National Heart, Lung, and Blood Institute

Division of Intramural Research

Annual Report

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SUMMARY REPORT OF THE SCIENTIFIC DIRECTOR

Introduction

The Division of Intramural Research, NHLBI, conducts clinical research on normal and pathophysiologic function of the cardiac, pulmonary, blood and endocrine systems, and basic research on normal and abnormal cellular behavior at the molecular level. The research activities of the 18 Laboratories and Branches range from structural organic chemistry to cardiac surgery (see Appendix). Major areas of interest include: the mechanisms of gene regulation, retroviral-mediated gene transfer, and, ultimately, gene therapy; the molecular basis of lipoprotein dysfunctions and the atherogenic process; the molecular basis of diseases of the alveolar structures of the lung, and the design of new therapeutic modalities; the cellular and molecular events underlying ischemic heart disease and myocardial hypertrophy; biochemical events associated with aging and certain pathologic processes; molecular, structural and developmental aspects of muscle and nonmuscle contractile systems; the biochemistry and physiology of calcium channels; molecular and cellular processes for the conversion of metabolic energy into useful work; the molecular basis of transmembrane signalling; the pathophysiology of renal function at the cellular and molecular levels; the biochemistry of trace nutrients; enzyme kinetics, metabolic regulation and protein chemistry; the cellular and molecular basis of toxicities induced by drugs and other foreign compounds.

The ultimate goal of gene therapy for human diseases provides a focus for the activities of several research groups (Molecular Hematology, Clinical Hematology, Pulmonary). Candidate diseases include several that are caused by genetic deficiencies (e.g. severe combined immunodeficiency, severe beta thalassemia, sickle cell anemia, emphysema associated with alpha-1 antitrypsin deficiency, hypercholesterolemia due to low density lipoprotein receptor gene deficiency), and several others for which successful treatment might result from, or be facilitated by, gene therapy (e.g cancer, AIDS, bone marrow failure).

The Division of Intramural Research has long been interested in understanding the molecular properties of the plasma lipoproteins, their biosynthesis, their role in lipid transport, and the molecular and morphological defects associated with disorders of lipid metabolism and atherogenesis (Molecular Diseases, Pathology). Also, several aspects of myocardial ischemia are under study including the energy metabolism of the ischemic and postischemic heart and brain (Cardiac Energetics, Surgery), the possibility that angina in the absence of coronary artery disease may, in some cases, be related to a generalized dysfunction of smooth muscle, and, in others, to abnormalities of endothelial function (Cardiology); and the potential use of growth factors to enhance development of collateral coronary circulation (Cardiology). Several Branches are collaborating on diverse studies of hypertrophic cardiomyopathy: identification of its genetic basis (Clinical Hematology, Cardiology); the possibility of the involvement of abnormal calcium metabolism (Cardiology); and improved operative treatment (Surgery).

Several Laboratories are investigating related aspects of the processes of transmembrane signal transduction. Studies include the cleavage of phosphatidylinositol by the multiple isoforms of phospholipase C of mammalian brain (Biochemistry); the mechanisms for control of the synthesis, assembly and operation of the guanine nucleotide-binding (G) proteins (Cellular Metabolism); the role of the G-proteins and phosphoinositide pathways in mediating the antigen-induced release of histamine from mast cells (Chemical Pharmacology); and the role of cyclic AMP in regulating protein synthesis (Biochemical Genetics).

The regulation of intracellular calcium ion concentration and its physiologic consequences provides another focus of activity. Interests include the regulation of the expression of the gene for one of the voltage-sensitive calcium channel subunits of neuronal cells (Biochemical Genetics); the fundamental role of calcium ions in regulating mast cell secretion (Chemical Pharmacology); and the possibility that abnormal calcium channel activity may be involved in microvascular angina and hypertrophic cardiac myopathy (Cardiology), and in the toxicities induced by maitotoxin and doxorubicin (Chemical Pharmacology).

The contractile proteins of muscle and nonmuscle cells are studied by several Laboratories. Investigations include the mechanism by which actin assembles to form the thin filaments of muscle and the microfilaments of non-muscle cells (Cell Biology); the structure, regulation and expression of muscle and non-muscle myosins (Cell Biology, Molecular Cardiology), and the functional roles of actomyosin in nonmuscle cells (Cell Biology, Molecular Cardiology). Related studies extend to the organization of the membrane-cytoskeleton complexes of neuronal cells that include voltage-sensitive sodium channels and the acetylcholine receptor (Biochemical Genetics). Through the use of non-invasive NMR and other spectroscopic techniques, the complex interaction between energy conversion processes and muscle contraction of the living heart is being studied (Cardiac Energetics), while the molecular basis of energy conversion, i.e. mitochondrial electron transport and oxidative phosphorylation, is investigated by others (Cell Biology).

The Division of Intramural Research has long been in the forefront in the development and application of new physical techniques to biomedical research and clinical support systems. Currently, this includes studies of the structure of peptides and proteins by time-resolved fluorescence spectroscopy (Technical Development), plasma desorption mass spectrometry and nuclear magnetic resonance spectroscopy (Chemistry); and structural studies of smaller organic molecules of biological interest by similar techniques and X-ray crystallography (Chemistry). Previously, NMR spectroscopy led intramural scientists to the discovery that osmotic regulation in the renal inner medulla involved the formation of sorbitol, inositol, glycerophosphate and betaine (Kidney and Electrolyte Metabolism). The physiologic role of the conversion of glucose to sorbitol in the kidney is particularly interesting because the same reaction is involved in the pathology of diabetes in eyes, nerves and kidneys.

This brief synopsis should give the reader some feeling for the breadth and

vitality of the research activities of the Division of Intramural Research, NHLBI. Research prospers because of the excellence and dedication of the permanent staff, and the continued influx of able and ambitious post-doctoral fellows from many nations. Indeed, the training of young scientists in preparation for their own independent research careers is a major activity of the Division. Principally, they are trained by their direct involvement in the research, but also by their participation in the numerous formal and informal seminars that occur on the NIH campus, the availability of the FAES Graduate School program, and the multiple interactions that occur among many scientists in the NHLBI and the other institutes of the NIH.

Personnel, Budget and Facilities

The Division of Intramural Research has a staff of about 430 including about 230 doctoral-level scientists of whom about 75 are in tenured positions. An additional 65 post-doctoral fellows are supported by Institute funds, and there are approximately 100 guest researchers. This combined staff of about 600 occupies a total space of about 100,000 square feet (including offices, conference rooms, cold rooms, etc.) and has the use of 80 beds in the Clinical Center.

In fiscal year 1989, the operating budget (for all research costs exclusive of salaries, hospital costs, and overhead, but including capital equipment, renovations, and domestic and foreign travel) was $14,800,000. This compares to budgets of $15,450,000 in FY 1986, $14,360,000 in FY 1987, and $13,526,000 in FY 1988. Clearly, one of the major difficulties faced by the intramural research program is the constancy of research dollars during a period of escalating research costs. A significant increase in the operating budget is needed to maintain the excellence of the present intramural research program, and to encourage the new initiatives that are necessary to remain at the forefront of biomedical research.

Other significant problems that need to be addressed include the inability to provide adequate research space to meet the demands of new programs, and the large and growing differential between government salaries and those of academia and industry. The general decrease in the numbers of talented individuals seeking careers in biomedical research, together with the diminished competitive position of intramural NIH, has made it increasingly difficult to attract skilled, young investigators, and to retain mid-career scientists of high quality. This situation may be improved by recent increases in post-doctoral stipends, more active recruitment efforts, and imaginative approaches to providing the clinical sub-specialty training that is not now available.

There were a few specific developments this year that should be particularly noted. Dr. Edward D. Korn was appointed to succeed Dr. Jack Orloff as Director of the Division of Intramural Research. Dr. Korn will continue to be Chief of the Laboratory of Cell Biology. As a result of the imminent retirement of Dr. Robert Bowman, who will continue his distinguished career as a Scientist Emeritus, the decision has been made to abolish the Laboratory of

Technical Development. Its scientific staff will be incorporated into the Laboratory of Chemistry and the Laboratory of Cell Biology. Plans were completed for the acquisition of a 4 Tesla NMR instrument for clinical magnetic resonance imaging, and for the necessary enlargement of the present Clinical Center MRI facility that will house it (which should be operational in about one year). This shared facility is, and will continue to be, available to scientists from all of the intramural programs.

Specific Scientific Achievements in Fiscal Year 1989

Details of this year's accomplishments are provided in the approximately 250 individual projects reports prepared by the scientific staff, and that are available upon request. The major accomplishments of each of the 18 Laboratories and Branches are summarized in the reports of the Laboratory and Branch Chiefs that follow this summary report. This very brief introduction is not intended to be a distillation of, nor a comprehensive guide to, those reports. Rather, it represents a relatively arbitrary selection of a few specific topics from a very much larger number of accomplishments many of which could have served the purposes of this summary report equally well.

Gene and Gene Transfer Studies: Scientists in the Laboratory of Molecular Hematology, in collaboration with scientists in the National Cancer Institute, are now conducting the first gene transfer protocol in human patients to be approved by the NIH and the FDA. In these studies, human tumor infiltrating lymphocytes (TIL) are grown in tissue culture, infected with a retroviral vector carrying the neoR gene for neomycin resistance, and re-administered to patients with advanced malignant melanoma. Although the genetically engineerd TIL have no enhanced therapeutic potential, the ability to follow the fate of the cells carrying the neoR gene may lead to improvements in current protocols for adoptive immunotherapy of cancer patients. As the first use of gene transfer technology in humans, this study is an important and necessary step in the development of gene therapy.

The genetic defect of the Watanabe heritable hypercholesterolemic rabbit has been corrected in vitro by retroviral insertion of the gene for the low density lipoprotein receptor. Furthermore, the expression of the LDL receptor by implanted cells in vivo has been demonstrated. Similarly, very high levels of secretion of human tissue plasminogen activator (t-PA) have been obtained from sheep endothelial cells transduced with the human t-PA gene and grown on intracoronary stents in vitro (Molecular Hematology).

Progress in gene replacement therapy for hemoglobin disorders has been hampered by the inefficiency of retroviral transfer of the globin gene into bone marrow hematopoietic stem cells, and the low level of expression of the transferred gene. Recent experiments by the Clinical Hematology Branch show that interleukin-3 is essential for the culture of mouse stem cells in vitro and that interleukin-6 augments the effects of IL-3. The growth factors increased retroviral-mediated gene transfer by 10-fold in the mouse cells. This experience has been found to be directly applicable to gene transfer in non-human primate stem cells which is much less efficient than in mouse stem cells.

Emphysema is predominantly due to a hereditary deficiency in the protease inhibitor alpha-1 antitrypsin leading to proteolytic dissolution of the lung parenchyma. This year at least 9 different alleles have been characterized with respect to the molecular alteration (DNA and protein) and physiologic consequences of the alteration (Pulmonary). Some mutations result in decreased secretion of an unstable protein, some to increased accumulation of the protein in the liver (resulting in hepatic damage), others are null mutants in which no protein is synthesized. A method has been developed to screen for the Z mutation, the most common form of alpha-1 antitrypsin deficiency. Retroviral transfer of human alpha-1 antitrypsin cDNA into T-lymphocytes has produced secreting cells that, when transplanted into mice, augment serum lung levels of alpha-1 antitrypsin (Pulmonary).

Regulation of Gene Expression and Translation: The promotor region of the eukaryotic translation factor gene (eIF-2) has been sequenced and analyzed, and influenza virus was found to encode a gene product that blocks the activity of an eIF-2-protein kinase which, if not inhibited, would phosphorylate eIF-2 and prevent viral protein synthesis (Molecular Hematology).

A powerful enhancer that controls beta globin gene expression (and which is required for the increase in globin synthesis that occurs during erythroid maturation) has been identified and localized to a 20 base pair segment of the cis-acting elements lying within the "locus activating" region of the gene (Clinical Hematology). A modular structure of the human gamma globin gene promotor has been described with multiple interacting cis-acting and trans-acting factors including one positive promoter that seems to interact directly with general components of the transcriptional complex (Clinical Hematology).

It has been found (Biochemical Genetics) that elevation of cAMP levels in cultured NG108-15 cells results in a 2-to-3-fold increase in mitochondrial DNA and a 5-fold increase in RNA transcribed from mitochondrial DNA. Cyclic AMP had previously been found to cause the appearance of functional voltage-sensitive calcium channels in these cells, and now has also been found (synergistically with phorbol esters) to elevate neuropeptide levels as much as 200-fold in rat pheochromocytoma cells. These factors, and nerve growth factor and glucocorticoids, seem to act by affecting the rates of transcription of neuropeptide Y RNA. Similarly, cAMP and glucocorticoids cooperatively activate transcription of the preproenkaphalin gene in brain cells.

The trace element, selenium, was previously found to be present in several enzymes in the form of selenocysteine and selenomethionine (selenium replacing sulfur) and in lysine- and glutamate-tRNAs (Biochemistry). Recent work shows that selenocysteine is biosynthesized by a novel pathway in which a unique serine-tRNA is phosphorylated on the serine hydroxyl group which is then replaced by selenium to form selenocysteine-tRNA, i.e. the conversion of serine to selenocysteine occurs on tRNA. Selenium incorporation into the polynucleotide chain of specific tRNAs occurs through an ATP-dependent

substitution of selenium for sulfur in a 5-methylaminomethyl-2-thiouridine residue in the "wobble position" (Biochemistry).

Contractile Proteins: Definitive evidence has now been obtained that the regulation of the enzymatic activity of conventional, filamentous myosins (myosin II class) of amoebae by heavy chain phosphorylation occurs through a global effect on the conformation of the myosin filament, and not at the level of the individual molecule (Cell Biology). Although it is clear that heavy chain phosphorylation, discovered in amoebae (Cell Biology), also occurs in mammalian muscle and non-muscle cells, for example upon antigen-activation of histamine and serotonin release from rat basophilic leukemic cells (Molecular Cardiology and Chemical Pharmacology), the effects of this phosphorylation on mammalian myosin II activity in vitro or myosin II function in vivo are not known. Indeed, the role of myosin II in this secretory process is not clear, although there is a strong temporal connection between the increase phosphorylations of the heavy and light chains by protein kinase C and myosin light chain kinase and the antigen-induced, calcium ion-dependent secretion (Molecular Cardiology, Chemical Pharmacology).

The non-filamentous myosins (myosin I class) have been found to be tightly bound to the plasma membrane, and to be specifically localized at the leading edges of lamellipodia and pseudopodia of locomoting amoebae and at the tips of phagocytic cups (Cell Biology). These regions contain actin filaments but are devoid of conventional myosin II which suggests that actomyosin I contributes to the motile force at the leading edge of migrating cells previously thought to be due solely to actin polymerization. Enzymes of the myosin I class are now known also to occur in mammals so these studies may lead to new insights into the molecular basis of macrophage and fibroblast motility, neuronal growth cone development, and other motile activities of mammalian cells. Consistent with this idea, studies of the 110-kD protein/calmodulin complex from intestinal brush border (Molecular Cardiology) show it to be a mechanochemical enzyme of the myosin I class first described in amoebae (Cell Biology).

Energy Metabolism: Myocardial contraction (a specialized form of cell motility) depends on the conversion of the chemical energy released by actomyosin-catalyzed hydrolysis of ATP to the mechanical movement of the interacting actin and myosin filaments. Recent studies with perfused hearts in vitro (Cardiac Energetics Laboratory in collaboration with the USSR Cardiology Research Center) suggest that an increase in oxygen delivery may be at least partially responsible for the increase in oxidative phosphorylation that accompanies increased contractile activity. In agreement with this hypothesis, in vivo stress tests indicate that cardiac levels of high energy phosphates, as monitored by non-invasive NMR, do not change until coronary blood flow becomes inadequate to support oxidative phosphorylation (Cardiac Energetics).

The effects of modern cardiopulmonary bypass techniques on heart and brain metabolism have been assessed by determining high energy phosphate levels and intracellular pH simultaneously through the use of non-invasive NMR spectroscopy (Surgery, Cardiac Energetics). No changes were detected at 37°,

or 26°, but at 18° the energy status of the brain increased, indicating a protective effect of deep hypothermia.

Basic studies continue on the process of oxidative phosphorylation, i.e. on the precise mechanism of coupling of ATP synthesis to electron transport (Cell Biology). It is believed that the transport of electrons generates a membrane potential that drives ATP synthesis. A simple system has now been developed in which membrane potential and pH gradients can be generated in artificial phospholipid membrane vesicles (liposomes), and their effects on cytochrome oxidase and/or ATP-synthesizing enzymes incorporated into the membranes studied.

