarose). An analogous cGI PDE is found in sarcoplasmic reticulum from human hearts; specific inhibitors of this PDE are being studied in clinical trials for treatment of certain kinds of cardiac failure. Following limited proteolysis (trypsin or endoproteinase LysC digests) of the purified platelet cGI PDE and separation of peptides by HPLC, partial sequence of a number of peptides was obtained. Antibodies have been raised against the purified PDE as well as against a synthetic peptide corresponding to a known sequence. Purified IgG fractions cross-react with cGI PDEs from human platelets and cardiac sarcoplasmic reticulum, and rat adipocytes. Based on amino acid sequences from several peptides, oligonucleotide probes have been constructed and are being utilized for screening of human erythroleukemic, cardiac, and fat cell cDNA libraries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00653-01 CM

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of a Highly Conserved 23 kDa Basic Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Russ Price, Ph.D.	Senior Staff Fellow	CM, NHLBI
Others:	Maria Nightingale	Chemist	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mech.	CM, NHLBI
	Martha Vaughan, M.D.	Chief	CM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the isolation and characterization of cDNA clones for ADP-ribosylation factors, ~20 kDa GTP-binding proteins, a human brain cDNA containing a partial open reading frame encoding 48 amino acids, of which 10 were basic, was isolated. This peptide was unusual based on its predicted pI of 11.7. Oligonucleotide probes specific for this sequence hybridized with two abundant mRNAs in HL-60 cells, one of which appeared to comigrate with an ARF3 mRNA. Because of the unusual nature of this peptide as well as the size and abundance of the mRNAs, studies were performed to identify and investigate the relationship between these mRNAs. The cDNA fragment was used to isolate overlapping clones from dibutyryl cAMP-differentiated HL-60 and bovine retinal cDNA libraries. A putative open reading frame was identified that encodes a 23.6 kDa protein (p23, 203 amino acids) with a predicted pI of 11.6. The deduced amino acid sequence is highly conserved between human and bovine, exhibiting 97% identity; they are 99% identical if conservative substitutions are included. The human and bovine nucleotide sequences exhibit 91% identity across the coding region. Clones from both species contain a poly-adenylation signal (AATAAA) 4 nucleotides downstream (3') from the termination codon as well as a poly(A)+ tail. Other clones, however, extended beyond the point of poly(A)+ addition. In one bovine clone, the 3'-untranslated region extended 442 nucleotides beyond the polyadenylation signal to end in a poly(A)+ tail; a second polyadenylation signal was found 21 nucleotides before the poly (A)+ tail. Hybridization of poly(A)+ RNA from various bovine tissues and brains of several mammalian species with cDNA and oligonucleotides specific for the coding region identified two abundant mRNA species of 1.2- and 0.8-kb. Oligonucleotide probes specific for the extended 3'-untranslated region hybridized with the 1.2-kb, but not the 0.8-kb, mRNA. Both mRNAs were observed in all mammalian tissues and species. These studies indicate that a ubiquitous, basic 23.6 kDa protein is highly conserved among mammals and is encoded by two mRNA species that appear to differ in the site of polyadenylation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00937-08 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of mast cell degranulation: PI breakdown and calcium signal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Michihiro Hide — Vist. Associate — LCP NHLBI

Other: Hydar Ali — Vist. Associate — LCP NHLBI

COOPERATING UNITS *(if any)*

Dr. Joel Moss, LCM, NHLBI

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The antigen-induced breakdown of membrane phospholipids appear to be mediated by a Gz-like protein and is associated with an increase in concentration of cytosolic Ca2+ ([Ca2+]i) and exocytosis in RBL-2H3 cells. All these responses were dependent on influx of Ca2+ through a nonselective voltage-independent cation channel that could carry Na+, Ca2+, Mn2+ and Sr2+ but not Zn2+ and La3+. The influx of Ca2+ allowed an increase in [Ca2+]i and replenishment of intracellular Ca2+ stores. Influx through this channel was enhanced in cells treated with cholera toxin - which activates a Gs-like G-protein - and suppressed in cells depolarized with high [K+]o. Other studies indicated that adenosine receptors in RBL-2H3 cells are coupled to the inositol phospholipid-specific phospholipase C via a G-protein that is inhibited by both pertussis toxin and cholera toxin. In addition, ADP-ribosylation studies revealed the presence of cytosolic protein (kDa, 97) as a substrate for endogenous ADP-ribosylating activity. This activity is enhanced by adenosine receptor stimulation. As reported elsewhere (see report Z01 00990 04 LCP) the responses of antigen are selectively down regulated and those to adenosine up regulated in dexamethasone-treated cells. Mapping of mRNA for G-proteins in these cells revealed the expected array of G-proteins and a decrease of mRNA for Gzα in dexamethasone treated cells, to indicate that this is the most likely G-protein coupled to the IgE receptor and phospholipase C. The studies in total indicated the recruitment of several G-proteins in activate RBL-2H3 cells which in turn activated various phospholipases, ion channels and possibly some other mechanisms to provide the necessary signals for secretion by these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00962-08 LCP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological Studies on the Mechanism of Halothane Induced Hepatotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Jackie L. Martin, M.D., PRAT Fellow, LCP, NHLBI

Others: Andrea Perrone, B.S., Special Volunteer, LCP, NHLBI
Lynn E. Butler, Ph. D., IRTA Fellow, LCP, NHLBI
Neil R. Pumford, Ph.D., IRTA Fellow, LCP, NHLBI
David Thomassen, Ph.D., Special Volunteer, LCP, NHLBI
John W. George, Chemist, LCP, NHLBI
Lance R. Pohl Pharm.D., Ph.D., Chief, Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Dr. Brian Martin, NSB, NIMH; Dr. Michael A. Beaven, LCP, NHLBI; Dr. Heloisa Gonzaga, LCP, NHLBI

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacological Chemistry Branch

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 2.45	PROFESSIONAL: 0.95	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous research has demonstrated that antibodies in the sera of halothane hepatitis patients react with trifluoroacetylated rat liver microsomal proteins of 100 kDa, 80 kDa, 63 kDa, 59kDa, 58 kDa, 57kDa, and 54 kDa. These findings suggest that similar trifluoroacetylated proteins are the immunogens responsible for the formation of the patients' antibodies and the subsequent development of hepatitis. This year the 58 kDa protein has been purified from liver microsomes of halothane treated and untreated rats. The amino acid sequences of the N-terminal of the protein and of several tryptic peptides showed 100% homology with the encoded sequence of a cDNA isolated from a rat basophilic leukemia cell (RBL-1) that corresponded to phosphoinositide specific phospholipase-C (PI-PLC) isozyme I (ã). Moreover, an antibody raised in rabbits against the 58 kDa protein was used to show by immunoblotting that a 58 kDa was present in RBL-2H3 cells, further suggesting that the 58kDa protein is PI-PLC isozyme I (ã). These results indicate that PI-PLC is a potential target of reactive metabolites of drugs and as a consequence might either become immunogenic or have its biological activity altered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00967-08 LCP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Regulation of cytochrome P-450 turnover

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Neme, title, laboratory, and institute effiliation)*

P.I.: Yoichi Osawa, Ph.D., Staff Fellow, LCP, NHLBI
Others: Lance R. Pohl, Pharm.D., Ph.D., Chief, Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Dr. Robert Highet LCH, NHLBI; Dr. Brian Martin, NSB, NIMH; Angela Murphy, LC, NHLBI; Dr. Adriaan Bax, LCP, NIDDK; Amina Woods and Drs. Ling Chen, Robert J. Cotter, and Steward Finney, The Johns Hopkins Sch.Med.; Drs. John Yates, Patrick Griffin, and Donald Hunt, The University of Virginia

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacological Chemistry Branch

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously showed that radical metabolites of various xenobiotics and endogenous compounds can lead to the covalent bonding of the heme prosthetic group of hemeproteins to their apoproteins. In the case of myoglobin, the covalently altered protein has been shown to have increased reductive activity and rapidly reduced molecular oxygen to presumably the toxic metabolite superoxide anion radical. It was also shown by peptide mapping and mass spectrometry that the protein-bound adduct formed in the reaction of bromotrichloromethane with myoglobin consisted of a heme-CCl2 moiety bound to the proximal histidine residue. This year we have obtained 1D and 2D proton NMR and C13 NMR spectra of this product and have identified for the first time the site of covalent attachment of a protein to its heme prosthetic group in a heme-protein adduct. In this case, the proximal histidine residue was found to be covalently bonded to a vinyl substituent of the heme moiety. We have also investigated whether bromotrichloro- methane reacts similarly with other hemeproteins. Hemoglobin formed two heme adducts to its ▪-subunit. One of them was similar to that of myoglobin and contained a heme-CCl2 moiety whereas the other had a covalently bound heme-CCl3 moiety. Cytochrome P-450 and chloroperoxidase, but not horseradish peroxidase, formed covalently altered heme products when they were incubated with bromotrichloromethane. It is believed that the reaction of bromotrichloromethane with hemeproteins can be used to determine the amino acid residues at the active site of many hemeproteins by labeling these residues with activated heme moieties. Moreover, since the covalently altered hemeproteins may also have altered catalytic properties, it is possible that the metabolism-based activation of hemeproteins by radical metabolites of xenobiotics and endogenous compound may play a role in variety of tissue injuries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00973-06 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical mechanisms of mast cell degranulation: Potentiating pathways

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Heloisa M.S. Gonzaga	Visiting Fellow	LCP	NHLBI
Others:	Michael A. Beaven	Deputy Chief	LCP	NHLBI
	Wilford Saul	Chemist	LCP	NHLBI

COOPERATING UNITS *(if any)*

Drs. K.P. Huang and F. Huang, ERRB, NICHHD

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies indicated that the activation of phospholipase C and protein kinase C transduce necessary signals for exocytosis in antigen-stimulated RBL-2H3 cells. Recent work now indicates that activation of phospholipase D may provide an additional source of messenger molecules in these cells. Phospholipase D appeared to be activated via a rise in [Ca2+]i, protein kinase C and synergistically by both mechanisms. The activation of phospholipase D, in cells stimulated with either antigen or Ca2+ ionophore, resulted in sustained increases in phosphatidic acid and diglycerides. Blockade of conversion of phosphatidic acid to diglycerides with propranolol, suppressed exocytosis in A23187- and antigen-stimulated cells. Unlike phospholipase C, phospholipase D was not down regulated by the activation of protein kinase C with phorbol myristate in antigen-stimulated cells. As these cells still exhibit exocytosis, the activation of phospholipase D may provide necessary signals for secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00983-05 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*

Mechanism of MPTP-induced cell death

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Neme, title, laboretory, and institute affilietion)*

P.I.:	R. Krishnan Kutty	Sr. Staff Fellow	LCP	NHLBI
Other:	Gopal A. Krishna	Section Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Drug Tissue Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a well known inducer of Parkinson's disease in primates. We have shown earlier that MPTP and its metabolite, MPP+ (1-methyl-4-phenyl pyridinium ion) produced cell death in cultures of neuroblastoma x glioma hybrid cell line NG 108-15 and that this system could serve as a model for studies of the neurodegenerative disease. Since Aiuchi et al.(Neurochem.Int.12: 525, 1988) has shown that tetraphenylborate (TPB) increases the accumulation of MPP+ in mitochondria and thereby potentiates its inhibitory effect on the respiration, we have studied the effect of TPB on MPTP-induced death in NG 108-15 cells. The release of [14C]prelabeled adenine nucleotides were used as a measure of cell death. The concentration of MPTP required to cause a 50% release of adenine nucleotides from the cells during an incubation period of 24 hrs was reduced from 1500 μM to 15 μM, when TPB (100 μM) was added to the medium. The cell death was preceded by a depletion of cell ATP and a 50% depletion was observed when NG 108-15 cells were treated with 1500 μM of MPTP for 12 hrs. However, the concentration of MPTP required was markedly reduced to 0.3 μM in the presence of TPB (100 μM). These results indicate that the potentiation of MPTP-induced neuronal cell death by TPB is possibly caused by an increased loss of cell ATP. Thus, loss of cell ATP is a major factor in the cell death resulting from MPTP treatment. In support of this conclusion, high concentrations of glucose in the medium protects NG 108-15 cells against the MPTP-mediated depletion of cell ATP and the subsequent cell death. It is thus possible to postulate that a reduction in cell ATP may also be responsible for the cell death in dopaminergic neurons in Parkinson's disease. Moreover, the interaction between TPB and MPTP with devastating effects on neuronal cells may indicate that more than one environmental agent may be responsible for Parkinson's disease in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00985-05 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymatic reactions of purified cytochrome P-450 isozymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Henry A. Sasame	Chemist	LCP	NHLBI
Other:	James R. Gillette	Chief, Lab.	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. Michael Boyd, NCI

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A pneumotoxin, 4-ipomeanol, is currently being tested in humans as a therapeutic agent for the treatment of lung cancer. The rationale for its use is based on the fact that 4-ipomeanol undergoes activation by an isozyme of cytochrome P-450 to a reactive metabolite that causes destruction of pneumocytes. It appears now that an isozyme of cytochrome P-450 in rat lung responsible for the activation of 4-ipomeanol is present not only in lung but also in kidney of mouse. Interestingly the activity of the enzyme in mouse kidney microsomes is dependent on androgens. The very low level activity of the enzyme present in female mouse kidney was markedly increased by the treatment of mice with testosterone propionate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00987-04 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Active Sites of Cytochromes P-450

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.	Kenneth Korzekwa	Staff Fellow	LCP	NHLBI
Others:	Susan Smith	Vist. Fellow	LCP	NHLBI
	James R. Gillette	Chief, Laboratory	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. Frank Gonzales and Toshifumi Aoyama (LMC, NCI)

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cytochrome P-450s are a family of isozymes capable of oxidizing a wide variety of both endogenous and exogenous compounds. Two characteristics of these enzymes make it possible for a limited number of isozymes to metabolize a vast and varied array of exogenous chemical compounds. The first is the generally broad substrate and regio-specificity presumably due to relatively nonspecific substrate binding characteristics and multiple binding orientations. The second is a versatile active oxygenating species that is capable of oxidizing a variety of functional groups. These characteristics are being explored with the ultimate goal of predicting how changes in composition and structure of drugs will alter metabolic pathways. While most cytochrome P-450s have low substrate specificity, isozymes used for the metabolism of endogenous substance can be very specific. An example of a high specificity isozyme is aromatase, the enzyme responsible for the conversion of androgens to estrogens. This project describes our attempts at defining the binding influences responsible for certain drug metabolizing P-450 isozymes and the electronic and protein interactions responsible for the unusual mechanism of the third oxidation of aromatase. Methods used in the project include recombinant DNA techniques, determination of enzyme kinetics, and molecular modelling techniques. We have studied the metabolism of testosterone by clones, chimeras and single point mutants of P-450IIA1, IIA2 and P-450b using expressed P-450s provided by Dr. Frank Gonzales and his associates (LMC,NCI). These studies have revealed that 1) modification of a few amino acid residues in critical positions can markedly affect not only the turnover number and pattern of metabolites, but also the enzyme stability, 2) regions of the polypeptide involved in binding are different for different families of isozymes and 3) the tertiary structure of P-450cam may be a valid model for mammalian P-450s.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HL 00990-04 LCP