Signal Transduction: Three quite dissimilar forms of the enzyme phospholipase C have been purified from bovine brain and their cDNAs cloned and sequenced (Biochemistry). The existence of multiple isoforms of this enzyme suggests a possible mechanism for the diversity of responses in different cell types to the same external signalling molecule. This concept was supported by the observation that phospholipase C-gamma (but not the alpha and beta isoforms) is phosphorylated by the tyrosine kinase activities of the epidermal growth factor and the platelet-derived growth factor receptors, each of which is activated by interaction with the respective growth factor (Biochemistry).

The high-affinity cAMP-phosphodiesterase of fat cell membranes is activated by insulin and isoproterenol, and mediates the actions of these effectors by regulating cellular levels of cAMP. It has now been found that both effectors cause serine phosphorylation of the diesterase in vivo, a reaction that might be involved in the regulatory events (Cellular Metabolism).

The G-proteins play an important role in the regulation of adenylate cyclase systems, visual excitation, and ion channels. The activity of the G-proteins are modulated by ADP-ribosylation of a cysteine resdiue near the C-terminus. Structural studies on the G_{oalpha} subunit modified by in vitro mutagenesis have confirmed and extended the known requirements for this reaction (Cellular Metabolism). Through the use of monoclonal antibodies specific for an epitope defined by the N-terminal 18 amino acids of G_{talpha}, the importance of this region for the interaction of the alpha subunit with the beta and gamma subunits has been indicated (Cellular Metabolism). Finally, the multiple forms of G_{oalpha} in neuronal tissue have been found to be the result of alternate splicing of transcripts of a single gene (Cellular Metabolism).

Antigen activation of mast cell secretion occurs through both phosphoinositide-dependent and phosphoinositide-independent processes with three effects: a rapid increase followed by a slow decay in intracellular calcium ion concentration, a slow sodium-dependent increase in pH, and activation of protein kinase C (Chemical Pharmacology). The phosphoinositide-dependent signal may be activation of protein kinase C, which would be consistent with the previously mentioned antigen-induced phosphorylation of myosin II. Through studies of the effects of adenosine analogues, it now seems that G-proteins also have an important role in the process of antigen-induced mast cell secretion, probably in part by activation of phospholipase C (Chemical Pharmacology).

The hypothesis that microvascular angina, a syndrome in which angina occurs in the absence of coronary artery disease, may be due to a narrowing of the pre-arteriolar coronary arteries is supported by the observation that 30-50% of such patients have other symptoms consistent with generally hyperactive smooth muscle. Symptoms are improved by calcium channel blockers suggesting that the vasoconstriction is mediated by elevated cytosolic calcium levels (Cardiology). Elevated cytosolic calcium levels may also contribute to the pathogenesis of hypertrophic cardiomyopathy as patients were found to have an increased concentration of voltage sensitive calcium channels (dihydropyridine binding sites) but normal densities of beta adrenergic receptors (Cardiology).

Metabolism and Enzymology: The Molecular Disease Branch has continued its various studies of lipoprotein structure and metabolism. Current findings of interest include: lipoprotein(a) is catabolized more slowly in patients with familial hypercholesterolemia than in normal controls, apparently because of a deficiency in the LDL receptor; two different mutations in the apoC-II gene have been identified that lead to defective synthesis of this apolipoprotein, which is required for activation of lipoprotein lipase, and the first mutant lipoprotein lipase gene has been characterized; the liver was found to synthesize and secrete the two HDL apolipoproteins, apoA-I and apoA-II, but the intestine synthesizes only apoA-I; the structural relationship between apoB-100 and apoB-48 has been established, the stop codon that leads to the formation of the latter rather than the former is introduced at the RNA level by a unique RNA-editing mechanism; several genetic variants of apoE have been characterized.

Control of the cellular accumulation of the osmolytes sorbitol, inositol, glycerophosphorylcholine and betaine is being studied in tissue culture of renal medullary cell lines (Kidney and Electrolyte Metabolism). Sorbitol accumulates in cells grown in hyperosmotic medium because of an increase in aldose reductase activity resulting from an increase rate of synthesis of aldose reductase; aldose reductase mRNA is greatly increased in these cells. These observations were confirmed in rats with congenital diabetes insipidus in vivo, and with normal rats, by manipulating the extracellular NaCl concentration. In contrast, increase in intracellular inositol occurs only when inositol is present in the culture medium and is due to increased uptake resulting from increased synthesis of sodium-dependent transporters. Betaine accumulation is similar to inositol accumulation, while the accumulation of glycerophosphorylcholine results from a combination of increased synthesis, increased uptake, and decreased catabolism.

Previous studies by the Biochemistry Laboratory had demonstrated that metal-catalyzed, oxygen radical-mediated damage to proteins marked enzymes for proteolytic degradation, and was implicated in the altered forms of enzymes that accumulate during aging. New evidence obtained by ischemia-reperfusion studies of gerbil brains suggests that the tissue damage is due to oxygen-free radicals produced during the re-perfusion phase. A similar mechanism appears to be responsible for demyelination in brain following hyponatremia. Interestingly, a single injection of bacterial endotoxin, which raises catalase and superoxide dismutase levels, protects rats against oxygen

toxicity. Evidence supporting the notion that metal-catalyzed oxidation involves a specific reaction at metal-binding sites on the protein has been obtained.

The myocardial toxicity of the anti-neoplastic agent doxorubicin was previously shown to be considerably decreased by simultaneous administration of the metal-chelator ICRF-187, suggesting that the cardiotoxicity is due to metal-catalyzed oxidation involving oxygen free-radicals (Pathology). It was found that ICRF-187 significantly protected beagle dogs when administered with doxorubicin over a 90-week period allowing doses at least 3-times larger than those that produced severe cardiomyopathy in control dogs (Pathology). Based on these studies, a study undertaken with New York University showed that ICRF-187 provided cardioprotection in human patients with metastatic breast cancer treated with doxorubicin.

Superoxide production by elevated levels of NADH-oxidase in alveolar macrophages may be involved in the etiology of chronic fibrotic disorders of the lung (Pulmonary). Aerosol administration of glutathione may be a feasible therapeutic approach in such situations.

Additional Topics of Interest: (1) In a laboratory model, extracorporeal membrane lung gas exchange has been shown to prevent and reverse the highly lethal acute respiratory failure caused by the use of mechanical ventilators (Technical Development). (2) Halothane-induced fulminant hepatitis in humans is mediated by an immune reaction, and the sera of such patients have been shown to contain antibodies to several trifluoroacetylated liver microsomal proteins of which 5 have been purified, characterized, and, in two instances, the cDNAs cloned (Chemical Pharmacology). (3) Studies on the interaction of herpes viruses and flavivirus with T-cells, hematopoietic cells and bone marrow, together with the discovery that non-A non-B hepatitis virus has the properties of a flavivirus support a unifying theory for bone marrow failure due to virus infection involving a combination viral anti-proliferative effects and toxic immune response (Clinical Hematology). B19 parvovirus, the cause of fifth disease, has been the subject of several recent advances: the polymerase chain reaction has been used to develop a very sensitive assay to detect the virus in sera; a genetically engineered cell line has been created that produces empty viral capsids that may be used for vaccine development; several patients have been successfully treated with immunoglobulin therapy with full restoration of erythropoiesis (Clinical Pharmacology).

ANNUAL REPORT OF THE CARDIOLOGY BRANCH
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The experimental interests of the Cardiology Branch focus on 1) elucidating the physiologic and cellular mechanisms responsible for the development of microvascular angina (MVA), including the possible roles of endothelial dysfunction and of aberrations of cytosolic calcium handling; 2) examining the cellular and molecular mechanisms responsible for the pathogenesis of hypertrophic cardiomyopathy; 3) the role of growth factors in causing angiogenesis and myocardial hypertrophy; 4) the use of growth factors to facilitate collateral growth in ischemic heart disease; 5) developing new techniques for opening coronary obstructions.

DYSFUNCTION OF THE CORONARY MICROVASCULATURE AS A CAUSE OF MYOCARDIAL ISCHEMIA

Angina occurring in the absence of coronary artery disease (CAD) or vasospasm of the large coronary arteries (Syndrome X) has been a diagnostic dilemma. Over the past 5 yrs we have explored the concept that this common disorder is due to dysfunction of the small intramural coronary arteries.

Microvascular coronary dysfunction and angina: We previously demonstrated that about 70% of pts with angina-like pain, but normal large coronary arteries, had inadequate increases in coronary flow and decreases in coronary resistance in response to pacing-induced stress, abnormalities exacerbated by ergonovine. The chest pain was associated with diminished myocardial lactate consumption and other findings consistent with myocardial ischemia, including exercise-induced LV dysfunction. Coronary vasodilator reserve was low not only in response to metabolic stimuli (pacing-induced increase in MVO_2), but also to dipyridamole, a potent dilator of coronary arterioles. Further analyses suggested that the flow limitation is due to narrowing of the small pre-arteriolar coronary arteries; therefore, we now refer to this syndrome as microvascular angina (MVA).

Evidence of a diffuse disorder of smooth muscle: Because abnormal esophageal motility was found in 30-50% of pts with MVA, we hypothesized the syndrome involves a general increase in smooth muscle tone. This concept was substantiated by the finding that the vasodilator response of the forearm vessels to ischemia was also blunted. Because dyspnea is common in MVA pts, we determined whether there also is dysfunction of bronchial smooth muscle causing airway obstruction. Methacholine inhalation decreased FEV_1 20% from baseline in the majority of MVA pts, a response observed rarely in controls. Likewise, while peak expiratory flow rate immediately following exercise increases in nls, it decreased in MVA pts. Hence, dyspnea in MVA pts seems partly to be due to hyper-reactivity of bronchial smooth muscle, results further substantiating the hypothesis that MVA involves a generalized disorder of vascular and nonvascular smooth muscle function.

Aberrations of cytosolic calcium as a cause of MVA: Voltage-dependent calcium channel blockers, verapamil, nifedipine and diltiazem, improve symptoms in most MVA pts. Some, however, are unresponsive to these agents. Lidoflazine, a calcium antagonist that blocks calcium entry at sites other than the voltage-dependent channel, improved effort tolerance and chest pain in these pts. Moreover, the drug improved the abnormally low coronary flow and high coronary resistance present during pacing and after dipyridamole. Thus, the elevated coronary resistance in MVA is not fixed but is due, at least in part, to reversibly elevated vasoconstrictor tone. The salutary effects of these different calcium antagonists also suggest that the vasoconstrictor influences may be mediated by elevated cytosolic calcium levels.

Cardiac sensitivity in MVA pts: The severity of chest pain does not correlate well with the presence and magnitude of myocardial ischemia in MVA pts. We tested the hypothesis that increased myocardial sensitivity to pain may contribute to cardiac pain. We found that when the RV was electrically paced just above control heart rate, MVA pts experienced pain, whereas controls did not. This suggests that heightened intracardiac nociception may contribute to the genesis of the pain syndrome in Syndrome X.

POSSIBLE NORMAL AND ABNORMAL ENDOTHELIAL AND MICROVASCULAR MECHANISMS CONTRIBUTING TO THE CONTROL OF SYSTEMIC AND CORONARY VASCULAR RESISTANCE

Impaired endothelium-dependent systemic vascular relaxation as a contributing cause of hypertension: Endothelium regulates vascular tone by secreting substances that modulate the activity of surrounding vascular smooth muscle. Acute and chronic hypertension impairs endothelium-mediated vasodilatation in experimental animals. To determine whether pts with hypertension have an endothelium-dependent abnormality in vascular relaxation, we studied the forearm vascular response of hypertensive pts and normal subjects to intra-arterially infused acetylcholine (Ach), an endothelium-dependent vasodilator, and to nitroprusside, an endothelium independent dilator. The vasodilator response to nitroprusside was unaltered in hypertensive pts but that to Ach was attenuated. Since Ach can inhibit norepinephrine secretion by sympathetic nerve endings, and thus theoretically vasodilate through this mechanism, we are evaluating pts after the local infusion of an alpha blocker. The vasodilator response to Ach does not seem to be altered in the alpha blocked state. Thus endothelium-mediated dilatation is impaired in hypertensive pts, a defect that may exacerbate or causally contribute to the hypertensive process.

Endothelium-dependent vasodilatation of coronary resistance vessels in man: Endothelium lining the large coronary arteries (CA) exerts a vasodilator action; whether endothelium also dilates coronary resistance vessels in man, and therefore regulates coronary flow, is unknown. We examined the effects of intracoronary Ach, which stimulates endothelium to release EDRF, on epicardial CA diameter and coronary flow and resistance in non-CAD pts. Epicardial CA did not change with Ach, but coronary flow increased and CVR decreased. Thus, the endothelium may regulate coronary resistance and flow in man; it is therefore possible that abnormalities of endothelial function of coronary resistance vessels may contribute to some myocardial ischemic syndromes.

Microvascular dysfunction as a cause of angina in pts with hypertension: Pts with systemic arterial constriction and hypertension frequently have angina-like chest pain, despite normal epicardial vessels. To determine whether the angina is due to an associated constriction of the small coronary arteries, we measured coronary vasomotor responses to atrial pacing before and after iv ergonovine in hypertensive pts with angina but without CAD or cardiac hypertrophy. Pacing-induced increase in coronary flow was less in hypertensive pts with angina compared to normotensive controls; ergonovine increased coronary resistance in hypertensive pts, but not in normals. Thus, hypertensive pts with angina but without CAD may have myocardial ischemia due to elevated coronary microvascular resistance that can be exacerbated by vasoconstrictor stimuli.

HYPERTROPHIC CARDIOMYOPATHY

Cellular and Molecular Mechanisms Responsible for the Pathogenesis of HCM

Abnormalities in cardiac calcium regulation: Because hearts of HCM pts are hyperdynamic and exhibit impaired relaxation, and because these abnormalities and pts symptoms respond to calcium antagonists, we hypothesized that abnormal cytosolic calcium regulation may be present in HCM. To test this concept we measured the density of dihydropyridine binding sites in the hearts of HCM pts undergoing

surgery, and found these sites (and presumably the density of voltage sensitive calcium channels) are increased in HCM pts, whereas beta adrenergic receptor density is unchanged. These results suggest that a specific increase in voltage sensitive calcium channels may play a role in the pathophysiology of HCM.

Abnormalities in cardiac NE kinetics: It has long been proposed that abnormal NE kinetics may be present in HCM, mainly because pts exhibit a hyperdynamic LV and because their symptoms respond to beta blocking agents. We examined cardiac NE kinetics and found that the production rate of NE (AV difference across the heart times coronary flow) was elevated in HCM. The increased NE spillover was not due to increased secretion by the nerve terminal, but to impaired neuronal uptake of NE. This defect probably increases NE levels at the neuroeffector junction, and thereby contributes to the pathophysiology of HCM, including myocardial hypertrophy, hyperdynamic LV, hyperplasia of the medium small coronary arteries, and myocardial ischemia due to vasoconstriction of these small coronary arteries.

Evidence favoring a proliferative disorder: The LV of HCM pts exhibits myocardial hypertrophy, excess scar tissue, hyperplasia of the smooth muscle present in small coronary arteries, and excessive angiogenesis. These findings are compatible with the hypothesis that HCM may be a primary proliferative disorder. We are presently examining the validity of this hypothesis.

Acidic and basic fibroblast growth factors (aFGF, bFGF) are angiogenic peptides and mitogens for fibroblast, smooth muscle and endothelial cells. They have been identified in many tissues of mesenchymal original, but previous to our studies, not in the heart. Two years ago we purified and characterized growth factors in human, dog, and rat heart that proved to be aFGF and bFGF. We also found these growth factors in hearts from HCM pts. Because of this finding and the possible abnormality in calcium regulation in HCM, we determined whether the mitogenic responses to FGF were associated with increases in cytosolic calcium. We found bFGF accelerated cell proliferation (rat aortic smooth muscle cells), and induced a dose-dependent sustained increase in intracellular calcium concentration. This suggests a possible inter-relation between abnormalities in the production or, or response to, FGF and abnormalities in cytosolic calcium regulation. Current studies involve the chronic infusion of FGF into the coronary artery of dogs to determine whether changes in cardiac tissue occur similar to the abnormalities present in HCM.