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical mechanisms of mast cell degranulation: Studies with disrupted cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: Dolores Collado-Escobar	Vist. Assoc.	LCP	NHLBI
Others: Michael A. Beaven	Deputy Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Cellular Pharmacology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prolonged exposure of rat basophilic leukemia (RBL-2H3) cells to dexamethasone resulted in global suppression of various stimulatory events in response to antigen and a global enhancement of the same stimulatory events to the adenosine analog, N-(ethylcarboxamide)adenosine (NECA). We had previously shown that antigen and NECA both activate phospholipase C but by different mechanisms; cells that had been treated with cholera or pertussis toxin, for example, responded to antigen but not to NECA with the release of inositol phosphates, increase in levels of cytosolic Ca^{2+}, and secretion. Because the toxins still inhibited the responses to NECA in dexamethasone-treated cells, the effects of dexamethasone may have been exerted at the level of receptor/G-protein coupling rather than at the level of effector systems. Additional evidence for this was the following: 1) NECA-induced hydrolysis of the inositol phospholipids was still enhanced after permeabilizing and washing the cells; 2) the response to the G-protein stimulant GTPγS was also enhanced in permeabilized, dexamethasone-treated cells and 3) kinetic studies suggested that the enhanced responsiveness to NECA was attributable in part to an increase in receptor number. The suppressive action of dexamethasone on antigen induced hydrolysis of inositol phospholipids, however, was readily lost by permeabilizing RBL-2H3 cells. The results indicate, therefore, that treatment with dexamethasone leads to changes in receptor-coupling mechanisms that are either resistant to (i.e., NECA-mediated responses) or reversed by (i.e. antigen-medicated responses) cell permeabilization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00991-04 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Signal generation and secretion of mediators in rat basophil leukemic cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Hydar Ali	Vist. Assoc.	LCP	NHLBI
Others:	Michael A. Beaven	Deputy Chief	LCP	NHLBI
	Wilford Saul	Chemist	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda,MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.0	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

3-Isobutyl-1-methylxanthine (IBMX) and certain related xanthines inhibited the generation of inositol phosphates, the increase in levels of free cytosolic Ca2+ ([Ca2+]i) and the secretion of 5-hydroxytryptamine in a rat tumor mast cell line (RBL-2H3 cells) in response to dinitrophenylated BSA (DNP-BSA). This inhibition did not correlate with the potency of these compounds as antagonists of adenosine receptors or as phosphodiesterase inhibitors. Instead, the inhibition appeared attributable to competitive antagonism of DNP-BSA binding to the DNP-specific IgE. Thus, the xanthines inhibited binding of [125I]DNP-BSA to cell bound DNP-specific IgE with the same rank order of potency as their inhibition of responses to antigen. The extent of inhibition by xanthines was inversely related to the proportion of receptors that were occupied by DNP-specific IgE. IBMX did not inhibit the responses to any other stimulants which included aggregated ovalbumin or concanavalin A in cells that were primed with ovalbumin-specific IgE or oligomers of IgE and antibody to IgE receptors in cells that were left unprimed. The effects of xanthine on DNP-BSA responses were markedly dependent on the chemical structure. IBMX was much more potent than theophylline. Because DNP-specific IgE is widely used in studies of antigen-induced stimulation of mast cells some reinterpretation of earlier studies in which xanthines have been utilized appears necessary.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00993-04 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phosphorylation of myosin heavy and light chains in stimulated basophils

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Oksoon Choi	Special Volunteer	LCP	NHLBI
Other:	Michael A. Beaven	Deputy Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. Robert S. Adelstein and Dr. Anna Koffer, LMC, NHLBI

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous studies indicated that IgE-mediated stimulation of rat basophilic leukemia (RBL-2H3) cells resulted in the secretion of histamine and the phosphorylation of the heavy (200 kDa) and light (20 kDa) chains of myosin. In the case of the 20-kDa myosin light chain, all of the phosphate was confined to a single site, which could be identified as the serine residue phosphorylated by MLCK in both turkey gizzard smooth muscle and human platelet myosin. Following RBL-2H3 cell stimulation there was de novo phosphorylation of a serine site that had previously been shown to be phosphorylated by protein kinase C in turkey gizzard and human platelet myosin. A similar scenario was observed for the myosin heavy chain. Unstimulated cells contained myosin heavy chains that yielded a number of phosphorylate tryptic peptides. However, following antigen stimulation, a single new tryptic phosphopeptide appeared. This peptide appeared to be one whose phosphorylation was catalyzed by protein kinase C. The stoichiometry of phosphorylation of these sites was substantial (> 0.5 moles of phosphate/myosin, light and heavy chain) and was closely correlated with the extent of exocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00994-03 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of anthracycline-induced cardiotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Giovanni Santostasi	Special Volunteer	LCP	NHLBI
Other: Gopal A. Krishna	Section Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Drug Tissue Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium channel blocking drugs have been reported to reduce survival rate of laboratory animals treated with the cardiotoxic antitumor anthracyclines. In order to elucidate the mechanisms of this clinically relevant interaction, cell toxicity of the anthracyclines, doxorubicin and daunorubicin, was evaluated in primary cultures of cardiac myocytes, isolated from neonatal rats. Low concentrations of extracellular calcium and addition of calcium entry blockers (nifedipine or flunarizine) potentiated myocardial toxicity of anthracyclines as assessed by the release of lactate dehydrogenase from the cells. Accumulation of anthracycline in the cardiomyocytes was increased by calcium entry blockers (nifedipine, flunarizine and verapamil) and by low extracellular calcium; efflux of [3H]-daunorubicin from myocardial cells was inhibited by nifedipine. These data are consistent with the presence in myocardial cells of a membrane transporter which accelerates the efflux of anthracyclines; whether this carrier is related to the glycoprotein GP170, which induces the outward transport of chemotherapeutic drugs from tumor cells, remains to be elucidated. Calcium channel blockers are known to combine with the GP170 and prevent the efflux of cytotoxic agents from multi-drug resistant tumor cells. In myocardial cells, however, doxorubicin retention was not affected by R(+)-verapamil, which lacks calcium channel blocking activity, and was reduced by the calcium channel agonist, Bay K-8644. Calcium channel blocking activity is thus required in order to increase the accumulation of anthracyclines in cardiomyocytes. These data indicate that anthracycline-induced cardiotoxicity may be potentiated during the course of calcium channel blocking therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00999-02 LCP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 chereters or less. Title must fit on one line between the borders.)*

Trifluoroacetylated 63 kDa Protein as Possible Immunogen in Halothane Hepatitis

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Neme, title, laboretory, and institute affiliation)*

P.I.: Lynn E. Butler, Ph.D., IRTA Fellow, LCP, NHLBI
Others: Jackie L. Martin, M.D., PRAT Fellow, LCP, NHLBI
David Thommasen, Ph.D., Special Volunteer, LCP, NHLBI
Lance R. Pohl, Pharm.D., Ph.D., Chief, Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Brian Martin, Ph.D.,NSB, NIMH

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacological Chemistry

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 1.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sera from halothane hepatitis patients have been shown to contain antibodies that react with several trifluoroacetylated proteins (100 kDa, 80 kDa, 63 kDa, 59 kDa, 57 kDa, and 54 kDa) purified from the livers of halothane treated rats. These findings suggest that similar trifluoroacetylated proteins are the immunogens responsible for the formation of the patients' antibodies and perhaps the subsequent development of hepatitis. This year a nearly full-length cDNA clone of the 63 kDa protein has been isolated from a Lambda gt11 rat liver cDNA library. Based upon the encoded sequence of this clone and the sequences of the N-terminal and several internal peptides of the purified protein, 63 kDa has been identified as calreticulin. Genomic DNA preparations from rat liver were probed with a fragment encompassing the cloned open reading frame. Strong homology was found to a single genomic region, with limited homology to a second region detected after prolonged autoradiography, indicating that the 63 kDa protein is not part of a multi-gene family. Immunoblotting of several tissues with the anti-63 kDa antibody indicated that the protein was present in all tissues of body, with highest concentrations being in the liver, testes, and adipose tissue. Calreticulin is a calcium binding protein of the endoplasmic reticulum in non-muscle cells and the sarcoplasmic reticulum in muscle cells. Calreticulin is thought to have a role in calcium homeostasis by acting as a storage reservoir of calcium. Since the present study has shown that calreticulin can be a target of the reactive metabolites of drugs, this raises the possibility that the calcium binding capacity of this protein can be altered by drugs and as a result play a role in the toxicity produced not only by halothane, but also by other drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01000-02 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Theoretical models for cytochrome P-450 Mediated Hydrogen Atom Abstraction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Kenneth Korzekwa	Staff Fellow	LCP	NHLBI
Others:	Susan Smith	Vist. Fellow	LCP	NHLBI
	James R. Gillette	Chief, Laboratory	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesa, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ubiquitous superfamily of enzymes, the cytochrome P-450s, continue to be the focus of many diverse research efforts. Interest in these heme containing monooxygenases stem from their ability to catalyze the oxidation of a wide variety of lipophilic endogenous (i.e. steroids, prostaglandins and fatty acids) and exogenous (drugs and environmental contaminants) compounds. Because of their importance in drug metabolism and toxicity, predictive models for cytochrome P-450 oxidations could be extremely useful. We have used molecular modeling techniques to develop a predictive model for cytochrome P-450 hydrogen abstraction reactions. Of several model oxygen radicals studied, the p-nitrosophenoxy radical has the most appropriate transition state symmetry for use as a model for P-450 mediated hydrogen atom abstractions. Using this model, a linear correlation was observed between H and a combination of H(R) and either the modified Swain-Lupton resonance parameter or the ionization potential of the radical formed. The latter relationship gave an estimated standard deviation of the predicted H of 0.8 kcal/mol. This suggests that it may be possible to obtain an estimate of the relative ability for any carbon-hydrogen bond to undergo P-450 mediated hydrogen atom abstraction by calculating the relative stability and ionization potential of the resulting radical. In addition reactants and products for 54 hydroxylation and desaturation reactions were modeled and used to predict the relative tendency for each reaction to occur.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03563-04 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endogenous calcium channel modulator

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: Ingeborg Hanbauer	Pharmacologist	LCP	NHLBI
Others: Mariagrazia Grilli	Special Volunteer	LCP	NHLBI
A. Gilbert Wright, Jr.	Chemist	LCP	NHLBI

COOPERATING UNITS *(if any)*

Angela Murphy, LC-NHLBI; Joe Davis, LB-NHLBI; Yoichiro Ito, LC-NHLBI
Lewis Pannell, LCB, NIDDKD

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A low molecular weight material has been isolated from rat brain and purified by counter current chromatography and Glyco-PAK N HPLC technique. The degree of purity of the active material is suitable for consecutive analysis by gas chromatography-mass spectrometry. The calcium channel modulating activity was established by the inhibition of nitrendipine binding and the blockade of calcium current through L- and T-type channel in whole cell patch clamp studies of primary cultures of neurons and in dissociated cardiac myocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03567-03 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of dopamine reuptake system

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Ingeborg Hanbauer	Pharmacologist	LCP	NHLBI
Mariagrazia Grilli	Special Volunteer	LCP	NHLBI
A. Gilbert Wright, Jr.	Chemist	LCP	NHLBI

COOPERATING UNITS *(if any)*

Italo Mochetti, Dept. Anatomy, Georgetown University, Washington,DC

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In primary cultures of mesencephalic neurons the expression of the dopamine transport and release system as well as specific binding sites of dopamine uptake blockers are developmentally linked to axonal outgrowth. In neurons cultured for five days the potassium-evoked dopamine release is mediated through activation of N-type calcium channel, while L- or T-type channels appear to be inactive. In contrast, veratridine-evoked dopamine release was not altered by L-, N- or T-type calcium channel blockers, but was inhibited by tetrodotoxin and zinc. Since veratridine elicits membrane depolarization by prolonging sodium channel opening it is suggested that the veratridine-evoked dopamine release is not mediated through voltage-dependent calcium channels, but that during depolarization calcium may enter the cells through the sodium transport system. In addition, in cultured mesencephalic neurons cholecystokinin-specific mRNA is expressed, supporting the hypothesis that cholecystokinin may play the role of a cotransmitter in dopaminergic neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL04401-01 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The metabolism of testosterone by expressed P-450IIA1 and P-450IIA2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Susan Smith	Vist. Fellow	LCP	NHLBI
Others:	Kenneth Korzekwa	Staff Fellow	LCP	NHLBI
	James R. Gillette	Chief, Lab	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. Frank Gonzales (LMC, NCI)

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A group of monooxygenase systems collectively known as cytochrome P-450 catalyzes the oxidation of many xenobiotics, procarcinogens and steroids. Because of importance of these enzymes in drug inactivation and toxicity, a long term goal of the Laboratory has been to develop methods for predicting the metabolic profile of a new drug or xenobiotic for the various cytochrome P-450's. The development of predictive methods would involve: a) an understanding of the factors that govern the binding orientations of the substrates and b) the susceptibility of various functional groups of the substrates to be oxidized.

Studies in the laboratory have developed a model to predict the relative tendency of a functional group to undergo oxidation. In order to test this model experimentally it was necessary to have a source of P-450 isozymes. In collaboration with the Laboratory of Frank Gonzales in NCI, we have employed preparations of P-450IIA1 expressed in Hepatoma G2 cells using Vaccinia virus as a vector. However, the maximum level or expression achieved with this system was about 0.2% of the total cellular protein. In addition, P-450IIA1 was also expressed in Spodoptera frugiperda cells infected with the recombinant baculovirus containing the cDNA for P-450IIA1. The activity of these preparations could be increased nearly six-fold by the addition of hemin, mesoporphyrin IX or deuteroporphyrin IX. P-450IIA2 has also been expressed in hepatoma G2 cells using Vaccinia virus as a vector. Although P-450IIA1 and P-450IIA2 are 90% identical in their amino acid sequences, their patterns of testosterone metabolites are markedly different. P-450IIA1 forms predominantly 7α-hydroxytestosterone, whereas P-450IIA2 forms predominantly 15α-hydroxytestosterone and a hither to unidentified metabolite. During the past year we have identified the unknown metabolite as 12α-hydroxytestosterone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL04402-01 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular biology of naphthalene hydroxylase in mice

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Henry A. Sasame	Chemist	LCP	NHLBI
Other:	James R. Gillette	Chief, Lab.	LCP	NHLBI