The genetic defect in HCM: Epidemiologic studies employing echocardiography have determined that HCM appears to be transmitted as an autosomal dominant trait in approximately 50% of HCM pts. We therefore initiated a collaborative study with Drs. Neal Epstein and Arthur Neinhuis to determine the chromosomal locus or loci of the genes responsible for hypertrophic cardiomyopathy. Over 150 members of 5 families have been evaluated by physical exam, EKG, and echocardiogram; their peripheral blood samples have been obtained and DNA extracted. Southern blots using more than 60 probes spanning greater than 40% of the genome have been performed. Having analyzed these data with linkage analysis programs, we are presently examining promising regions in greater detail.

Sudden Death in HCM: Identification and Treatment of Pts at High Risk

Natural history and Holter studies: The precise incidence of sudden unexpected death in HCM relates to pt selection. We found that middle-aged pts with no or mild symptoms had an excellent long-term prognosis; these pts usually had mild and localized LV hypertrophy. Also, particularly marked hypertrophy (wall thickness $\geq$25 mm in $\geq$ 2 of 4 LV segments) was 10 times more common in sudden cardiac arrest (SCA) pts than in controls. Mild hypertrophy (wall thickness $\leq$17 mm in one segment) was present in only 1 adult pt with SCA. To identify more precisely HCM subgroups at high risk of SCA, we analyzed the long-term follow-up data of pts who on routine Holter monitoring had VT. Survival of VT pts was worse than that of the no VT pts

(approximately 80% versus 95% at 5 years); however, the large majority of VT pts survived and some pts without VT died. Moreover, in a study of SCA survivors, over half of both young and middle-aged SCA survivors had no VT on Holter. Hence, VT alone was not highly sensitive in detecting pts with SCA, and its specificity was low. We therefore initiated EP studies to determine whether the data so derived would provide prognostic and therapeutic value.

EP studies: We found a high correlation between inducibility of VT and the pts' presenting clinical status. Thus, almost 80% of survivors of SCA were inducible, as were 50% of pts with a history of syncope, 30% of pts with presyncope, 20% of asymptomatic pts with VT on Holter, and 10% of asymptomatic pts without VT on Holter. Moreover, while there was an association between inducibility of VT in the EP lab and Holter VT, there were disparities. The majority of SCA survivors or pts with syncope with no Holter VT were induced in the EP lab, a finding suggesting that Holter is not as sensitive as EP studies in uncovering an electrically unstable ventricle. On the other hand, the large majority of pts who had only presyncope or no symptoms, but who had VT on Holter, were not inducible in the EP lab, suggest that Holter VT is a nonspecific finding in minimally symptomatic pts. EP studies in SCA survivors demonstrated likely mechanisms of SCA in all 24 pts studied, thereby providing critically important therapeutic information. Pts in whom ventricular instability could not be controlled pharmacologically had an automatic defibrillator (AICD) implanted. There have been no deaths in the 12 pts with an AICD, and 3 appropriate discharges have occurred in 2 pts. Two pts had pacemakers, and one was treated with accessory pathway ablation. Thus, although ventricular instability is the major mechanism responsible for SCA in HCM, there are multiple causes, many of which require EP study for proper identification. Holter monitoring can provide relevant information, although its sole use provides information that is relatively insensitive and nonspecific for detecting the pt at risk of SCA. Our results presently suggest that all HCM pts be screened with echo and Holter monitoring studies, and it should be ascertained whether the pt has had syncope or a family history of SCA. EP studies should be carried out in all pts with 1) prior SCA, 2) syncope, 3) malignant family history, or 4) marked LVH on echo. We are examining whether VT on Holter, in the absence of any other risk factor, is an adequate indication for EP study.

ANGIOGENESIS IN MYOCARDIAL ISCHEMIA AND INFARCTION

Increases in certain solid tumor cell populations must be preceded by an increase in the capillaries that converge upon the tumor to supply it with blood. This observation led to studies seeking to identify and ultimately to inhibit those factors responsible for neovascularization (and therefore tumor expansion). Five years ago we adopted an analogous but opposite approach: to use angiogenic factors to promote, rather than to inhibit, blood vessel growth in ischemic myocardium. We therefore initiated studies to determine the role of various growth factors in angiogenesis, and to determine whether it is possible to potentiate angiogenesis in ischemic myocardium.

Cellular biology of angiogenic factors in myocardium: As previously noted, we demonstrated that aFGF and bFGF are present in dog, rat and normal human heart. While many laboratories are pursuing the regulation of these factors and their receptors at the genetic level, we have begun to examine their physiologic and pathophysiologic regulation. For example, while many cells synthesize these factors in vitro, it was not known which cells synthesize them in vivo. We developed specific immunocytochemical methods, and demonstrated in tissue sections that myocytes contain these growth factors; endothelial and smooth muscle cells do as well, but in much smaller amounts. In collaboration with the Laboratory of Chemoprevention, NCI, we found transforming growth factor-beta$_1$ and its mRNA in

normal heart tissue, and have localized the peptide to myocytes and, to a lesser extent, to smooth muscle and endothelial cells.

We then determined whether changes in the levels and/or in the distribution of these growth factors occur during myocardial infarction. We found that bFGF mRNA and its peptide are increased in endothelial cells sprouting and dividing in response to myocardial infarction. These findings occurred in both infarcted and in presumably ischemic but non-necrotic regions, suggesting that ischemia-induced coronary collateral development may be caused, at least in part, by synthesis and/or release of FGF. We also found loss of TGF $beta_1$ immunoreactivity with myocardial infarction. If TGF $beta_1$ diffuses out of the impaired tissue, it may serve as a chemotactic factor for monocytes, neutrophils, and fibroblasts. TGF $beta_1$ has potent growth inhibiting properties; hence, its loss from the infarct zone may release capillary endothelial cells from inhibition, such that they can migrate into the center of the wound. Later in the evolution of infarction TGF $beta_1$ immunoreactivity appeared outside new capillary tubes, suggesting that TGF $beta_1$ may play a role in their differentiation. Fibronectin and laminin immunostaining also change during myocardial infarction; in vitro fibronectin expression is associated with endothelial migration and mitosis, whereas laminin expression is associated with subsequent vessel remodeling. Currently, immunohistochemical studies of these four peptides and of aFGF (a methodology we have just developed) is being applied to a model of canine myocardial ischemia, and to several models of myocardial hypertrophy.

Attempts to enhance coronary collateral development: CAD pts who develop refractory ischemic symptoms and are not candidates for initial or repeat bypass surgery pose a major therapeutic problem. We are assessing the potential for extra-cardiac blood vessels to supply the heart with nutritive flow through the development of new colateral connections, and the potential of angiogenic substances to stimulate collateral growth. For these studies we have devised a dog model of chronic ischemia, and as an extra-cardiac vessel, have implanted the internal mammary artery (IMA) into the LAD zone (Vineberg operation), which is rendered ischemic by an ameroid occluder placed around the LAD. IMA to LAD collaterals formed after 8 weeks, and provided 20% of collateral perfusion to the LAD territory. The circumflex coronary artery was responsible for 30% of collateral flow. Simultaneous occlusion of both vessels caused systolic dysfunction in the LAD territory, while occlusion of either artery alone had no effect, proving that the flow provided by the IMA with its newly formed collaterals was functionally important.

In our next series of studies we are determining whether the collateral flow supplied by either extra-cardiac or native blood vessels can be enhanced pharmacologically. In the first of these studies heparin was infused into a tube situated in the distal IMA and connected to an implanted pump; this preparation provided for continuous local infusion to the site of collateral formation. Although heparin alone is not angiogenic, it potentiates the mitogenic activity of aFGF. In addition, heparin has a high binding affinity for bFGF and aFGF, and protects them from inactivation. Since these factors are present in the heart, and since our and other studies suggested a potential role of bFGF in angiogenesis, we reasoned that exposure of ischemic tissue to exogenous heparin might facilitate the release of stored angiogenic factors, potentiate their activity, augment their stability, and thereby increase their capacity to promote angiogenesis. During maximal vasodilatation IMA to LAD zone conductance comprised, on average, 6% of total conductance in saline treated dogs, and 18% in heparin treated dogs. IMA to LAD zone conductance was highest in the high dose heparin group, intermediate in the low dose heparin group, and lowest in the untreated group, suggesting a dose-response relationship. Although these results did not reach statistical significance, the 7 dogs in which IMA -derived collaterals were responsible for the greatest percentage

of overall collateral flow were in the heparin-treated group. While not definitive, the data suggest that heparin infusion promotes vessel growth between an extra-cardiac blood source and the coronary circulation, facilitating the angiogenic response to myocardial ischemia. Thus, these results indicate that extra-cardiac blood vessels can establish biologically important connections with the native coronary circulation, and they suggest that it may be possible, by pharmacologic means, to increase their potential for collateral development to ischemic myocardium.

TRANSCATHETER CARDIOVASCULAR THERAPEUTICS

Over the past year, we made considerable progress in our continuing effort to further understand underlying pathophysiologic concepts, material science engineering, and clinical applications of new device development for transcatheter therapeutics in pts with coronary and peripheral artery disease.

Morphologic analysis of coronary artery disease: Transmural vessel wall architecture was analyzed from 21 pts with symptomatic coronary atherosclerosis and from 9 pts without known CAD. The average thickness of the media was reduced by 30% in pts with symptomatic CAD, and focal media thinning was accentuated in areas of greatest local plaque accumulation. This has important implications with regard to recanalization ablative techniques (lasers or atherectomy devices), since atheroma removal may be associated with a high risk of vessel perforation. Moreover, we confirmed previous observations that during advancing CAD compensatory dilatation of the vessel wall develops, which usually preserves lumen area and prevents flow limiting narrowing. In pts who did have severe focal lumen compromise, the narrowing was not caused by the expected increase in plaque volume, but rather by the absence of compensatory enlargement of the vessel. These observations may have important implications in the pathogenesis of coronary atherosclerosis.

Guidance and control systems: An improved optical detection system for laser-excited fluorescence spectroscopy has been tested during in vitro necropsy tissue experiments and in various clinical settings (during open heart surgery and during cardiac catheterization). Broad band ultraviolet optics provide improved lower wavelength resolution of fluorescence spectra, thereby achieving improved sensitivity and specificity of plaque recognition. This will enhance our capacity to perform safe laser angioplasty. We have also completed a series of in vitro and in vivo validation studies, using a prototype miniaturized (1.5 mm diameter) ultrasound catheter (25 MHz), which permits high resolution cross-sectional images of large and small vessels. In vitro studies established a striking correlation between ultrasound vessel wall geometry and cross-sectional area compared with quantitative comparable histologic evaluations from necropsy specimens.

Laser angioplasty: Previously we developed a comprehensive fluorescence-guided laser angioplasty system utilizing real-time fluorescence spectroscopy to direct a pulsed dye laser for tissue ablation. Utilization of this device in pts with peripheral vascular disease (femoropopliteal occlusions) resulted in an 81% successful recanalization rate in those pts who could not be treated by standard balloon angioplasty. Emphasis is now being placed on developing a multifiber over-the-wire laser catheter for large mass tissue removal, which could be used as a stand-alone catheter to achieve definitive recanalization in patients with coronary and peripheral artery disease.

Atherectomy devices: In vivo and in vitro studies using a new rotational atherectomy device have helped define necessary oeprating parameters for future clinical studies. The catheter-based device causes micro pulverization of obstructing atheroma into small size particulate debris. Studies in our laboratory have characterized the number and size distribution of ablation particles for a given mass of tissue removal. Importantly, microsphere flow studies in dogs after

injection of incremental doses of particles generated from human necropsy atherosclerotic specimens has helped to define the tolerable limits of particulate debris in the coronary microcirculation.

Endovascular prosthetic devices (stents): Intracoronary stent implantation will likely become a useful adjunct to standard PTCA in the treatment of CAD pts. We have seeded stents with genetically engineered endothelial cells, incorporating marker and tPA genomes, in an effort to develop a metallic stent design that would resist platelet adhesion and early thrombus formation. In addition, fluid mechanics studies were performed using a pulse duplicator system to simulate human anatomic situations, which has helped to understand turbulence characteristics and sheer stress factors when stents are placed in vessels. We are also developing a new non-metal polymer stent design which may be less thrombogenic, is biodegradable, utilizes a unique delivery system concept, and can be developed in various shapes that would permit large vessel stenting in situations of acute and chronic aortic dissections. Clinical studies have included the use of a balloon expandable metal tubular slotted stent in the treatment of severe stenoses in 35 pts with previous restenosis episodes after standard PTCA. Thus far, in cooperation with an international multicenter study, we have helped to define parameters associated with subacute thrombotic closure, operational factors for improved stent delivery, and pharmacologic treatment that might favorably influence acute and chronic biological responses.

CORONARY ARTERY DISEASE

Indications for coronary bypass surgery (CABG) in asymptomatic or mildly symptomatic pts with CAD: Great controversy surrounded the use of CABG for the asymptomatic or minimally symptomatic pt with CAD. Several years ago it was routinely advised, by many experts, that once the diagnosis of multivessel CAD was established, CABG was indicated. In a series of long-term follow-up studies, the Cardiology Branch led the way to a more rational strategy for use of CABG surgery in the treatment of pts with chronic stable angina. Our data demonstrated that in the mildly symptomatic CAD pt with normal rest LV function, it is only the pt with 3 VD and inducible ischemia who has a poor prognosis on medical therapy. More recently, we extended these observations to pts with less severe coronary angiographic abnormalities, but with LV dysfunction; the likelihood of a serious cardiac event occurring in pts with 1 or 2 vessel disease and resting EF ranging from 20-40% is very high if EF decreases with exercise, whereas prognosis is excellent if EF increases.

The next important question we addressed is how should pts initially identifed as having an excellent prognosis be followed longitudinally? To determine whether subsequent changes in EF with time signify important changes in coronary anatomy, we studied 90 mildly symptomatic pts with 2 coronary angiograms spanning 2-12 yr (mean 5 yr), and correlated the presence or absence of change with serial radionuclide angiograms (RNA) performed every 1-2 yrs. We found that progression of CAD was rare in the absence of alterations in EF at rest or with exercise; such changes in LV function indicated a high likelihood of a new total coronary occlusion or development of left main disease, even in the absence of progressive symptoms. Thus, RNA may be used not only in initial risk stratification of CAD pts, but also in serial evaluation to determine when repeat coronary angiography is necessary.

Do repeated episodes of reversible ischemia cause LV dysfunction in mildly symptomatic CAD pts? These data allowed us to address another issue that has been raised in mildly symptomatic pts with CAD and inducible ischemia. We found that it was rare for LV function at rest to deteriorate with time in the absence of a new 100% coronary artery obstruction, even in pts with repeated evidence of recurrent episodes of myocardial ischemia. Thus, recurrent myocardial ischemia, in and of

itself, does not appear to predispose to the development of LV dysfunction in the absence of anatomic progression of CAD in humans.

Detection of ischemic versus infarcted myocardium in pts with LV dysfunction: Many exercise-induced thallium perfusion defects in ischemic myocardium do not normalize on redistribution images, even when it is suspected that the myocardium is ischemic rather than infarcted. We hypothesized that late re-injection of thallium may facilitate delayed thallium uptake and better distinguish between ischemic versus infarcted tissue. We studied 73 CAD pts by exercise thallium SPECT imaging. Immediately after the redistribution images, an additional 1 mCi thallium was injected at rest, and images were re-acquired. Our results suggest that the late re-injection of the thallium and repeat imaging at rest, improves the detection of severely ischemic but still viable myocardium. This was substantiated in two subgroups of pts. 1) We re-studied 18 pts after myocardial revascularization. Prior to revascularization, 24 myocardial segments had persistent thallium defects on redistribution imaging, of which 17 (71%) improved or normalized after thallium re-injection study. All 17 segments that had normal thallium uptake after reinjection had normal thallium uptake on the post-revascularization study, along with improved LV wall motion. In contrast, most segments with persistent thallium defects after re-injection had, after revascularization, abnormal thallium uptake with associated persistent segmental wall abnormality. 2) Another 12 pts were studied in concert with PET imaging to evaluate blood flow with ^{15}O-water and exogenous glucose utilization with ^{18}F-fluordoxyglucose (FDG). All segments with increased thallium uptake after re-injection also had an increased ratio of FDG:^{15}O uptake, compatible with underperfused but viable myocardium. Thus, it appears that when thallium uptake occurs after reinjection, even if a defect is "fixed' after a standard redistribution study, it indicates myocardium that is viable and severely ischemic, rather than scarred.