COOPERATING UNITS *(if any)*

Ida Owens and John Ritter, CH; M. Negishi, NIEHS

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Naphthalene, a commonly used industrial solvent, causes pneumotoxicity in mice but not in rats. The mechanism appears to depend on the enzymatic activation of naphthalene by cytochrome P-450 present in mouse lung. In the past we have isolated a cytochrome P-450 from mouse liver designated P-450m50b that metabolizes naphthalene almost solely to only on epoxide, namely (1R,2S)-naphthalene 1,2-oxide. In order to characterize this protein, polyclonal anti-P-450m50b antibodies were raised in rabbit and used to screen cDNA libraries. Ten positive clones were isolated. A cDNA from mouse lung cDNA library containing a complete protein reading frame was sequenced. This clone was expressed in both COS cells and yeast. The expressed enzyme displayed the naphthalene hydroxylase activities. The metabolic profiles of naphthalene conjugates and hydroxybiphenyl expressed in this clone transformed yeast microsomes confirmed unequivocally that this clone is naphthalene hydroxylase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04403-01 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Eseroline, a metabolite of physostigmine, induces neurotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: R. Krishnan Kutty
Other: Gopal A. Krishna

COOPERATING UNITS *(if any)*

Dr. Satu M. Somani, Prof. of Pharm., Southern Illinois University School of Medicine, Springfield, IL

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NHLBI-IR, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The toxic effects of physostigmine, an anticholinesterase drug, and its metabolite eseroline were investigated in three neuronal cell culture systems; mouse neuroblastoma N1E-115, rat glioma C6 and neuroblastoma-glioma hybrid NG 108-15. Leakage of lactate dehydrogenase and release of [^{14}C]adenine nucleotides were used as parameters of cell death. Physostigmine and eseroline (0.5 mM) elicited a time-dependent leakage of lactic acid dehydrogenase (LDH) from all three cell types. An increased release of [^{14}C]adenine nucleotides was also detected from cells when they were prelabelled with [^{14}C]adenine. Eseroline was comparatively more toxic than the parent compound, physostigmine. Eseroline elicited a dose- and time dependent leakage of LDH and release of adenine nucleotides from the neuronal cells. A nonneuronal cell line, rat liver ARL-15, was comparatively the most resistant cell type to eseroline toxicity. The concentrations of eseroline needed for 50% release of adenine nucleotides or 50% leakage of LDH from NG-108-15 and N1E-115 cells in 24 hr ranged from 40 to 75 μM. The concentrations of eseroline needed to obtain similar responses in C6 and ARL-15 cells were much higher and ranged from 80 - 120 μM. Phase contrast microscopy showed extensive damage to three neuronal cell lines at concentration of eseroline as low as 75 μM. The loss of ATP from N1E-115 cells exceeded 50% when they were treated with 300 μM eseroline for 1 hr - at which time the leakage of LDH was not detectable. It seems that eseroline causes neuronal cell death by a mechanism involving loss of cell ATP. The formation of eseroline may contribute to the toxic effect of physostigmine

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04404-01 LCP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Trifluoroacetylated 80 K Protein as a Possible Immunogen in Halothane Hepatitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Neil R. Pumford, Ph.D., IRTA Fellow, LCP, NHLBI
Others: Jackie L. Martin, M.D., PRAT Fellow, LCP, NHLBI
David Thomassen, Ph.D., Special Volunteer, LCP, NHLBI
Lance R. Pohl, Pharm.D., Ph.D., Chief, Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Brian Martin, Ph.D., NSB, NIMH

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacological Chemistry

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.15	PROFESSIONAL: 1.15	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sera from patients with halothane hepatitis contain antibodies directed against liver microsomal proteins (100 kDa, 80 kDa, 63 kDa, 59 kDa, 58 kDa, 57 kDa, and 54 kDa) covalently modified by the trifluoroacetyl halide metabolite of halothane. These altered proteins (neoantigens) are believed to cause an immune-mediated hepatotoxicity. In this project, the 80 kDa protein was purified from liver microsomes of halothane treated and untreated rats by ionic exchange chromatography. An antibody was raised against the protein in rabbits and was used to determine the subcellular localization and organ distribution of the protein. Quantitative immunoblotting studies revealed that the highest levels of the protein were found in the microsomal fraction of the liver, followed by approximately 50% lower amounts in the nuclear, mitochondrial, and plasma membrane fractions. Very low levels of the protein were detected in the cytosol fraction of the liver. The protein was detected in all tissues studied with the highest levels found in the liver, fat, and testes, while moderate levels were measured in all other tissues tested except for the heart and skeletal muscle which had low levels of the protein. When the amino acid sequences of several internal peptides produced by either tryptic or mild acid hydrolysis of the protein were compared to sequences of proteins in several data bases, it was discovered that the 80 kDa protein showed greater than 95% sequence homology with two cDNA clones. One of the clones encoded for ERp 72, an endoplasmic reticulum protein with unknown function. The other clone corresponded to deoxycytidine kinase, the rate-limiting enzyme in the activation of many important anticancer and retroviral drugs, including the anti-AIDS drug AZT.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04405-01 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Trifluoroacetylated 100 K Protein as a Possible Immunogen in Halothane Hepatitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: David Thomassen, Ph.D., Special Volunteer, LCP, NHLBI

Others: Lance R. Pohl, Pharm. D., Ph.D., Chief, Section, LCP, NHLBI

Jackie L. Martin, M.D., PRAT Fellow, LCP, NHLBI

COOPERATING UNITS *(if any)*

Brian M. Martin, Ph.D., NSB,NIMH

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacological Chemistry

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical use of the inhalation anaesthetic halothane is associated with a rare, but often fatal hepatitis, which is apparently immune-mediated. In recent years, the mechanism of the disease has been shown to involve an antibody response against a specific set of liver proteins that are covalently modified by the reactive trifluoroacetyl chloride metabolite of halothane. This project has focussed on the isolation and characterization of the 100 kDa protein, to which the majority of patients produce antibodies. The antigen was identified as endoplasmin, a highly conserved glycoprotein with a probable role in calcium homeostasis and intracellular protein transport. In cell culture, we established that the expression of endoplasmin can be induced by hyperphysiologic temperatures. The significance of this finding stems from the emerging role of so-called "heat-shock" stress proteins in autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus and in immunity against certain chemically induced tumors. These findings suggest that stress proteins may also have an underlying role in drug allergic reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04406-01 LCP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Trifluoroacetylation of Tissue Proteins by Chlorofluorocarbon Replacements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Jackie L. Martin, M.D., PRAT Fellow, LCP, NHLBI
Others: Lance R. Pohl Pharm.D., Ph.D., Chief, Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

James W. Harris, B.S., University of Rochester, Rochester, N.Y.
M.W. Anders, D.V.M., Ph.D., University of Rochester, Rochester, N.Y.