Myocardial ischemia during ambulatory monitoring: Pts with CAD exhibit a circadian variation in the frequency of ischemic episodes and in the apparent ease with which ischemia is precipitated (ischemic threshold). Last year we found that diurnal changes in ischemic threshold correlated with changes in minimal forearm vascular resistance (determined following forearm ischemia). If changes in forearm resistance parallel changes in coronary resistance, these findings suggest that alterations in coronary resistance may account for alterations in ischemic threshold. We are now carrying out an investigation to determine if variations in alpha adrenergic tone, or variations in endothelium-dependent smooth muscle relaxation, are responsible for these diurnal changes in vascular resistance. The study consists of noninvasive measurements of forearm blood flow by plethysmography under baseline conditions, after 5 minutes of forearm ischemia, and after the intra-arterial infusion of nitroprusside, phentolamine, and acetylcholine. In each subject, three studies are performed at different times of the day (early morning, afternoon, and evening). Preliminary results suggest that the circadian variation in vascular resistance correlates with the variation in the vasodilator response to acetylcholine, but not to that of either phentolamine or nitroprusside, suggesting that endothelial modulation of vascular tone could account for the diurnal variation observed in systemic (and possibly coronary) vascular tone, and consequently in the ischemic threshold observed in CAD pts.

We have also related the frequency of daily myocardial ischemic episodes, as recorded by Holter monitoring, to the ischemic threshold (determined by treadmill stress testing) in CAD pts who were asymptomatic or only mildly symptomatic, to determine whether ambulatory ECG monitoring contained prognostic information that could not be ascertained by treadmill stress testing. Ischemic threshold was defined as the time of exercise at onset of 1 mm ST segment depression. Pts with a negative exercise stress test rarely exhibited ischemic episodes on Holter

monitoring. In pts with positive stress tests, the magnitude of daily myocardial ischemic episodes was predicted by the ischemic threshold -- pts who developed ischemia at low levels of exercise had frequent episodes of ischemia on Holter monitoring, whereas pts with a high ischemic threshold had no or rare such episodes. We also found that the sensitivity of ambulatory ECG monitoring is markedly affected by resting LV function as well as the resting ECG. Thus, pts with subnormal resting EF had a low prevalence of ischemic episodes, even in the subgroup developing more severe dysfunction with exercise. This subgroup is known to have a poor prognosis. Moreover, despite similar frequency of positive treadmill exercise tests, less than one quarter of pts with R wave amplitude in lead V5 <5 mm had ambulatory ischemic episodes. Also, pts with anteroseptal Q waves had a lower prevalence (33%) of ambulatory ischemic episodes compared to pts with inferior, anterolateral, or apical Q waves (prevalence 52%, 53%, 63% respectively). Thus, resting LV systolic dysfunction, as well as R wave amplitude and Q wave location on the ECG, importantly influence the detection of ambulatory ischemic episodes, factors which may substantially diminish the sensitivity of ambulatory ECG monitoring in identifying CAD pts with poor prognosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04175-03 CB

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.)*

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
Cedric D. Sheffield, M.D.	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:	PROFESSIONAL:	OTHER:
3.2	2.2	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.)*

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 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) is occluded gradually over a 2 week period by an ameroid constrictor. The internal mammary artery (IMA) is implanted into the region supplied by the LAD, with the supposition that collaterals will develop between the IMA and the territory normally perfused by the LAD. By assessing maximal myocardial blood flow with radiolabeled microspheres and monitoring regional contraction in the LAD area, the conductance from the IMA to the LAD vascular bed can be determined, and its functional importance assessed. In a group of 23 dogs, IMA to LAD collaterals formed after 8 weeks provided 20% of collateral perfusion to the LAD territory. The circumflex coronary artery, a major provider of collateral flow to the LAD region, was responsible for 30% of collateral flow overall. Simultaneous occlusion of both vessels caused systolic dysfunction in the LAD territory, while occlusion of either artery alone had no effect, proving that the flow provided by the IMA with its newly formed collaterals was functionally important. An additional study strongly suggested that administration of heparin augmented the formation of vessels between the IMA and the coronary circulation. Thus, we have shown that the potential exists for vascular connections to develop *de novo* between the systemic artery and the LAD area, that functionally important flow occurs, and that it may be possible to enhance this flow pharmacologically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04179-02 CB

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.)*

Ambulatory silent ischemia and noninvasive tests in coronary artery disease

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

Arshed A. Quyyumi, M.D.	Senior Investigator	CB	NHLBI
Julio Panza, M.D.	Guest Researcher	CB	NHLBI
Jean Diodati, M.D.	Senior Staff Fellow	CB	NHLBI
Timothy S. Callahan	Project Coordinator	CB	NHLBI
B. William Choi, M.D.	Guest Researcher	CB	NHLBI
Robert O. Bonow, M.D.	Senior Investigator	CB	NHLBI
Vasken Dilsizian, M.D.	Senior Staff Fellow	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch		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	2	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.)*

Silent ischemia during normal daily activities may have adverse prognostic significance in coronary artery disease (CAD). Our studies were performed to investigate the relation between the presence and magnitude of silent ischemia and the abnormalities in the conventional tests which have established prognostic value in CAD. We studied 124 CAD patients after withdrawal of antianginal medications with 48 hour ambulatory ST segment monitoring, treadmill exercise, exercise radionuclide ventriculography and thallium scintigraphy. Transient episodes of ST segment depression (ischemia) during ambulatory monitoring were often silent (>80%). They occurred only in patients with ST depression during treadmill exercise and the duration of ambulatory ischemia correlated with the duration of treadmill exercise to onset of ischemia. R wave amplitude and the presence and location of Q waves on the resting EKG importantly influenced the occurence of silent ischemia. During radionuclide ventriculography, many high risk patients with severe coronary disease and LV dysfunction at rest and exercise did not have ambulatory silent ischemia during monitoring whereas 40% of low risk patients with no exercise LV dysfunction had episodes. Similarly, with thallium scintigraphy, more patients without reversible thallium defects (but a positive treadmill exercise test) had episodes of ambulatory silent ischemia compared with patients who had reversible thallium defects. Thus, ambulatory ST segment depression occurs in patients with an early positive exercise test; however many patients with extensive inducible ischemia during radionuclide studies do not have episodes and this may be related to resting LV function, low R wave magnitude and Q waves on the resting EKG.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04181-02 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of Microvascular Endothelium - Dependent Reactivity

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
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, MD

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.)*

Studies have shown that the endothelium regulates vascular tone by modulating the activity of vascular smooth muscle. It has also been demonstrated that the endothelium plays an important role in atherosclerosis and animal models of hypertension.

In a previous investigation, we have shown that patients with essential hypertension have a normal vascular response to sodium nitroprusside (a direct smooth muscle relaxing agent) but an impaired response to acetylcholine (an endothelium-mediated vasodilator). This suggests the presence of an abnormal endothelium-dependent vascular relaxation in hypertensive patients.

To rule out the possibility that these findings are the result of a different inhibition of norepinephrine release from presynaptic adrenergic terminals (another proposed mechanism of acetylcholine-induced vasodilation), we are studying the response of the forearm vasculature to the intraarterial infusion of acetylcholine and phentolamine (an alpha adrenergic antagonist) both separately and in combination, in normal volunteers of both sexes.

Preliminary results show that the infusion of both drugs in combination results in greater vasodilation then the one observed when either drug is used alone. This suggests different mechanisms of action for phentolamine and acetylcholine and further supports our initial observation that the impaired vasodilator response to acetylcholine we observed in hypertensive patients is due to an abnormal endothelium-dependent vascular relaxation in these patients and cannot be accounted for solely by the effect of acetylcholine on sympathetic nerves.

We plan to study hypertensive patients under the same methodology and compare the results between the two groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04195-02 CB

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.)*

Circadian variation in ischemic threshold and vascular resistance in pts with CAD

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

Arshed A. Quyyumi, M.D.	Senior Investigator	CB	NHLBI
Julio Panza, M.D.	Guest Researcher	CB	NHLBI
Jean Diodati, M.D.	Senior Staff Fellow	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch		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:
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 have previously demonstrated a circadian variation in the ischemic threshold measured as the heart rate at the onset of 1mm ST segment depression during exercise in patients with coronary artery disease such that the threshold was lower in the morning and night compared to the rest of the day. A similar and inverse circadian variation was demonstrated in post-ischemic forearm vascular resistance which was found to be higher in the morning and night compared to the rest of the day. In order to investigate whether it is an increase in afterload from an increased peripheral vascular resistance during the morning hours that was lowering the ischemic threshold or an increase in coronary vascular resistance at this time of the day, we have undertaken a study to measure pacing induced ischemic threshold where accurate blood pressure and heart rate measurements can be made together with forearm blood flow measurements at different times of the day. Seven patients have been studied and the results are preliminary at this time.

To determine the underlying mechanisms for the observed circadian variation in peripheral vascular resistance, we have performed intra-arterial infusions of phentolamine, acetylcholine and nitroprusside at different times of the day and measured forearm blood flow. This will help evaluate if alpha sympathetic tone or endothelial mediated vasodilation is important in determining the circadian variation in vascular resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04808-02 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Growth Factors Involved in Myocardial Angiogenesis and Hypertrophy

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

Ward Casscells, M.D.	Senior Investigator	Stephen E. Epstein, M.D., Chief,
Edith Speir	Biochemist	Cardiology Branch, NHLBI
Shashi Shrivastav, Ph.D.	Special Volunteer	
Zu-Xi Yu, M.D.	Special Volunteer	Section on Experimental Physiology
Michio Chiba, M.D.	Special Volunteer	and Pharmacology, CB, IR, NHLBI
C. Phillip Nesbitt	Special Volunteer	
Tomoya Iino, M.D.	Special Volunteer	
Sadatoshi Biro, M.D.	Special Volunteer	

COOPERATING UNITS *(if any)*

Section on Ultrastructural Pathology, Pathology Branch, IR, NHLBI, Laboratory of Chemoprevention, NCI

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NHLBI, 10/7B04

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, the Section on Experimental Physiology and Pharmacology has focused on the in vivo physiology and pathophysiology of growth factors and extracellular matrix glycoproteins in the heart. In particular, we have developed methods with which we have studied the expression of basic fibroblast growth factor, transforming growth factor beta-1, fibronectin and laminin in myocardial infarction. These in vivo studies have helped to clarify a number of controversies that arose in vitro. We have considerable evidence that these growth factors are involved in myocardial angiogenesis, healing, and hypertrophy. Currently we are extending these studies to other members of the FGF and TGF beta families.

Because in vivo studies are essentially descriptive and not readily amenable to experimental manipulations, we are also trying to model ischemia in vitro in cultured capillary endothelial cells and adult rat cardiac myocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04811-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Compensatory Coronary Dilatation and Media Thinning in Progressive Atherosclerosis

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

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COOPERATING UNITS *(if any)*

Biostatistics Branch, NHLBI
Biomedical Engineering and Instrumentation Branch, DRS

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.)*

Careful morphologic analysis of transmural coronary artery pathology and vessel wall geometry would provide important insights for new transcatheter techniques to recanalize obstructed coronary and peripheral vessels. Specifically, plaque eccentricity, global and regional thickness of the underlying media, and cross sectional dimensions of plaque and vessel area would be important to determine in patients with severe atherosclerosis. We examined the coronary architecture from 21 neocropcy patients with severe atherosclerosis and 9 control patients with minimal atherosclerosis and no cardiac symptoms using a computerized video planimetry system of transverse histologic sections. We found attenuation of the media consistently in patients with progressive atherosclerosis with areas of focal thinning in regions of greatest plaque accumulation. In addition, compensatory vessel wall dilatation appears to be a uniform response to progressive plaque accumulation. However, those individuals with greatest lumen compromise lacked adequate compensatory vessel wall dilatation which appeared to be responsible for cross sectional narrowing. Thus, absolute plaque area was not greatly increased in regions of severe stenosis compared with contiguous more normal vessel segments. This finding has important implications in the pathophysiology of hemodynamically significant and clinically active coronary artery disease. If such observations are corroborated, angiographically significant and clinically symptomatic coronary artery disease is due largely to absent or diminished compensatory vessel dilatory responses and not focal increase in plaque deposition. These findings will have important implications in further understanding current pathophysiology and in devising treatment strategies including ablative recanalization techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 04812-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Slotted and Tubular Balloon Expandable Stents Across Vein Graft Anastomotic Sites

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
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Yaron Almagor, M.D.	Senior Staff Fellow	CB	NHLBI
Antonio L. Bartorelli, M.D.	Research Fellow	CB	NHLBI
Renu Virmani, M.D.	Chief	PB	AFIR

COOPERATING UNITS *(if any)*

Arm Forces Institute of Pathology Lab/Branch

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.)*

Restenosis at aortocoronary anastomotic sites is a common and difficult problem after standard percutaneous transluminal coronary angioplasty. Endovascular prosthetic devices (stents) have been a proposed solution to maintain an expanded internal scaffold which would resist radial compression and improve anastomotic site patency. The development and testing of such a device would importantly improve long term clinical results after vein graft implantation in the arterial system to bypass stenotic or occluded coronary and peripheral vessels. The current study utilizes an animal model in which reversed saphenous veins are placed end to side in femoral arteries and allowed to mature for eight weeks. Two forms of metallic stents (tubular slotted and coiled designs) were compared after balloon catheter expansion and implantation at proximal or distal anastomotic sites. Follow-up angiography and pathology analysis were performed at different time-points (3, 8, and 24 weeks). From the study we concluded that implantation of stents in vein graft at anastomotic sites results in excellent patency, rare thrombosis, occasional migration which requires careful attention to stent-vessel wall sizing factors, and rapid endothelialization which is almost always complete between 8 and 24 weeks. We believe that these studies indicate that stent implantation across the anastomotic sites may be a practical solution with favorable long term host responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04813-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Ostial Renal Artery Stent Implantation

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
Yaron Almagor, M.D.	Senior Staff Fellow	CB	NHLBI
Richard F. Neville, M.D.	Research Fellow	CB	NHLBI
Antonio L. Bartorelli, M.D.	Research Fellow	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:
.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.)*

Renal artery stenosis is an important clinical problem which often results in systemic hypertension and contributes to chronic renal failure with loss of renal mass. Current techniques to treat renal artery stenosis have been generally unsuccessful especially when the area of narrowing is at or close to the renal artery ostium. Current balloon angioplasty techniques can achieve initial favorable responses but chronic recurrence (within the first six months) due to treatment site restenosis occurs in greater than 70% of individuals. Expandable intravascular prostheses (stents) have been proposed as a catheter-based solution to the problem of chronic restenosis in patients with ostial renal artery narrowing. We evaluated the acute deployment factors and chronic healing responses after implantation of a metallic tubular-slotted balloon expandable stent in normal sheep renal arteries. We found that careful adjustment of operator technique is required to correctly implant balloon expandable stents in the renal ostium without migration or extension into the aorta. Vasospasm was occasionally encountered but thrombus formation was absent and rapid endothelialization was uniform after correct positioning of stents in the renal artery ostium. We conclude that this study has helped to provide useful operational and pathological insights concerning the use of stents as a treatment modality in patients with renal artery stenosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 04812-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Slotted and Tubular Balloon Expandable Stents Across Vein Graft Anastomotic Sites

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
Richard F. Neville, M.D.	Research Fellow	CB	NHLBI
Yaron Almagor, M.D.	Senior Staff Fellow	CB	NHLBI
Antonio L. Bartorelli, M.D.	Research Fellow	CB	NHLBI
Renu Virmani, M.D.	Chief	PB	AFIR

COOPERATING UNITS *(if any)*

Arm Forces Institute of Pathology Lab/Branch

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.)*

Restenosis at aortocoronary anastomotic sites is a common and difficult problem after standard percutaneous transluminal coronary angioplasty. Endovascular prosthetic devices (stents) have been a proposed solution to maintain an expanded internal scaffold which would resist radial compression and improve anastomotic site patency. The development and testing of such a device would importantly improve long term clinical results after vein graft implantation in the arterial system to bypass stenotic or occluded coronary and peripheral vessels. The current study utilizes an animal model in which reversed saphenous veins are placed end to side in femoral arteries and allowed to mature for eight weeks. Two forms of metallic stents (tubular slotted and coiled designs) were compared after balloon catheter expansion and implantation at proximal or distal anastomotic sites. Follow-up angiography and pathology analysis were performed at different time-points (3, 8, and 24 weeks). From the study we concluded that implantation of stents in vein graft at anastomotic sites results in excellent patency, rare thrombosis, occasional migration which requires careful attention to stent-vessel wall sizing factors, and rapid endothelialization which is almost always complete between 8 and 24 weeks. We believe that these studies indicate that stent implantation across the anastomotic sites may be a practical solution with favorable long term host responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04813-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Ostial Renal Artery Stent Implantation

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
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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)

☐ (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.)*

Renal artery stenosis is an important clinical problem which often results in systemic hypertension and contributes to chronic renal failure with loss of renal mass. Current techniques to treat renal artery stenosis have been generally unsuccessful especially when the area of narrowing is at or close to the renal artery ostium. Current balloon angioplasty techniques can achieve initial favorable responses but chronic recurrence (within the first six months) due to treatment site restenosis occurs in greater than 70% of individuals. Expandable intravascular prostheses (stents) have been proposed as a catheter-based solution to the problem of chronic restenosis in patients with ostial renal artery narrowing. We evaluated the acute deployment factors and chronic healing responses after implantation of a metallic tubular-slotted balloon expandable stent in normal sheep renal arteries. We found that careful adjustment of operator technique is required to correctly implant balloon expandable stents in the renal ostium without migration or extension into the aorta. Vasospasm was occasionally encountered but thrombus formation was absent and rapid endothelialization was uniform after correct positioning of stents in the renal artery ostium. We conclude that this study has helped to provide useful operational and pathological insights concerning the use of stents as a treatment modality in patients with renal artery stenosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04814-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Fluorescence-Guided Laser Angioplasty in Peripheral Vascular Disease

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
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Robert F. Bonner, Ph.D.	Senior Investigator	BEIB	NHLBI
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COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Lab Branch

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.)*

A new dual laser system utilizing fluorescence spectroscopy as a guidance modality for pulsed dye laser tissue ablation has been studied in 70 patients with femoropopliteal occlusions as part of a cooperative multi-center evaluation. This device, originally conceived and developed as a product of joint NIH-industry research is now being used in 10 centers in the United States and France. The system is unique among laser angioplasty devices in that real-time guidance of the treatment laser is provided utilizing florescence spectroscopic identification of proposed target sites which should greatly improve recanalization efficacy and reduce the frequency of transmural perforations. The patients treated in this study had femoropopliteal occlusions of varying lengths which could not be treated successfully using standard balloon angioplasty techniques. In this patient cohort, primary recanalization using the single fiber laser device was successful in 81% of patients followed by successful balloon angioplasty in 74% of patients. Their were no major complications and florescence spectroscopy was helpful in identifying thrombus, in situations of flush main channel occlusions terminating in a large branch collateral vessel, and under circumstances when eccentric fiber position approached underlying media threatening vessel wall perforation. We believe that this advanced laser angioplasty system with real-time guidance capabilities will be an important tool in the treatment of coronary and peripheral vascular disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04815-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Seeding of Intravascular Stents with Genetically Engineered Endothelial Cells

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

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Richard F. Neville, M.D.	Research Fellow	CB	NHLBI
James A. Zwiebel, M.D.	Research Staff Fellow	MHB	NHLBI
Scott M. Freeman, M.D.	Research Staff Fellow	MHB	NHLBI
W. French Anderson, M.D.	Chief	MHB	NHLBI

COOPERATING UNITS *(if any)*

Molecular Hematology 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 ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Stents are being used as a clinical device to maintain an effective internal scaffold within vascular structures. However, the ultimate utility of stent implantation maybe limited by adverse early and late biological responses. Both subacute thrombotic closure after stent placement and neointimal proliferation have resulted in untoward clinical results in some patients. In an effort to improve versatility and function of stents molecular biology techniques are being considered to reduce surface thrombogenicity and evoke more favorable biological responses. Since animal studies have indicated that rapid stent endothelialization markedly reduces early thrombus formation, this study was undertaken to seed metallic stents with genetically engineered endothelial cells incorporating the gene for either bacterial B-galactosidase or human tissue plasminogen activator. This work, supervised and performed in close collaboration with the Molecular Hematology Branch, indicated that seeding stents with genetically engineered endothelial cells was indeed feasible. These preliminary findings suggest the possibility that modification of the stent surface with partial or complete endothelial cell coverage might result in an environment less susceptible to thrombus formation with the potential for local delivery of pharmacologic substances which might ultimately improve stent function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04816-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fluorescence-Guided Laser Angioplasty Using Multifiber Laser Catheters

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
Antonio L. Bartorelli, M.D.	Research Staff Fellow	CB	NHLBI
Robert F. Bonner, Ph.D.	Senior Investigator	BEIB	NHLBI
Richard F. Neville, M.D.	Research Fellow	CB	NHLBI
Yaron Almagor, M.D.	Research Fellow	CB	NHLBI

COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Branch

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.)*

Previously, we had developed a fluorescence-guided angioplasty system utilizing fluorescence spectroscopy to direct a pulse dye laser for selective atheroma removal. However, this device was limited in clinical application because the delivery system comprised a single small diameter laser fiber wire which resulted in primary channel recanalization but required subsequent balloon angioplasty for definitive luminal expansion in all cases. Therefore, it became necessary to develop a larger catheter-based system which would provide definitive atheroma removal with large surface area recanalization of stenotic or occluded coronary and peripheral vessels. Concentric and eccentric multifiber design catheters with a working through lumen for guide-wire placement were tested to asses fluorescence spectroscopic sensing and tissue ablation parameters. These catheters were 4.5 French for coronary use and 6.5 French for peripheral artery use. Trackability and flexibility were compatible with easy in vivo transcatheter application. After extensive in vitro testing utilizing human ecropsy atherosclerotic material, we concluded that a multifiber fluorescence-sensing laser catheter still provides reliable tissue recognition and accurately guides plaque ablation by a 480 nM pulse dye laser. Parameter studied included changes in computer algorithms for composite sensing of heterogeneous sites, photobleaching phenomena, florescence changes associated with thermal tissue effects, and requirements to elicit a homogeneous large area crater from multiple small fibers. These basic evaluations have provided the impetus for several design changes which we expect will result in a working catheter design for clinical studies in the near future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04817-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Coronary Stent Implantation in Patients After Multiple PTCA Restenosis

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
Yaron Almagor, M.D.	Research Staff Fellow	CB	NHLBI
Fayaz A. Shawl, M.D.	Chief	IC	WAH
Antonio L. Bartorelli, M.D.	Research Fellow	CB	NHLBI
Richard F. Neville, M.D.	Research Fellow	CB	NHLBI
Richard O. Cannon, III, M.D.	Senior Investigator	CB	NHLBI

COOPERATING UNITS *(if any)*

Washington Adventist Hospital, Washington, D.C.

LAB/BRANCH
Cardiology Branch

SECTION
Cardiovascular Diagnosis Section

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.)*

Metallic intracoronary stents have been proposed as a treatment alternative for patients with episodes of recurrent restenosis after standard balloon angioplasty (PTCA). Assuming a component of restenosis pathophysiology is elastic recoil of the vessel segment, a permanent implantable scaffold should retain expanded lumen dimensions and prevent hemodynamically significant narrowing of the vessel. To test the hypothesis that intracoronary stent implantation prevents restenosis in patients after standard PTCA, we studied the acute and long-term responses in a group of patients (n=21) who have had at least 2 episodes of previous restenosis. Intracoronary stents were placed to cover sites of previous restenosis after standard primary angioplasty. Although some degree of neointamal proliferation was present in most follow-up angiograms (at 4-6 months after stent implantation), clinical restenosis was not observed and angiographic restenosis was noted only in 1 of 10 patients during the follow-up period. Although this represents a preliminary report, we are encouraged that stent implantation in the setting of recurrent restenosis after standard PTCA maybe a beneficial treatment modality to improve long-term vessel patency and maintain clinical stability.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04818-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Flow Turbulence Induced by Coronary Stents

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
Jon A. Peacock, M.D.	Research Staff Fellow	CB	NHLBI
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Antonio L. Bartorelli, M.D.	Research Fellow	CB	NHLBI
Yaron Almagor, M.D.	Research Staff Fellow	CB	NHLBI

COOPERATING UNITS *(if any)*
Biomedical Engineering and Instrumentation Branch

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)

☐ (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 metallic intravascular stents have been placed in patients with peripheral and coronary artery disease, there are no data describing fluid mechanics changes which would predict for favorable or adverse clinical responses. We have hypothetical concerns that placement of multiple stents (either in tandem or non-tandem fashion) may affect flow and large side-branches especially in the setting of either proximal or distal disease. To define quantitative parameters examining flow turbulence and wall sheer stress across stented endovascular surfaces would be helpful in predicting future design modifications and in anticipating potential adverse anatomic or clinical stent placement variables. Therefore, an in vitro pulse duplicator system was developed which reproduces coronary flow waveforms using a glycerin and water mixture equivalent to blood viscosity as the test fluid. Hot film probes and laser doppler techniques were used to assess turbulence and wall shear stress in these artificially constructed vascular tree models before and after stent implantation. These studies demonstrated that multiple stents and especially disease in a vessel segment proximal to the site of stent placement results in significance flow instability which might correlate with adverse short-term responses, including increase thrombogenicity and deleterious chronic affects such as intimal proliferation which might result in chronic restenosis. Further modeling techniques are being used to expand upon this design concept such that we can make more definite analyses and predictions of flow turbulence after stent implantation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04819-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Intravascular In Vivo Catheter-Based Ultrasound

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
Antonio L. Bartorelli, M.D.	Research Fellow	CB	NHLBI
Richard F. Neville, M.D.	Research Fellow	CB	NHLBI
Yaron Almagor, M.D.	Research Staff Fellow	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:
.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.)*

Standard imaging modalities of small vessels have inherent limitations and cannot characterize cross-sectional wall geometry or transmural vessel wall pathology. Therefore, the use of miniaturized intravascular ultrasound has become an emerging imaging technique to characterize artery wall morphology and to monitor transcatheter procedures. We are working with a variety of miniature prototype flexible catheters (1.5mm) capable of generating high radial resolution ultrasound images after placement within the vessel. To study the feasibility and utility of this new imaging system, arteries were imaged in sheep and vessel wall dimensions were correlated with contrast angiography. In addition, ultrasound examination of metallic stents after implantation was performed in an effort to define ultrasound factors which might help in assessing the adequacy of stent implantation. We found a striking correlation between angiographic and ultrasound vessel wall diameter but noted important additional information from ultrasound including cross-sectional area, lumen geometry, transmural thickness and appearance, and pulsatile changes during the cardiac cycle suggesting vessel wall compliance. Metallic stents were easily visualized and several factors relating to stent placement might be of clinical importance. The presence or absence of intraluminal filling defects, the adequacy of circumferential stent expansion, and stent artery wall contact are all variables which can be identified easily using ultrasound techniques. Therefore, in this preliminary in vivo study we feel encouraged that catheter-based ultrasound imaging techniques will be an important modality to help characterized vessel wall geometry and pathology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 04820-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Subacute Thrombotic Events After Coronary Stent Placement

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

Martin B. Leon, M.D.	Senior Investigator	CB	NHLBI
Yaron Almagor, M.D.	Research Staff Fellow	CB	NHLBI
Antonio L. Bartorelli, M.D.	Research Fellow	CB	NHLBI
Richard O. Cannon, III, M.D.	Senior Investigator	CB	NHLBI
Richard Schatz, M.D.	Director	CR	AHI

COOPERATING UNITS *(if any)*

Arizona Heart Institute

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.)*

The use of balloon expandable metallic intracoronary stents is gaining acceptance as a potential future adjunct to standard angioplasty to 1) prevent abrupt closure, 2) improve acute angioplasty results, and 3) prevent chronic restenosis. However, in a small (5-10%) number of patients immediately after stent implantation, subacute thrombotic closure results in important adverse clinical consequences. Therefore, an understanding of the clinical spectrum and predicted factors of subacute thrombotic events after coronary stent placement would be extremely important. We retrospectively analyzed operational, equipment, pharmacologic, and other treatment variables which might contribute to subacute thrombotic events after stent implantation. The clinical spectrum of subacute thrombotic events includes early onset (range 2-11 days) of acute chest pain with ECG evidence of evolving acute transmural infarction resulting in total closure of the stent treatment site requiring a combination of local thrombolysis and mechanical recanalization using guidewire and balloon techniques. The majority of patients develop either Q-wave or non Q-wave infarctions, but there have been no deaths thus far in the 157 patients analyzed, including 12 subacute thrombotic events. Predicted factors for thrombotic closure included a higher platelet count, small stent size, multiple stent placements, angiographic evidence of thrombus formation immediately before or after stent placement, and the absence of systemic anticoagulation with coumadin. We believe that further critical analysis of this data set will help to define a variety of situational and treatment variables which will importantly reduce the frequency of thrombotic events after stent implantation in the coronary arteries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04821-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Nicardipine on Effort Tolerance in Microvascular Angina

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

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, 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.)*

A subset of patients with angina pectoris despite angiographically normal coronary arteries have limited coronary flow reserve as a consequence of dysfunctional small coronary arteries, a syndrome we have called microvascular angina (MVA). Although calcium channel blockers are useful therapy in the majority of MVA patients, approximately 1/3 continue to have angina. Eighteen MVA patients symptomatic despite trials of commercially available calcium channel blockers participated in a drug trial using a new dihydropyridine derivative, nicardipine. Compared to baseline study off all medications, MVA patients demonstrated approximately one minute improvement in exercise duration and two minute prolongation of time to onset of angina during treadmill exercise testing using the Bruce protocol on nicardipine 30 mg three times daily for three weeks, without any change in myocardial oxygen consumption at peak exercise or peak expiratory flow rate. Sixteen patients subsequently participated in a randomized, double blind study of nicardipine 30 mg three times daily and identical appearing placebo, each for one month. Two patients developed generalized macular rashes on nicardipine and were withdrawn from this phase of the study. The remaining fourteen patients demonstrated no significant differences in treadmill exercise duration and myocardial oxygen consumption or symptom end-point between nicardipine and placebo phases. Thus nicardipine was of demonstrable benefit to only a minority of MVA patients who were symptomatic failures on conventional calcium channel blockers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04822-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Airways Obstruction and Exercise Capacity in Patients with Heart Disease

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

Richard O. Cannon, M.D.	Senior Investigator	CB	NHLBI
William H. Schenke	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, 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.)*

Effort dyspnea is common in patients with heart disease and is generally considered to be due to left ventricular systolic or diastolic dysfunction. To investigate a possible contribution of airways obstruction, maximum oxygen consumption was measured during, and peak expiratory flow rate measured before and immediately following treadmill exercise in 25 patients with coronary artery disease, 39 patients with microvascular dysfunction (microvascular angina) and 17 normal controls, all non-smokers. Rest and exercise ejection fraction was measured in patients by radionuclide angiography. Compared to normal volunteers, both men and women with microvascular angina and coronary artery disease had reduced maximum oxygen consumption during exercise. In women, the exercise peak expiratory flow rate was also significantly less than men, demonstrating an actual fall in peak expiratory flow rate compared to that measured at rest. The maximum oxygen consumption correlated with peak expiratory flow rate during exercise for both men and women, but did not correlate with the change in ejection fraction. Thus increased airways resistance independent of left ventricular systolic function contributes to exercise limitation in many patients with heart disease, particularly women. This may result from pulmonary congestion as a consequence of diastolic dysfunction. However, the data are also compatible with reflex bronchial changes during exercise, a phenomenon not seen in normal controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04823-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Significance of 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, M.D.	Senior Investigator	CB	NHLBI
Robert O. Bonow, M.D.	Senior Investigator	CB	NHLBI
Patrick O'Gara, M.D.	Visiting Fellow	CB	NHLBI
James E. Udelson, M.D.	Visiting Fellow	CB	NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cardiology Branch

SECTION
Cardiovascular Diagnosis Section

INSTITUTE AND LOCATION
NHLBI, 9000 Rockville Pike, Building 10, Room 7B15, 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.)*

Thallium scanning has been used during exercise to identify regions of relative myocardial hypoperfusion and myocardial ischemia in patients with coronary artery disease. Our group has previously found that the majority of patients with hypertrophic cardiomyopathy (HCM), both symptomatic and asymptomatic, have thallium myocardial perfusion defects during exercise that reperfuse with subsequent rest. To ascertain whether reversible thallium perfusion defects identify HCM patients with inducible myocardial ischemia, 22 HCM patients (18 men, 4 women; average age 44) with normal epicardial coronary arteries underwent treadmill exercise thallium scanning and, during the same week, measurement of arterial and great cardiac vein lactate and oxygen content, great cardiac vein flow and left ventricular end-diastolic pressure at catheterization. Seven of the patients were restudied after operation for left ventricular outflow obstruction. In a total of 29 patient-studies, 19 showed one or more reversible thallium scanning defects. Of these 19 patients, 16 had myocardial lactate extraction less than or equal to 0 mmol/liter during rapid atrial pacing, metabolic evidence of severe myocardial ischemia. In contrast, only 2 of 10 patients without thallium scanning defects had metabolic evidence of myocardial ischemia during rapid atrial pacing. Patients with apparent cavity dilatation (indicative of endocardial hypoperfusion) on exercise thallium scanning were found to have significantly higher left ventricular diastolic pressures at termination of pacing compared to those without this perfusion defect. Thus, symptomatic HCM patients with thallium scanning defects are commonly found to have evidence of myocardial ischemia during rapid atrial pacing, indicating that thallium scanning can identify patients with inducible myocardial ischemia. Compressive effects of left ventricular filling pressures on the endocardium probably contribute to worsening ischemia and apparent cavity dilatation because of endocardial hypoperfusion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04824-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Effect of Surgery for Hypertrophic Cardiomyopathy on Exercise Hemodynamics

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

Richard O. Cannon, M.D.	Senior Investigator	CB	NHLBI
Stephen E. Epstein, M.D.	Chief	CB	NHLBI
Charles L. MacIntosh, M.D.	Senior Surgeon	SB	NHLBI

COOPERATING UNITS (if any)
Surgery Branch, NHLBI

LAB/BRANCH
Cardiology Branch

SECTION
Cardiovascular Diagnosis Section

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.)