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacological Chemistry Branch

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As a result of ozone depletion by chlorofluorocarbons the elimination of these compounds has been proposed and replacements are being sought. The addition of hydrogen atoms to the chlorofluorocarbon molecules should allow substantial degradation of these compounds in the lower atmosphere, with little depletion of stratospheric ozone. HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane) is a compound currently under investigation as a chlorofluorocarbon replacement. Because of its structural similarity with halothane (2-bromo-2-chloro-1,1,1,-trifluoroethane), we investigated the metabolism of HCFC-123 in male Fischer 344 rats. Rats were placed in inhalation chambers. Group 1 received air for 2 hours, group 2, 1.3% halothane for 2 hours, while groups 3 and 4 received 1.1% and 0.7% HCFC-123, respectively, for 2 hours. All rats were killed 15 hours post-exposure and liver microsomal and cytosol fractions were prepared. Following sodium dodecyl sulfate polyacrylamide gel electrophoresis, constituent polypeptides from each fraction were transferred to nitrocellulose membranes and probed with hapten specific anti-trifluoroacetyl protein serum. Both compounds were found to produce identical patterns and similar levels of trifluoroacetylated proteins in both fractions, with the microsomal fraction showing the highest level of trifluoroacetylated proteins. This finding raises the possibility that susceptible individuals repeatedly exposed to HCFC-123 may become sensitized and develop hepatitis similar to that produced by halothane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04407-01 LCP

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Theoretical isotopes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: James R. Gillette	Chief, Lab.	LCP	NHLBI
Others: Kenneth Korzekwa	Staff Fellow	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. William Trager, University of Washington

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The term cytochrome P-450 represents a family of heme-containing enzymes that collectively catalyze the oxidation of virtually an infinite number of endogenous and exogenous compounds. Each of the enzymes has its own substrate specificity, but they possess the unusual characteristic of forming several different metabolites either by parallel reactions or in sequence. Although the formation of several different products implies the existence of different orientations of the substrate within the active site of the enzyme, the mechanisms by which these different orientations are achieved may be markedly diverse. Studying the isotope effects on the metabolism of different substrates is a highly useful approach for elucidating various features of the enzymatic cycles. To this end, we have derived enzyme kinetic equations for several plausible models by which a cytochrome P-450 enzyme may form several metabolites from a single enzyme. The equations illustrate the usefulness of estimating isotope effects not only on isotope-sensitive pathways, but also on isotope-insensitive pathways and on total metabolism in differentiating between different mechanisms of product formation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01266-08 KE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Control of epithelial cell volume

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Kenneth R. Spring	Res. Physiologist	LKEM, NHLBI
Others:	Bruno Flamion	Visiting Associate	LKEM, NHLBI
	Timothy J. Furlong	Visiting Associate	LKEM, NHLBI
	Haim Garty	Special Volunteer	LKEM, NHLBI
	Carter Gibson	Electrical Engineer	LKEM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Kidney and Electrolyte Metabolism

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, Bethesda, MD 20892

TOTAL MAN-YEARS: 4.3	PROFESSIONAL: 4.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Large quantities of salt and water move across epithelial cells. These cells are able to maintain a constant volume by balancing solute entry and exit. The mechanisms for epithelial cell volume regulation are under investigation in this laboratory. Optical and microelectrode studies have been performed on the gallbladder of Necturus, on the renal medullary collecting tubule of the rabbit, the toad urinary bladder, on cultured renal papillary epithelium.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01282-04 KE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Solute and Water Transport in Renal Epithelia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Mark A. Knepper Chief, Renal Mechanisms Section

Others:

M. Flessner	Medical Staff Fellow	P.A. Wright	Visiting Fellow
Y. Terada	Visiting Fellow	S.P Lankford	Special Volunteer
S.M. Wall	Senior Staff Fellow	R. Mejia	Mathematician
C.-L. Chou	Senior Staff Fellow	M.B. Burg	Chief
R. Packer	Guest Worker		

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Kidney and Electrolyte Metabolism

SECTION

Renal Mechanisms Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, NIH, Bethesda MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.2	8.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The kidney contains several distinct epithelia that, in their aggregate function, are responsible for formation of the urine. We are studying the roles of these epithelia in the regulation of the excretion of water, urea, ammonium, bicarbonate, sodium, potassium, and chloride. The general approach is to dissect the epithelia from the kidney and to study their functions in vitro. The data are analyzed and integrated using mathematical models of transport in the kidney. Experiments in the cortical collecting duct of rat showed a large fraction of active NaCl absorption occurs via a thiazide-sensitive electroneutral transport pathway which is regulated by atrial natriuretic factor (ANF). Studies are underway, using the polymerase chain reaction in single microdissected rat nephron segments, to localize along the nephron the specific mRNA that codes for the ANF receptor-guanylate cyclase protein. Isolated perfused tubule studies have demonstrated the presence of a vasopressin-regulated urea transporter in the apical and basolateral membranes of the rat inner medullary collecting duct. In preliminary studies, the transporter has been expressed in Xenopus oocytes by injecting them with mRNA from rabbit renal inner medulla (collaboration with M. Hediger, Boston). We have developed and validated a high time-resolution spectrofluorometric method to measure urea transport in perfused tubules. Studies are continuing to map the NH_3, NH_4^+, and bicarbonate permeabilities along the nephron with a long term goal of understanding the mechanism by which acid-base excretion is regulated by the kidney. Mapping of the activities of ammoniagenic enzymes along the nephron have been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01283-03 KE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of organic osmolytes in renal cells.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.	M. Burg	Chief		
Other:	T. Moriyama	VF	J. Ferraris	GW
	A. Garcia-Perez	SF	C. Williams	Biol. Lab. Tech.
	H. Murphy	Chemist	R. Robey	GW
	B. Cowley	GW	K. Zablocki	VF
	M. Kwon	GW		
	J. Handler	Scientist Emeritus		

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Kidney and Electrolyte Metabolism

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
9.0	9.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The osmolality of the blood in the renal inner medulla is high and varies with the urinary concentration. Both NaCl and urea are elevated. The medullary cells evidently survive and function in this adverse environment. The present studies are concerned with understanding the mechanisms involved. When cells are stressed by a high salt environment, they generally accumulate osmotically active organic solutes ("osmolytes") in order to maintain a favorable internal milieu, while regulating their volume. We identified the organic osmolytes in renal inner medullary cells as glycerophosphorylcholine (GPC), betaine, sorbitol, and inositol, and showed that the osmolyte levels varied with urine concentration (and, presumably, medullary salt concentration). We are now using renal cell cultures and living animals to study the mechanism and control of osmoregulatory accumulation of these organic osmolytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 01665-15 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth and Differentiation of Smooth Muscle and Nonmuscle Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sachiyo Kawamoto, M.D., Ph.D., Visiting Associate, LMC, NHLBI
Robert S. Adelstein, M.D., Chief, LMC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Myosin is found in all eukaryotic cells and appears to be involved in diverse cellular motile processes, such as cytokinesis. Recently, this laboratory and another laboratory (Katsuragawa et al., Eur. J. Biochem. 184: 611-616, 1989) have isolated two different cDNAs which encode two different chicken nonmuscle myosin heavy chains (MHCs). In order to understand the biological significance of MHC isoforms in nonmuscle cells, we studied the expression of two types of MHCs in a number of chicken tissues at different stages of development and quantitated the relative contents of mRNA encoding the MHC isoforms in a number of tissues, using RNA blot analysis with two specific oligonucleotide probes. Our results show that the relative content of mRNA encoding MHC-A and MHC-B differs in a tissue-dependent manner. The relative content of mRNA encoding MHC-A vs MHC-B varies from greater than 9:1 in spleen and intestinal epithelial cells, to 6:4 in kidney and 2:8 in brain. Using SDS-polyacrylamide gels, we have separated two nonmuscle MHC isoforms (196 and 198 kD) which can be distinguished from each other and from the gizzard smooth muscle MHCs by peptide mapping. Spleen and intestinal epithelial cells contain almost exclusively 196 kD MHC, whereas brain contains predominantly 198 kD MHC. Kidney contains both 196 and 198 kD MHCs. These results suggest that MHC-A mRNA encodes the 196 kD polypeptide and MHC-B mRNA encodes the 198 kD polypeptide. We also studied the effect of serum on MHC mRNA expression in cultured chicken embryo fibroblasts as well as on primary cultures of aorta smooth muscle cells. Serum stimulation results in a three-fold increase in the mRNA encoding MHC-A and a three-fold decrease in mRNA encoding MHC-B at 6 h. The maximum changes in both MHC-A and B mRNA expression occur during G1 phase. Actinomycin D, an inhibitor of transcription, abolished the serum-induced changes in both MHC-A and B mRNAs, suggesting that these changes are regulated at the transcriptional level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01785-11 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Myosin and Caldesmon Phosphorylation in Nonmuscle Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James R. Sellers, Ph.D., Research Biologist, LMC, NHLBI
Joann M. Hettasch, Ph.D., Staff Fellow, LMC, NHLBI
Estelle V. Harvey, Biologist, LMC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.95	0.70	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Caldesmon is an actin-binding protein which may modulate actin-based cellular processes. These include activities such as cytokinesis, shape change, cell motility, cell to cell interactions, adhesion to substrata and secretion. In vitro studies have shown that caldesmon can inhibit the actin-activated MgATPase activity of myosin and that this inhibition can be reversed by calmodulin binding to caldesmon. Caldesmon is a substrate for various kinases and has been shown to be phosphorylated during smooth muscle contraction. It is also phosphorylated when human platelets are treated with phorbol esters.