Surgery for obstructive hypertrophic cardiomyopathy (septal myotomy/myectomy or mitral valve replacement) improves symptoms in many, but not all patients with hypertrophic cardiomyopathy. To assess which hemodynamic changes before and following surgery best correlate with exercise benefit following surgery, 14 patients with hypertrophic cardiomyopathy underwent treadmill exercise testing (Bruce protocol) with measurement of maximum oxygen consumption before and 6 months following surgery. The post-operative exercise study demonstrated improved exercise duration but with only a marginal improvement in maximum oxygen consumption. The improvement in maximum oxygen consumption following surgery compared to the preoperative study correlated directly with the magnitude of reduction in left ventricular outflow gradient and left ventricular end-diastolic pressure. The 5 patients with no improvement in maximum oxygen consumption, including 2 with greater than 50 mmHg gradient reduction, had less than 5 mmHg reduction in left ventricular end-diastolic pressure. Seven of the 9 patients with increased post-operative maximum oxygen consumption had a gradient reduction greater than or equal to 65 mmHg; the 2 with lesser gradient reduction had greater than 8 mmHg reduction in left ventricular end-diastolic pressure. Thus, benefit in exercise capacity following surgery may be determined as much by the reduction in the left ventricular filling pressures as by the magnitude of gradient reduction following surgery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04825-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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, M.D.	Senior Investigator	CB	NHLBI
Arshed Quyyumi, M.D.	Senior Investigator	CB	NHLBI
Lameh Fananapazir, 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, 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.)*

The presence and severity of chest pain in patients does not always correlate with the presence and magnitude of myocardial ischemia. To test the hypothesis that increased myocardial sensitivity may contribute to cardiac pain, 3 groups of patients with chest pain and one group with heart disease but without a clinical history of chest pain were studied during diagnostic cardiac catheterization. We determined whether or not their typical chest pain was of non-ischemic etiology by probing the right atrium and right ventricle with a 6F pacing wire, and pacing from both sites at 5 beats per minute greater than their basal heart rate with a pacing stimulus starting at 1 milliamps, and increasing to 10 milliamps. Patients with chest pain syndromes but normal coronary arteries demonstrated a high incidence of typical chest pain provoked by pressure or pacing stimulation of the right heart (14 out of 16 patients) as did a smaller number of patients with hypertrophic cardiomyopathy (6 out of 16). In contrast, none of 14 coronary disease patients and none of 6 patients with valvular heart disease but without a history of chest pain demonstrated endocardial sensitivity. We conclude that in patients with chest pain despite normal coronary arteries, heightened intracardiac nociception may be of importance to the genesis of their pain syndrome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04826-01 CB

PERIOD COVERED: October 1, 1988 - September 30, 1989

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

Investigation and 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, MRCP (UK), MD
Senior Investigator
Director of Clinical Electrophysiology Laboratory
Cardiology Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Cardiology Branch

SECTION: Clinical Electrophysiology

INSTITUTE AND LOCATION: NHLBI, 9000 Rockville Pike, Building 10, Room 7B15, 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.)*

Sudden cardiac arrest and syncope are serious complications in hypertrophic cardiomyopathy (HCM) patients. Hemodynamic and electrophysiologic (EP) studies were therefore performed in 155 HCM patients to risk stratify patients, determine the nature and mechanisms of their arrhythmias and assess therapeutic strategies for arrhythmia control and prevention of sudden cardiac death and syncope.

EP abnormalities, often multiple, were found in 78% of patients and provided valuable insights into the causes of cardiac arrest and syncope. Significant EP findings that guided therapy were atrial arrhythmias, His-Purkinje conduction disease and ventricular tachycardia (VT). Although VT accounted for most episodes of cardiac arrest, a variety of other mechanisms - sinus node disease, heart block and myocardial ischemia and supraventricular tachycardia resulting in severe hypotension, amenable to therapy were recorded in the remaining patients. The findings of polymorphic VT (PVT) as the cause of an episode of sudden cardiac arrest, inducibility of sustained PVT in 75% and 50% of cardiac arrest survivors and syncope patients, respectively, but only in 20% of asymptomatic patients, and intracardiac electrographic recordings during induced PVT indicated that PVT is an important reentrant VT in HCM patients.

Frequent aggravation of VT and distal conduction disease by antiarrhythmic drugs suggest that these drugs should not be used empirically. Type 1A antiarrhythmic agents - procainamide and quinidine were useful in the management of atrial arrhythmias but not VT. Therapy with type 1c drugs - flecanide and encanide was abandoned due to their proarrhythmic effects. In selected patients control of arrhythmias was achieved with amiodarone but some of these patients required a pacemaker. The automatic defibrillator was implanted in 15 patients with sudden cardiac arrest (N=13) and syncope (N=2) combined with surgical relief of left ventricular obstruction in 6 patients. There has been no deaths and 5 appropriate discharges have been recorded in 3 patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04827-01 CB

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.)*

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, Special Volunteer, CB, NHLBI
Beate H.B. Scheffknecht, Special Volunteer, CB, NHLBI
Stephen L. Bacharach, Ph.D., Physicist, DNM, CC
Alberto Cuocolo, M.D., Fogarty Fellow, 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:
2.0	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.)*

In many patients with coronary artery disease (CAD), impaired left ventricular (LV) systolic function at rest arises on the basis of regionally ischemic or hibernating myocardium rather than irreversibly infarcted myocardium. However, the identification of such potentially reversible LV dysfunction has been problematic. Many exercise-induced thallium-201 defects do not normalize on subsequent redistribution images, even when the underlying myocardium is viable rather than infarcted. We hypothesized that reinjection of Tl-201 at rest immediately after the standard 4-hour redistribution image would facilitate late uptake of Tl-201 and better distinguish between viable and infarcted myocardium. We studied 73 patients with CAD by standard exercise Tl-201 SPECT imaging. Of 179 abnormal myocardial segments during exercise, 64 (36%) were read as persistent or "fixed" defects on the redistribution image. However, 34 of these "fixed" defects (53%) demonstrated improved or normal uptake after reinjection of Tl-201. That the late uptake of Tl-201 demonstrated in this manner does represent viable myocardium is substantiated by data in two subgroup of patients. 1) In 18 patients restudied 3-6 months after revascularization, normal Tl-201 uptake both at rest and during exercise after revascularization, along with improved regional wall motion by radionuclide angiography, was observed in those myocardial segments with fixed defects on redistribution studies identified as viable by reinjection studies before revascularization. 2) In 12 patients studied by PET imaging with fluorodeoxyglucose (FDG) and ^{15}O water, segments with increased Tl-201 uptake after reinjection also had an increased ratio of FDG:^{15}O uptake, compatible with underperfused but viable myocardium. These data indicate that myocardial segments with improved Tl-201 uptake after reinjection of the isotope indeed represent viable but jeopardized myocardium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04828-01 CB

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.)*
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: Barry J. Maron, M.D., Senior Investigator, CB, NHLBI
Richard O. Cannon, M.D., Senior Investigator, CB, NHLBI
Vasken Dilsizian, M.D., Special Volunteer, CB, NHLBI
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:
1.5	1.4	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.)*

Many of the clinical manifestations of hypertrophic cardiomyopathy (HCM) result from impaired left ventricular (LV) diastolic function. Using radionuclide angiographic techniques, we have demonstrated that impaired LV relaxation and diastolic filling is evident in roughly 80% of HCM patients. We have also shown that verapamil therapy enhances LV relaxation, regional and global LV filling dynamics, and LV diastolic pressure-volume relations in the majority of patients with HCM. These improved indices of LV diastolic function by verapamil are significantly associated with reduced symptoms and improved effort tolerance. In contrast, beta blockers appear to have no important effects on LV diastolic performance in HCM. We have recently investigated the effects of beta adrenergic stimulation on LV diastolic properties. We demonstrated that isoproterenol increases the rate and extent of LV relaxation in patients with obstructive HCM, leading to improved pressure-volume relations,. Of note, improved diastolic function with isoproterenol occurred despite worsening of myocardial ischemia, which would be expected to reduce the rate and extent of LV relaxation and aggravate the LV pressure-volume relationship. We have also reported recently that exercise-induced myocardial ischemia (as assessed by thallium-201 SPECT) develops in over 2/3 of patients with HCM, and that this evidence of ischemia may be reduced (or prevented entirely) by verapamil therapy. Indices of LV diastolic function correlate with the extent and severity of myocardial ischemia, and both LV diastolic dysfunction and inducible ischemia are related significantly to the magnitude of LV hypertrophy as assessed by echocardiography.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04829-01 CB

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.)
Natural History of Mildly Symptomatic Coronary Artery Disease

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

COOPERATING UNITS (if any)

LAB/BRANCH: Cardiology Branch

SECTION: Nuclear Cardiology Section

INSTITUTE AND LOCATION: National Heart, Lung, and Blood Institute

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.)

We have previously demonstrated that left ventricular (LV)ejection fraction (EF) at rest and during exercise stratifies patients with mildly symptomatic coronary artery disease (CAD) according to the risk of multivessel CAD and of death during the course of medical therapy. To determine if subsequent changes in EF with time signify important changes in coronary anatomy, we studied 90 mildy symptomatic pts with two coronary angiograms spanning 2-12 yr (mean 5 yr) of medical therapy and with serial radionuclide angiograms at rest and during exercise every 1-2 yrs, after withdrawal of all anti-ischemic drugs. We observed that progression of CAD by coronary arteriography was rare in the absence of changes in LV function at rest or exercise. However, progressive CAD developed in the majority of patients manifesting either a reduction in resting EF or a deterioration in the EF response to exercise, even in the absence of progression of anginal symptoms. In these latter patients, there was also a high likelihood of the developnment of new total coronary artery obstructions or new left main stenoses.

These data allowed us to address another issue that has been raised in mildly symptomatic patients with CAD and inducible ischemia. We found that it was rare for LV function at rest to deteriorate with time in the absence of a new 100% coronary artery obstruction, even in patients with repeated evidence of recurrent episodes of myocardial ischemia. Thus, recurrent myocardial ischemia, in and of itself, does not appear to predispose to the development of LV dysfunction in the absence of anatomic progression of CAD in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 04830-01 CB

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.)*

Promotion of Myocardial Angiogenesis via Intracoronary Infusion of FGF

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 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:
2.2	1.2	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.)*

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 fibroblast growth factor (FGF) to effect myocardial angiogenesis in a canine model, and to direct this process to ameliorate myocardial ischemia. This polypeptide, available to us in large quantities, is a potent stimulator 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, the territory it supplies becomes dependent on collateral vessels, but myocardial infarction generally does not occur as long as the occlusion is gradual.

After 5 weeks, 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 FGF or placebo directly into the LCX. After 5 weeks, resting and maximal myocardial blood flow will again be quantitated. Thus, collateral function can be compared before and after treatment in both groups. Vessels will be examined morphometrically and various hematologic, biochemical, and immunologic parameters will be assessed in the 2 groups. Dogs will be monitored for any potential adverse effects of FGF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04831-01 CB

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.)*

Promotion of Myocardial Angiogenesis via Intracoronary Infusion of FGF

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 Fellow	CB	NHLBI
Matie Shou, M.D.	Visiting Fellow	CB	NHLBI
Stephen E. Epstein, M.D.	Chief	CB	NHLBI

COOPERATING UNITS *(if any)*

Veterinary Resources Branch, NIH

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

INSTITUTE AND LOCATION

NIH, NHLBI, Building 10, Room 7B03

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	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.)*

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 fibroblast growth factor (FGF) to effect myocardial angiogenesis in a canine model, and to direct this process to ameliorate myocardial ischemia. This polypeptide, available to us in large quantities, is a potent stimulator 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, the territory it supplies becomes dependent on collateral vessels, but myocardial infarction generally does not occur as long as the occlusion is gradual.

After 5 weeks, 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 FGF or placebo directly into the LCX. After 5 weeks, resting and maximal myocardial blood flow will again be quantitated. Thus, collateral function can be compared before and after treatment in both groups. Vessels will be examined morphometrically and various hematologic, biochemical, and immunologic parameters will be assessed in the 2 groups. Dogs will be monitored for any potential adverse effects of FGF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04832-01 CB

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.)*

Vasodilator reserve in asymptomatic hypertrophic cardiomyopathy

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

Arshed A. Quyyumi, M.D. Senior Investigator CB NHLBI
Richard O. Cannon, III, M.D. Senior Investigator CB NHLBI
Stephen E. Epstein, M.D. Chief, Cardiology Branch 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:
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.)*

Previous work has demonstrated that pts with chest pain and hypertrophic cardiomyopathy (HCM), or chest pain and normal coronary arteries, may have impaired vasodilator reserve compared to normal individuals. In order to determine if pts with chest pain and nonobstructive hypertrophic cardiomyopathy, who have previously been shown to have impaired vasodilator reserve, differ from individuals with nonobstructive hypertrophic cardiomyopathy who do not have chest pain, we compared myocardial metabolism and coronary vascular hemodynamics in 8 asymptomatic HCM pts with 26 nonobstructive HCM pts with chest pain. Great cardiac vein (GCV) flow, regional coronary vascular resistance, myocardial oxygen consumption and lactate extraction were measured at rest and after pacing at 150 bpm. Compared to symptomatic HCM pts, asymptomatic HCM pts had a greater increase in GCV flow and fall in coronary vascular resistance, for similar increases in myocardial oxygen demand with pacing. More symptomatic HCM pts (42%) had evidence of ischemia and produced lactate with pacing compared to asymptomatic HCM pts (14%). Thus, ischemia and angina in HCM appears to be due, at least partly, to impaired vasodilator reserve, but some asymptomatic HCM pts may also have ischemia which is silent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04833-01 CB

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.)*
The effects of endothelium-derived relaxant factor on coronary resistance vessels

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

Arshed A. Quyyumi	Senior Investigator	CB	NHLBI
Richard O. Cannon, III, M.D.	Senior Investigator	CB	NHLBI
Julio Panza, M.D.	Guest Researcher	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch		NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Cardiology Branch

SECTION
Experimental Physiology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD

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 have previously demonstrated that the forearm vasculature of hypertensive patients is less responsive to the effects of infused acetylcholine (Ach) which causes the endothelium to release endothelium-derived-relaxant factor (EDRF). In this study, we investigated the effect of Ach on the endothelium lining of coronary conductance and resistance vessels in patients with various chest pain syndromes.

The effects of intracoronary Ach on epicardial coronary diameter, flow and coronary resistance in 16 pts with normal epicardial coronary arteries and chest pain, and those with normal epicardial coronary arteries and hypertrophic cardiomyopathy were studied. Epicardial coronary artery diameter did not change significantly. However, GCV flow increased and coronary vascular resistance decreased incrementally to Ach. At 10^{-8} molar dose of Ach, GCV flow increased by 12±3%; at 10^{-7} molar, GCV flow increased by 34±6%; at 10^{-6} molar dose, it increased by 49±9% compared to baseline. This suggests that endothelium-dependent dilation of coronary resistance vessels occurs independent of epicardial coronary artery changes. Thus, it is conceivable that the endothelium may regulate coronary resistance and flow in man. It remains to be determined whether abnormalities of vasodilatation of resistance vessels my be secondary to endothelial dysfunction, and thus contribute to myocardial ischemic syndromes in some pts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04834-01 CB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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, MD	Senior Investigator	CB	NHLBI
Paolo Spirito, MD	Guest Researcher	CB	NHLBI
Jannet F. Lewis, MD	Guest Researcher	CB	NHLBI
Antonio Pelliccia, MD	Guest Researcher	CB	NHLBI

COOPERATING UNITS *(if any)*

Howard University Hospital; 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 04835-01 CB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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, MD	Senior Investigator	CB	NHLBI
Paolo Spirito, MD	Guest Researcher	CB	NHLBI
Jannet F. Lewis, MD	Guest Researcher	CB	NHLBI

COOPERATING UNITS *(if any)*

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. 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.

Annual Report of the Clinical Hematology Branch
National Heart, Lung, and Blood Institute
October 1, 1988 to September 30, 1989

The research of this Branch is directed toward understanding the underlying causes and developing effective treatment for major hematological disorders, including thalassemia, sickle cell anemia, and various syndromes of bone marrow failure and myelodysplasia. The scope of our work is broad and includes basic study of the molecular mechanisms of gene regulation and extends to applied clinical trials of specific therapeutic agents. Modern methods of molecular and cell biology, including recombinant DNA technology, are utilized in this comprehensive approach to disease mechanisms and therapy. The following are several highlights of progress made in our research during the past year.

Genetic Therapy for Hemoglobin Disorders

Gene replacement as therapy for severe beta thalassemia and sickle cell anemia is a direct goal of our research efforts. Required is the introduction of a globin gene into bone marrow hematopoietic stem cells and subsequent specific, high level expression of that transferred gene in red cell precursors. Retroviral mediated gene transfer is the most efficient method of gene transfer available. Prior studies had indicated that approximately 1 in 10 mouse hematopoietic stem cells could be infected by an appropriately designed retroviral vector, but similar strategies were completely unable to effect gene transfer into primate hematopoietic stem cells. Furthermore, the transfered human globin gene in mouse red cell precursors was expressed at a very low level. Our work is focused on the low efficiency of gene transfer and the low level of protein expression.

1) Use of Hematopoietic Growth Factors to Facilitate Retroviral Mediated Transfer: Survival and proliferation of hematopoietic stem and progenitor cells in vitro is required for efficient gene transfer. We have devised assays to systematically explore the effects of hematopoietic growth factors on these parameters. Interleukin-3 (IL-3) has been shown to be absolutely required to support mouse stem cells in vitro. The repopulating potential of bone marrow cultured for 4-6 days in the presence of IL-3 is ten-fold greater than bone marrow cultured without the growth factor. Interleukin-6 (IL-6) is ineffective alone but augments the ability of IL-3 to support early stem and progenitor cells. The multi-potential progenitor capable of giving rise to spleen colonies (CFU-S) can be induced to proliferate in vitro in the presence of IL-3 and IL-6. Retroviral mediated gene transfer into these cells was increased 10-fold by addition of growth factors to the medium. The two growth factors markedly increased the efficiency of gene transfer into repopulating stem cells.

These results are important for two reasons. First, we have identified IL-3 and IL-6 as important components of any culture medium designed to sustain stem cells during retroviral-mediated gene transfer. Second, we have devised an assay system in mouse that will be useful in a systematic effort to define optimal conditions for gene transfer. Our work in primates indicates that findings in the mouse system can be directly extended to higher animals.

2) Gene Transfer into Primate Stem Cells: We have succeeded in introducing genetic information into primate repopulating hematopoietic stem cells by applying knowledge gained from the mouse assays in constructing culture medium that included primate IL-3 and IL-6. We have devised a strategy for identifying retroviral producer clones that generate a concentration of retroviral particles orders of magnitude higher than those commonly used. Experience in the murine system had indicated that the rate of production of virus is one of the most important parameters in achieving successful infection. A concentration of viral particles of 10^7/ml of culture medium appeared adequate for the murine system. Coculturing producer clones of ecotrophic (mouse) and amphotrophic (broad specificity, including human) results in successive rounds of viral infection. The recombinant proviral genome is amplified. Subsequent screening and selection of individual clones has identified several that generate titers of more than 10^{10} viral particles/ml of culture. We have shown that concentrations of virus in this range will transfer genes into primate hematopoietic stem cells whereas those in the range of 10^7 will not. These findings are generally applicable to subsequent viral vector development since we have devised a general strategy for identifying "super high titer" producer clones and demonstrated their utility in achieving transfer into primate stem cells.

Regulation of Hemoglobin Switching

Patients with either severe beta-thalassemia or sickle cell anemia could benefit from increased production of fetal hemoglobin. At the gene level the switch reflects turn-off of the gamma globin genes and turn-on of the beta globin genes. If both beta genes are defective, the switch leads to the onset of hematologic disease. The beta and gamma genes are part of a multi-gene cluster on chromosome 11 that extends over 60,000 base pairs. This complex includes the epsilon globin gene that is at the 5′ end of the cluster. Next in line are the gamma globin genes (a duplicated locus) and the delta and beta genes.

Human globin genes exhibit tissue specificity in that they are expressed only in red cell precursors, developmental specificity in that individual genes are expressed at particular stages of ontogeny, and maturation specificity in that gene expression increases dramatically during progression from pro-erythroblasts to the later stages of erythropoiesis. Regulation of the globin genes is achieved by proteins that bind to DNA with sequence specificity (trans-acting factors). These proteins bind to cis-acting elements within and flanking the cluster that interact to modulate gene expression.

1) Identification of a Powerful Enhancer that Controls Globin Gene Expression during Erythroid Maturation: At the 5′ end of the beta-globin gene cluster, upstream from the epsilon gene, are found several cis-acting elements that appear to have critical roles in tissue specific expression of the globin genes. These elements, distributed over 15 kilobases of DNA, can be identified in isolated nuclei by their selective sensitivity to nuclease. Collectively these four hypersensitive sites are referred to as the "dominant control" or "locus activating" region (LAR). Naturally occuring deletion mutations in human chromosomes that remove the LAR but leave the globin genes intact nonetheless completely inactivate the cluster. Furthermore the LAR region confers position-independent and copy

number-dependent expression of human globin genes in transgenic mice at a level equivalent to that of the endogenous mouse genes.

We have begun to dissect the cis-acting elements within the human beta globin LAR. One of the hypersensitive sites had been shown to contain a powerful enhancer in transient assays. This enhancer increases promoter function regardless of its orientation or distance from the promoter. Deletion mapping analysis has allowed us to localize a 20 base pair segment. A remarkable discovery is that this enhancer is absolutely required for the increase in globin expression that occurs during erythroid maturation. One or two proteins bind to this region with sequence specificity. In cells that are induced to undergo erythroid maturation, a high molecular complex of these proteins appears to form on the 20 base pair enhancer element. Full enhancer activity in cells in which a globin gene has been stably integrated also requires one additional hypersensitive site.

The data outlined above are directly applicable to the design of retroviral vectors. A high level of globin gene expression following retroviral mediated gene transfer will depend on the incorporation of specific cis-acting elements within the retroviral vector. Our progress in mapping the active portions of the LAR can be directly extended to retroviral design as the minimal lengths of active cis-acting elements are defined.

2) Structure of the Gamma Globin Gene Promoter: By systematic mutagenesis in functional testing in a transient assay, we have defined a modular structure of the human gamma globin gene promoter. Several cis-acting elements interact with many trans-acting factors to achieve a high level of globin gene expression. One of these, an erythroid specific factor (NF-E1), has been shown in our experiments to be a powerful positive regulator of the gamma gene promoter and appears to interact directly with general components of the transcriptional complex. A non-deletion form of hereditary persistence of fetal hemoglobin is based on a mutation in one of the potential binding sites for this factor. We have shown that a modular promoter constructed from a cassette containing the region with which this protein interacts, mutated to contain the HPFH mutation, expressed more than 200-fold higher than the wild type gamma promoter in a transient assay.

Autocrine Mechanisms in Leukemogenesis

We have utilized retroviral-mediated gene transfer to introduce interleukin-3 (IL-3) or interleukin-6 (IL-6). Proliferative syndromes arise that reflect the biological spectrum of action of the growth factor and that resemble specific human hematological disorders. Our data suggest that both IL-3 and IL-6 confer a clonal proliferative advantage on hematopoietic cells, perhaps at the stem cell level. By making specific mutations of the IL-3 coding sequences we have shown that this growth factor can interact with its receptor in establishing an autocrine mechanism completely within the cell.

Discovery of a Gene Involved in DNA Mismatch Repair

For the past several years, we have characterized the human dihydrofolate reductase gene. This housekeeping gene is expressed in all cells. During the past year, we have discovered a new gene that is encoded

on the opposite DNA strand from the DHFR gene. The transcriptional start site for this new gene is only 100 base pairs 5' to that of the DHFR gene. Thus their promoters overlap and possibly are regulated by a common cis-acting element. We have molecularly cloned the complementary DNA for this divergently transcribed gene. A computer based search has identified a very high level of anology between this gene product and a bacterial enzyme involved in DNA mismatch repair. This is the first gene for a component of the human DNA repair system that has been molecularly cloned.

The major research and clinical interest of the Cell Biology Section of the Clinical Hematology Branch is directed toward the pathogenesis, pathophysiology, and effective therapy of bone marrow failure states. The hematology service, now located on 8 East, sees a large volume of patients with bone marrow failure syndromes, especially aplastic anemia, myelodysplasia and undiagnosed pancytopenia. In addition, the Branch serves as a reference center for physicians with questions concerning the management of patients with aplastic anemia and for research studies on parvovirus infection of patients.

B19 (Human) Parvovirus

B19 parvovirus was discovered in 1975 and associated with increasing numbers of human diseases. This virus is the etiologic agent of fifth disease, a common childhood rash illness which presents as an arthritis syndrome in adults; some cases of hydrops fetalis; and transient aplastic crisis of hematolytic disease. In studies previously performed in our laboratory, we demonstrated that the virus directly infects and kills hematopoietic progenitor cells. Using human bone marrow explants, the virus has been propogated uniquely in tissue culture, allowing a full description of its molecular biology. We have demonstrated that the virus could persist in humans and cause chronic anemia. Current studies have been directed first at developing a reliable, large-scale source of antigen to make available testing for the presence of antibody to this virus, and second to an understanding of the biology of persistent infection and the treatment of patients with chronic anemia due to this virus.

Testing for antibody to virus has been limited to reference facilities which have collected small quantities of serum from acutely infected patients which contain viral antigen. We have genetically engineered a mamallian cell line by transfection and expression of a portion of the genome of cloned B19 parvovirus. After amplification of the genome, this cell line has been shown to produce large quantities of the capsid proteins which self-assemble into empty capsid structures. Antibody to virus can be detected by immunofluorescence with these cells, and a lysate can completely replace serum as a source of antigen in a capture immunoassay. Large scale and safe production of viral antigens should be possible with this cell line, allowing the commercial development of kits for the detection of antibody to virus. In addition, our current work is directed at defining the packaging signals for genetic material in capsid structures in an attempt to utilize this cell line for gene therapy (see above). The cell line should provide a source of radioactively-labeled capsids for performance of cell surface binding studies.

In clinical studies we have continued to expand the spectrum of persistence. Chronic parvovirus infection has been demonstrated in three

patient populations: congential immunodeficiency syndrome, children with acute lymphocytic leukemia, and patients with AIDS. By in vitro immunologic studies, these groups have been shown to share a similar defect, the inability to produce a neutralizing antibody to parvovirus. Failure to produce neutralizing antibody can be detected in functional assays of erythroid progenitors and by immunoblot analysis. Children with leukemia and adults with AIDS do not produce any antibody. However, the defect in patients with congenital immunodeficiency is more subtle and restricted. In these patients, persistent parvovirus may be the major clinical nmanifestation of their underlying immunodeficient state. In addition, these patients do produce an antibody detected by capture immunoassay, suggesting a qualitative defect in antibody production, perhaps of a class-switch nature. The syndrome of parvovirus infection in patients with AIDS is sterotypical, in that patients present with pure red cell aplasia as the initial manifestation of underlying HIV infection.

Having identified an antibody defect in patients with persistent parvovirus infection, and also having shown that commerical immunoglobulin contain neutralizing to this virus, we have successfully treated patients with persistent parvovirus infection by immunglobulin infusion. Treatment of patients at NIH and directed treatment of patients elsewhere has consistently resulted in complete hematologic remissions in one case of congenital immunodeficiency syndrome, three cases of AIDS, and one patient with acute lymphocytic leukemia. In vitro studies have shown that disappearance of virus from serum is an early phenomena. These provide a novel mechanism for the efficiacy of immunoglobulin treatment and an important therapeutic advance in the approach to anemia.

Using the polymerase chain reaction, we have developed techniques for the assay of parvovirus in fixed tissue specimens as well as in sera. Early results suggested that parvovirus may be present in about 15% of patients with a diagnosis of idiopathic pure red cell aplasia. In addition, parvovirus has been detected by this methods in one patient with apparent Diamond-Blackfan syndrome and in some patients with myocarditis. It is apparent that the full clinical spectrum of parvovirus infection and the variety of the immune response have not yet been defined.

Herpesviruses and Flaviviruses as Agents of Bone Marrow Failure

In others studies in our laboratory, we have linked two other families of viruses to aplastic anemia. In the first, Epstein-Barr virus has been demonstrated in the bone marrow of some patients with aplastic anemia, including those in which the disease follows infectious mononucleosis. In vitro studies have suggested that the virus can directly infect hematopoietic cells, but that it is not cytotoxic to these cells except at very high infective doses. Virus infection has been associated both in clinical studies and in the laboratory with activation of the immune system, specifically, T-cell proliferation, activation, and production of cytotoxic lymphokines. Some patients with Epstein-Barr virus-associated aplastic anemia have been successfully treated by immunosuppression. In one of our patients atypical relapse in Diamond-Blackfan syndrome occurred after infectious mononucleosis. Virus were demonstrated by in situ hybridization in the patient's bone marrow. The patient had a transient response to Acyclovir but an enduring remission with Cyclosporine theapy.

The second family of viruses that has attracted attention are members of the Flaviviridae group. Arboviruses have long been associated with hematodepressive syndromes such as dengue. In recent studies we have shown that dengue and other flavivirus can be efficiently propagated in hematopoietic cells of the bone marrow and hematopoietic cell lines. Infection results in high production of virus as demonstrated by immunofluorescence and molecular studies. But cells infected with flavivirus are not killed, although their growth is significantly inhibited. Entrance of flaviviruses into hematopoietic cells is not enhanced by the presence of subneutralizing quantities of antibody, suggesting that here is unique receptor for this virus on hematopoietic cells. These results are of particular importance for two reasons. First, the immune response in vitro to flavivirus is similar to that observed in vivo with aplastic anemia, namely lymhocyte proliferation and lymphokine, particularly gamma interferon, release. Second, the non-A non-B hepatitis agent (hepatitis C) have recently been shown to be amember of the flavivirus family. This form of hepatitis has been linked to aplastic anemia. Therefore, bone marrow infection with hepatitis C may cause aplastic anemia, presumably by an immune mediated mechanism. If true, this finding would provide a basis for the epidemiologic observation of an increased incidence of aplastic anemia in the Far East compared to the West.

Treatment of Aplastic Anemia and Related Disorders

Anti-thymocyte globulin has been shown in studies conducted by the NHLBI and elsewhere to be effective treatment for aplastic anemia. Unfortunately, production difficulties have limited the quantities of ATG in the United States. We have developed an effective alternative regimen in Cyclosporine, which has resulted in response rates similar to those observed with anti-thymocyte globulin. Ongoing studies with monoclonal antibody directed against human T-cells have not been successful, possibly due to inadequate reduction of T-lymphocyte number. Current studies with GM-CSF have shown that occasional patients with aplastic anemia may respond to this treatment, but that it is ineffective in patients with established fungal infection and severe neutropenia and in the majority of patients on presentation. The relationship between the response to GM-CSF as a predictor for later improvement with immunosuppressive therapy will be the subject of a retrospective analysis. Efforts are underway to institute trials of interleukin-1 in patients with aplasia. Interleukin-1 is of special interest because of its action at an early stage of hematopoiesis and because we have demonstrated that interleukin-1 is absent in patients with severe aplastica anemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 02208-15 CHB

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.)*

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)*

Laboratory of Molecular Hematology, 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, 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.)*

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. Fifty patients, followed for 6 or more years, have been characterized with respect to chelation compliance, cardiac disease, endocrine dysfunction and liver iron concentration. Dramatic improvement in cardiac function has been documented in three patients, treated with intensive intravenous chelation. We have identified patients who have been poorly compliant and those who regularly used Desferal. Several poorly compliant patients have developed heart disease and died whereas the compliant group has remained clinically stable. Thus Desferal appears to prevent the development of heart disease and reverse established cardiac dysfunction in some patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 02307-10 CHB

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.)