We have examined the phosphorylation of caldesmon following treatment of platelets with physiological agonists such as thrombin, ADP and collagen and after treatment with the prostaglandin, PGI2, which raises cAMP levels. There is no increase in the level or sites of phosphorylation of caldesmon following treatment of platelets with thrombin, ADP or collagen, but treatment with PGI2 results in an increase in phosphorylation. Tryptic phosphopeptide maps show that this phosphorylation is primarily occurring at two sites which are also phosphorylated in vitro by cAMP-dependent protein kinase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01786-11 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Phosphorylation as a Regulatory Mechanism in Muscle Contraction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James R. Sellers, Ph.D., Research Biologist, LMC, NHLBI
Giovanni Cuda, Ph.D., Visiting Fellow, LMC, NHLBI
Seiji Umemoto, M.D., Ph.D., Visiting Fellow, LMC, NHLBI
Estelle V. Harvey, Biologist, LMC, NHLBI
William Anderson, Jr., Chemist, LMC, NHLBI

COOPERATING UNITS *(if any)*

P. Wagner, NCI

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.55	0.85	0.70

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To understand the mechanism of smooth muscle contraction and how actin and myosin interact in nonmuscle cells, we have been using various assays of myosin function. One of these assays involves the visualization in the fluorescent microscope of the movement of fluorescently-labeled actin filaments over a surface coated with myosin molecules. This movement is an active process which requires the presence of myosin and MgATP. The movement of actin filaments by smooth muscle and nonmuscle myosins is almost completely dependent upon phosphorylation of the myosin by myosin light chain kinase. Turkey gizzard smooth muscle myosin translocates actin filaments at about 5 times the rate of translocation obtained with human platelet myosin. Myosin from bovine brain appears to move actin filaments even more slowly than does platelet myosin. Heavy meromyosin, the soluble two-headed proteolytic fragment of myosin, can be prepared from platelet myosin. This heavy meromyosin can also translocate actin filaments in the motility assay at a rate similar to that obtained with the intact platelet myosin. Myosin can be bound to the glass surface as either filaments (consisting of a packed array of myosin molecules) or as monomers. Both forms translocate actin filaments at the same rate indicating that with smooth muscle and vertebrate nonmuscle myosin, filaments are not absolutely required for activity. This also raises the possibility that myosin filaments per se may not be necessary for motile functions in cells and may explain some of the difficulties that have been experienced in demonstrating the presence of myosin thick filaments in vertebrate nonmuscle cells. In order to better understand the role filaments play in the interaction of smooth muscle myosin with actin, we have cross-linked the myosin molecules within a filament with EDC. These cross-linked myosin filaments do not depolymerize under conditions that normally promote depolymerization of myosin and, thus, represent a mechanism for studying actin-myosin filament interactions under conditions where the filaments are unstable in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04205-08 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure, Function and Expression of Myosin Light Chain Kinase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gertrude L. Cornwell, Ph.D., Staff Fellow (PRAT, NIGMS), LMC, NHLBI (terminated 6/29/90)

Robert S. Adelstein, M.D., Chief, LMC, NHLBI
William Anderson, Jr., Chemist, LMC, NHLBI

COOPERATING UNITS *(if any)*

D. Lansing Taylor, Ph.D., Carnegie Mellon University, Pittsburgh, PA

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.95	0.85	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of injecting a calcium-calmodulin independent form of myosin light chain kinase (IMLCK) was studied using 3T3 fibroblasts. No obvious conclusions could be drawn as to whether the kinase had a consistent effect on cell shape or movement. These studies are continuing at Carnegie Mellon University in an effort to obtain consistent results with a greater number of cells. Treatment of 32P-labeled chicken embryonic fibroblasts did not appear to result in an increase in the phosphorylation of MLCK, but did result in a decrease in the phosphorylation of the 20 kDa light chain of myosin. When similar experiments were conducted with primary cultures of rat aortic smooth muscle cells, there was both an increase in MLCK phosphorylation and a decrease in the phosphorylation of the 20 kDa myosin light chain. However, no evidence was obtained for a causal relation between these observations, nor was the extent of phosphorylation quantitated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04207-05 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Cloning of the cDNA Encoding T-lymphocyte Myosin Heavy Chains

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Katsutoshi Yamakawa, M.D., Ph.D., Visiting Fellow, LMC, NHLBI
Robert S. Adelstein, M.D., Chief, LMC, NHLBI
Yvette A. Preston, Biologist, LMC, NHLBI

COOPERATING UNITS *(if any)*

O. Wesley McBride, DCBD, NCI

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myosin is a major contractile protein present in all nonmuscle eukaryotic cells, but the exact function of myosin in nonmuscle cells is still under study. Recent genetic experiments in Dictyostelium have shown that myosin heavy chains (MHCs) might play a role in cytokinesis, chemotaxis, cell development and specialized cellular functions such as secretion and capping. We have undertaken the cloning of nonmuscle MHC genes and cDNAs to study the function, regulatory mechanism and tissue specific expression of nonmuscle MHCs. The purpose of this study is to clone the cDNA for human nonmuscle MHCs in order to have new tools to examine the role of MHC in cell function.

We had already obtained cDNA clones (#302, HL-1, #707) from a human T-lymphocyte lambda gt10 library using different cDNA regional probes from the chicken intestinal epithelial cell MHC. Clone #302 contains a 1.3 kb insert that consists of 1200 nucleotides encoding the first 400 amino acids of a nonmuscle MHC as well as 5' untranslated nucleotides. HL-1 is a 1.9 kb cDNA clone isolated from the same library as #302 and encoding the same portion as that encoded by the 5' portion of pNMHCM2 in macrophages (C.G. Saez et al., Proc. Natl. Acad. Sci. USA 87: 1164-1168, 1989). There was a gap in the sequence of MHC-A between #302 and pNMHCM2. In order to fill in the sequence, we screened a human T-lymphocyte library using three different cDNA probes. Two cDNA clones (ha5-1, hacal) were isolated, which were 2.4 kb and 1.5 kb in size, respectively, and sequenced partially to complete the gap sequence. The gap sequence of nucleotides and amino acids was very homologous to that of the chicken cell MHC-A (83% identity in nucleotides, 98% identity in amino acids), as compared to human nonmuscle MHC-B (77% in nucleotides, 90% in amino acids).

GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04208-04 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning of the cDNA for a Nonmuscle Myosin Heavy Chain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Masayuki Takahashi, Ph.D., Visiting Fellow, LMC, NHLBI
Mary Anne Conti, Ph.D., Research Chemist, LMC, NHLBI (terminated 6/30/90)
Sachiyo Kawamoto, M.D., Ph.D., Visiting Associate, LMC, NHLBI
Robert S. Adelstein, M.D., Chief, LMC, NHLBI
Yvette A. Preston, Biologist, LMC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.35	2.05	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Having completed the cDNA cloning of the mRNA encoding the myosin heavy chain (MHC) A isoform in chicken intestinal epithelial cells, we are presently attempting to clone the cDNA encoding the MHC-B isoform. To accomplish this, we have been screening a chicken cerebellar library and have isolated a number of clones, including one that contains approximately 230 nucleotides of 5' untranslated mRNA. Our goal is to understand if the two MHCs are functionally different and whether their expression is regulated at the level of translation, transcription, or both.

We used our knowledge of the amino acid sequence of chicken intestinal epithelial cell MHC to locate the single serine residue phosphorylated in the 196 kDa MHC. The serine residue is located toward the end of the myosin rod, at residue 1915 out of 1959 residues. This serine is present in all vertebrate nonmuscle MHCs sequenced to date and evidence for its phosphorylation by protein kinase C has been found using myosin from human platelets, chicken intestinal epithelial cells and RBL-2H3 cells. Although many of the amino acids in this area are conserved in the smooth muscle MHC, the easily identifiable serine residue position is occupied by an alanine residue in the smooth muscle MHC. This explains our inability to phosphorylate the smooth muscle MHC of aortic cells with protein kinase C and suggests that there is at least one mechanism by which the activity of nonmuscle MHC may be regulated (reversible phosphorylation by protein kinase C), that is not available to smooth muscle MHCs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04210-03 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Myosin Phosphorylation and the Regulation of Contractile Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Christine A. Kelley, Ph.D., Staff Fellow, LMC, NHLBI
Robert S. Adelstein, M.D., Chief, LMC, NHLBI
William Anderson, Jr., Chemist, LMC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Vertebrate nonmuscle and smooth muscle myosins are regulated by phosphorylation of the 20 kDa light chains. However, the heavy chains of these myosins can also be phosphorylated. Our present goal is to understand the function of smooth muscle myosin heavy chain phosphorylation. We began by identifying the kinase(s) that phosphorylate smooth muscle myosin heavy chains in intact vascular cells. Of a variety of serine/threonine kinases assayed, only casein kinase II and calcium/calmodulin-dependent protein kinase II phosphorylated the smooth muscle myosin heavy chain to a significant extent in vitro. Two-dimensional maps of tryptic peptides derived from heavy chains phosphorylated in cultured vascular cells revealed one major and one minor phosphopeptide. Identical tryptic peptide maps were obtained from heavy chains phosphorylated in vitro with casein kinase II, but not with calcium/calmodulin-dependent protein kinase II. Of note, the 204 kDa smooth muscle myosin heavy chain, but not the 200 kDa heavy chain isoform, was phosphorylated by casein kinase II. Partial sequence of the tryptic phosphopeptides generated following phosphorylation by casein kinase II yielded the following: VIENADGS*EEEV. The S* represents the Ser(PO4) which is in an acidic environment, as is typical for casein kinase II phosphorylation sites. By comparison with the deduced amino acid sequence for rabbit uterine smooth muscle myosin (Nagai, R., Kuro-o, M., Babij, P., and Periasamy, M. (1989) J. Biol. Chem. 264, 9734-9737), we have localized the phosphorylated serine residue to the non-helical tail of the 204 kDa isoform of the smooth muscle myosin heavy chain. The ability of the 204 kDa isoform, but not the 200 kDa isoform, to serve as a substrate for casein kinase II suggests that these two isoforms can be differentially regulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04212-02 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of Vertebrate Myosin I

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James R. Sellers, Ph.D., Research Biologist, LMC, NHLBI
Estelle V. Harvey, Biologist, LMC, NHLBI
William Anderson, Jr., Chemist, LMC, NHLBI

COOPERATING UNITS *(if any)*

Paul Matsudaira, Assoc. Professor, Whitehead Institute, MIT
Kathy Collins, Graduate Student, MIT

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.2	0.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chicken intestinal brush border epithelial cells contain a calmodulin binding protein of 110 kDa which has been shown to be a myosin I type molecule. There are probably three calmodulin molecules associated with each heavy chain. This myosin does not form filaments, but does have an actin-activated MgATPase activity and other properties of the more conventional two-headed myosin molecules. We have shown that it is capable of translocating actin filaments in an in vitro motility assay. Motility and actin-activation of the MgATPase activity is inhibited at high calcium concentrations due to a dissociation of a fraction of the calmodulin molecules. Re-addition of calmodulin restores motility and actin-activation of the MgATPase activity. Tropomyosin binding to the actin filaments also inhibits motility and actin-activated MgATPase activity due to a large decrease in the affinity of actin-tropomyosin for myosin. Interestingly, immunofluorescent data demonstrates that myosin I does not co-localize to tropomyosin-rich regions of tissues culture cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04213-01 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interaction of Invertebrate Myosins With Actin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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William Anderson, Jr., Chemist, LMC, NHLBI

COOPERATING UNITS *(if any)*

Bechara Kachar, LMO, NIDCD, NIH

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the sliding actin in vitro motility assay, the movement of fluorescently-labeled actin over a surface coated with myosin is visualized in the fluorescent microscope. In all the cases reported to date, the underlying myosin surface is not directly visualized. It is usually assumed that the bound myosin filaments or monomers are randomly oriented. We have isolated large native thick filaments from molluscan muscle using a rapid and gentle method. These thick filaments are between 10 and 50 um long depending on the source of the muscle and can be directly visualized on the glass surface using video-enhanced differential interference contrast microscopy. This allows us to directly correlate the movement of the fluorescently-labeled actin filaments with their position on the native bipolar thick filament. Our observations show that actin can travel both toward and away from the center of the thick filament and that the polarity of actin determines the direction of movement. Actin filaments moving toward the center of the thick filament travel about 9 times faster than those traveling away from the center. The movement of actin away from the center of the thick filament is opposite that which usually occurs in muscle contraction and suggest that the heads of myosin must be very flexible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04214-01 MC

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effect of Retroviral Infection on Myosin Heavy Chain Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carrie Phillips, M.D., Medical Staff Fellow, LMC, NHLBI
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eukaryotic cells contain unique forms of myosin which may play a role in cell proliferation. Referred to as "nonmuscle" myosins, these proteins share a greater degree of amino acid identity with smooth muscle myosin, but a lesser degree of similarity when compared to striated muscle myosin. Our interest is in the identification of nonmuscle myosins from human cells and the expression of these proteins in lymphocytes infected with retroviruses.

Previous investigators have isolated two forms of mammalian nonmuscle myosin heavy chain (NMMHC) cDNA. These isoforms, from chicken and human cDNA libraries, have been designated "A" and "B". Using restriction enzyme digestion of a previously sequenced cDNA B clone, a probe has been generated near the 3' portion of the clone. A human T-cell (Jurkat) library was screened with this probe resulting in the isolation of new clones. This allowed for extension of NMMHC B sequence in the 3' direction. A second probe generated from this clone is currently being used to screen another T-cell library.

NMMHC A and B oligonucleotide probes have been synthesized in order to distinguish between the two isoforms. Southern analysis reveals that these A and B differential probes can selectively discriminate between the two isoforms. Human genomic DNA and Northern blots of human T-cell RNA are currently being probed. RNA from HTLV-I-infected T-cells has been prepared for Northern analysis with these differential probes. HTLV-I-infected cells have been chosen because retroviral genes appear to integrate into the host's genetic material upon infection, altering normal gene expression. If successful, this experiment may provide information about mRNA expression in cells responding to infection by retroviruses.