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: Timothy Browder, M.D., Senior Staff Fellow, CHB, NHLBI
Amanda Cline, Research Assistant, CHB, NHLBI
Bernhard Maier, Ph.D., Visiting Fellow, 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.)

The two major disorders that affect hemoglobin, sickle cells anemia and severe beta thalassemia may potentially be treated by gene replacement. The introduction of a structurally normal human beta globin gene into the cells of patients with one of these disorders could correction the deficiency of beta chain synthesis in thalassemia or replace the defective beta globin in the hemoglobin of patients with sickle cell anemia. Retroviral mediated gene transfer is the most efficient available means to introduce new genetic material into the eukaryotic genome. We are designing retroviral vectors to carry a human globin gene along with appropriate regulatory elements, into hematopoietic stem cells. We have shown that transfer of the human beta globin gene into mouse stem cells is feasible. Tissue specific expression of the human globin gene in red cells and their precursors has been shown. Secondary recipients of bone marrow from these primary animals continue to express the human globin gene documenting gene transfer into stem cells. Interleukin-3 (IL-3) and interleukin-6 (IL-6) enhance the survival and proliferation of primative hematopoietic progenitors and stem cells in culture and facilitate retroviral mediated gene transfer. Regulatory elements derived from the locus activating region of the human beta globin gene cluster will be added to the vector to achieve a higher level of gene expression in recipient animals. Using an amphotrophic producer clone that yields more than 10^{10} viral particles per ml of culture over 24 hours, we have achieved gene integration into primate hematopoietic stem cells. This is a necessary first step to extend our results with globin gene transfer from mouse to man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02310-09 CHB

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.)*

Characterization 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
Carol Geckle, Biologist, 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Transcripts initiated in the region immediately upstream from the human dihydrofolate reductase (DHFR) gene but transcribed from the opposite strand have been identified in human cells. We have isolated complementary DNA clones derived from the divergent transcripts and identified a 3.5 kb open reading frame in one of these clones. Computer assisted sequence analyses have shown that there is significant amino acid sequence homology between the divergently transcribed gene product and the bacterial mutS protein. The degree of identity is 25% in the entire sequence and 60% in a stretch of 119 amino acids in the carboxy terminal portion. The mutS protein is an essential component in the DNA mismatch repair system. therefore, it is highly likely that this human mutS-like protein (HMS protein) is one of the human DNA mismatch repair enzymes.

The DHFR gene and the HMS gene are organized in a head-to-head configuration separated by a short sequence. Promoter activity in a transient assay, using heterologous reporter genes, has been found within a 165 base pair DNA fragment oriented in either direction. Expression of the two divergent genes is regulated by a bidirectional promoter that may use common regulatory elements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02313-07 CHB

PERIOD COVERED

October 1988 to September 30, 1989

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)*

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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 interacts with several DNA binding proteins. The gamma globin gene promoter has been disected into several cis-acting elements. Each of these interacts with one or more proteins; one of these is erythroid specific and acts as a powerful positive regulator of gamma globin gene expression in test assays. The enhancer sequence immediately downstream from the gamma globin genes contains several sequences that bind this protein. The ultimate goal of this work is to understand in detail the mechanisms by which globin genes are regulated. This may be applicable to the treatment of human disease. Patients with either sickle cell anemia and thalassemia would benefit from reactivation of the gamma globin genes during adult life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02315-07 CHB

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.)*

Viruses and Aplastic Anemia

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

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COOPERATING UNITS *(if any)*

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LAB/BRANCH

Clinical Hematology Branch

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	-

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.)*

Bone marrow failure has clinical and laboratory features that have suggested a possible viral etiology. Illness may follow on a viral infection, in particular infectious mononucleosis or non-A non-B hepatitis. Patients have evidence of activation of their immune system similar to that observed in viral infections. There are also animal models of virus-induced bone marrow failure. Our efforts have concentrated on two families of virus, the herpesviruses (Epstein-Barr virus) and flavivirus (dengue). As an entension of our previous work on Epstein-Barr virus, we have shown that in vitro, using an autologous culture system, Epstein-Barr virus-infected cells incite an immune response of T-cells similar to that observed in vivo, consisting of lymphocyte activation, release of the lymphokine gamma interferon, and increased levels of the soluble form of the interleukin-2 receptor. High concentrations of Epstein-Barr virus are toxic to hematopoietic progenitor cells, and this toxicity may be mediated by the T-cell response. In a second major project we have explored the basis for bone marrow failure in dengue and other associated avbovirus hematodepressive diseases. We have demonstrated that dengue efficiently propagates in normal human bone marrow and also in several hematopoietic cell lines. Dengue is not cytotoxic to cells but alters their proliferative potential. Dengue RNA and proteins are easily detected in infected cells. Entry of virus into hematopoietic cells is not mediated by antibody, as is its entry into macrophages, suggesting that hematopoietic cells have a unique receptor for the flaviviruses. Dengue antigens are also known to induce an immune response consisting of lymphocyte activation and lymphokine release. The recent discovery that the non-A non-B hepatitis virus has the properties of a flavivirus supports a unifying theory for bone marrow failure due to virus infection, in which viral anti-proliferative effects are combined with toxic immune response to produce a marrow failure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02319-06 CHB

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.)*

B19 (Human) Parvovirus

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

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J. Ayub, M.D., Chemist, CHB, NHLBI
T. Shimada, M.D., Visiting Associate, CHB, NHLBI
S. Anderson, Medical Technician, CHB, NHLBI

COOPERATING UNITS *(if any)*

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Communicable Disease Center, Atlanta, Georgia
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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:
3.05	2.3	.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.)*

Our studies have intergrated laboratory features of the novel human pathogen, called B19 parvovirus, with descriptions of clinical diseases resulting from acute and chronic infection. This virus is the cause of fifth disease, a common childhood rash illness; transient aplastic crisis in patients with underlying hemolysis; an arthritis syndrome in adults; some cases of hydropsfetalis; and chronic bone marrow failure due to persistent infection. The virus is extraordinarily specific for human erythroid progenitor cells. In current studies, we have made two important technical advances. First, the polymerase chain reaction has been used to detect the virus with a very high degree of sensitivity in sera of infected individuals. Second, we have engineered a cell line which constitutively produces empty viral capsids, which will be useful in establishing assays for antibody and in the development of a vaccine. As a central reference laboratory, we have assayed a large number of clinical specimens from patients with a variety of illness. We have established that persistent infection with this virus is probably common, especially among children receiving immunosuppressive therapy for acute lymphocytic leukemia; in patients with underlying congential immunodeficiency; and as a presenting illness in patients with acquired immunodeficiency syndrome. Under our direction, several patients have received immunoglobulin therapy with full restitution of erythropoiesis. The site of sequestration of B19 parvovirus in chronic disease is uncertain. Studies in our laboratory with human macrophages have suggested that the virus can associate with this cell but does not replicate in monocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02320-06 CHB

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.)

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
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Griffin Rodgers, M.D., Senior Investigator, LCB, NIDDK
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LAB/BRANCH

Clinical Hematology Branch

SECTION

Molecular Biology

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 (e.g. erythropoietin) and agents that modify DNA or chromatin structure (e.g. 5-azacytidine or sodium butarate, respectively). We have completed a phase II clinical study of hydroxyurea. This agent increased fetal hemoglobin synthesis in 7 of 10 patients with sickle cell anemia. Animal studies have shown that erythropoietin augments HbF synthesis. A new clinical study has been designed combining maximal doses of hydroxyurea with erythropoietin. The significant response rate among sickle cell anemia patients to hydroxyurea has prompted implementation of a study of similar design in patients with thalassemia. Animal studies have shown that IL-3 and GM-CSF, particularly when given in combination, augment the HbF response to hydroxyurea. These data have implication with respect to the design of future clinical studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02330-03 CHB

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.)*

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 Staff Fellow, CHB, NHLBI

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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. The disease status has been ascertained by echocardiography and DNA collected from all relevant members in 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 identified a region on one human chromosome that appears likely to contain the HCM locus human DNA polymorphisms. Our clinical genetic studies have verified that many sporadic cases are likely to have a genetic bases. For example, we have identified a pair of genetically identical twins, one of whom has clinical HCM whereas the other has normal cardiac anatomy and function. In a second family, we have identified a "skipped" generation. Once a closely linked marker to the disease locus is identified it will be possible to do accurate presymptomatic diagnosis and devise refined diagnostic techniques. Furthermore, we should be able to determine whether HCM is a single or multilocus disorder. The ultimate goal is to identify the gene or genes that cause this disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02331-03 CHB

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.)*

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)*

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Timothy Browder, M.D., Senior Staff Fellow, CHB, NHLBI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI
Seiji Hayashi, M.D., COP, DCT, NCI
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

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.)*

Acquired immunodeficiency syndrome is caused by human immunodeficincy 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". There are a number of gene products that might be effective. Anti-sense RNA sequences are generated when portions of the HIV genome are reversed in their transcriptional orientation and expressed at high levels in T-lymphocytes. The anti-sense sequences can form intermolecular duplexes with the HIV genome, potentially blocking RNA processing, translational or replication. We have utilized retroviral mediated gene transfer to develop a system for transfering and expressing HIV anti-sense sequences in T-lymphocyte populations. Unfortuantely, the anti-sense methodology has proved ineffective in modifying HIV infectivity. At present we are exploring two alternative approaches. Expression of the HIV genome culminating in viral production requires action of several trans-acting protein factors that are encoded by HIV. Recently, it has been shown that a mutated form of one of the these factor will block the function of the normal factor. We are currently devising vectors to transfer and express this mutated gene product in T-lymphocytes to determine whether it will significantly inhibit HIV infectivity. A second approach is to express an interferon gene in lymphocytes under the control of the HIV promoter. HIV infection will then induce interferon production perhaps limiting viral replication and spread to adjacent cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02333-02 CHB

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 and Function of the Murine M-CSF 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., Medical Staff Fellow, CHB, NHLBI
Others: Elise Feinfold, Ph.D., Staff Fellow, CHB, NHLBI
Arthur W. Nienhuis, M.D., Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

Molecular Biology

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 receptor for the monocyte-macrophage specific growth factor (M-CSF) is a transmembrane glycoprotein that exhibits tyrosine kinase activity. We are investigating the mechanism by which M-CSF induces signal transduction. The receptor's primary structure has been interrupted at the junction between the external and transmembrane domains through insertion of either a polyglycine tract or a segment of alpha-helix. The specific question being addressed is whether ligand (growth factor) activation of the receptor as an enzyme (tyrosine kinase) occurs via an intramolecular or intermolecular mechanism. The inserted amino acid segments are designed to disrupt any intramolecular conformational signal while allowing intermolecular ligand induced associations to occur. We have found that the modified receptor molecules are capable of M-CSF binding and that the ligand induces both tyrosine auto-phosphorylation of the receptor and a mitogenic response in growth factor dependent cells. These results strongly favor an intermolecular mechanism of ligand induced receptor activation. In a second set of experiments, we have introduced transforming mutations into the M-CSF receptor using site directed mutagenesis of its cDNA. These mutant molecules, when introduced by retroviral mediated gene transfer into hematopoietic stem and progenitor cells, will provide the basis for determining the transforming potential of the activated C-FMS proto-oncogene product in vivo. Finally, the normal M-CSF receptor has been introduced into hematopoietic cell lines that do not usually display this molecule. Our purpose is to determine whether M-CSF will induce a monocyte-macrophage pattern of differentiation in these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02335-02 CHB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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: Cynthia Dunbar, M.D., Medical Staff Fellow, CHB, NHLBI
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Chris Walsh, M.D., Medical Staff Fellow, CHB, NHLBI
David Sachs, M.D., Chief, Immunology Branch, DCBD,NCI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

Immunology Branch, DCBD, NCI

LAB/BRANCH

Clinical Hematololgy Branch, NHLBI

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. Introduction of the interleukin-3 (IL-3) gene into hematopoietic stem cells results in a myeloproliferative syndrome that resembles chronic myelogenous leukemia. Analysis of more than 50 animals with this syndrome has shown that dysregulated expression of the IL-3 gene in cells bearing the receptor for this growth factor results in a substantial proliferative advantage for the abnormal clone but does not alter the self-renewal capacity of stem cells or the maturational characteristics of precursors. Introduction of the interleukin-6 (IL-6) gene by retroviral mediated gene causes a lymphoproliferative syndrome characterized by B-lymphocyte and plasma cell proliferation, hypergammaglobulinemia and decreased serum albumin. The Harvey Ras oncogene causes T-cell lymphomas of thymic origin. This highly malignant neoplasm involves liver, spleen and lymph nodes. These results illustrate the potential for use of defined genetic modifications to create specific neoplasms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02336-02 CHB

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.)*

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)*

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Gail Ozawa, Research Assistant, 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. These growth factors are produced by bone marrow stromal cells such as fibroblasts and lymphocytes. Their action on hematopoietic cells is mediated by high affinity cell surface receptors. There is substantial redundancy in the spectrum of activity of hematopoietic growth factors in that individual factors may act on mature and immature cells of several lineages and there are several factors that have overlapping spectra of activity. One approach to defining the physiological role of an individual growth factor is to create animals deficient in their capacity to provide that factor. We are attempting to use anti-sense RNA sequences specific for the mouse granulocyte macrophage-colony stimulating factor (GM-CSF) to achieve this purpose. The coding sequences for GM-CSF are placed in a reverse transcriptional orientation under the control of a strong promoter in a expression plasmid that includes a lymphocyte specific, dominant control region. The ability of these sequences to inhibit GM-CSF production by cultured lymhocytes will be determined. If the anti-sense strategy proves effective, transgenic animals will be created containing the active transcriptional unit. The lymphocytes of such animals should lack the capacity to produce GM-CSF. Study of these transgenic animals may provide an indication as to the physiological role of GM-CSF in hematopoiesis. A second active area of investigation is directed at the mechanism of signal transduction by hematopoietic growth factors. Each growth factor induces the expression of several "early" genes the products of which are thought to lie on a signal transduction pathway. Our general strategy is to express several of these genes at high level in cells to determine whether the requirement for growth factor is abrogated. In addition, we will determine whether a cascade is operative whereby one gene product induces the expression of other genes.

ANNUAL REPORT OF THE
HYPERTENSION-ENDOCRINE BRANCH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1988 through September 30, 1989

The Hypertension-Endocrine Branch has focused its activities on a number of the neurohumoral and vasoactive systems that control the circulation and are involved in the pathogenesis of hypertension.

I. Catecholamines and the sympathetic nervous system. Over the years, we have developed and applied techniques to measure sympathoadrenal activity in cardiovascular and neurologic diseases, especially hypertension. In particular, we have developed a radiolabelled tracer method which uses regional norepinephrine spillover to indicate regional sympathetic activity. In our preclinical studies this year we have validated this technique by direct comparison with directly recorded sympathetic nerve activity in intact animals. Using this technique we have found that centrally administered corticotropin releasing hormone (CRH) stimulated both the sympathoadrenal and the pituitary adrenocortical systems, but administration of a CRH antagonist did not prevent adrenal medullary responsiveness to insulin-induced hypoglycemia. This indicates that CRH is not the "master stress hormone", as has been proposed in the past. We have also developed a means to visualize cardiac sympathetic innervation and function in vivo using 18 F-fluorodopamine and positron emission tomographic scanning. Fundamental pharmacologic studies showed that after the injection of 18F-fluorodopamine storage vesicles in sympathetic nerve endings were radiolabelled. Analyses of PET time-activity curves provided noninvasive, in vivo qualitative assessments of both exocytotic release of NE and of uptake-1 activity in the heart. This PET imaging technique is a valuable new tool in the assessment of cardiac sympathetic innervation and function and can be applied both to man and experimental animals. In an attempt to understand the differences between salt resistant and salt sensitive hypertensive subjects, we have found that urinary DOPA and dopamine (DA) excretion increased markedly during dietary salt loading in rats, consistent with a role for the renal DOPA-DA system in maintaining sodium homeostasis. The increases could not be attributed to increased DOPA spillover into arterial blood and were present even in rats with denervated kidneys. Therefore, the kidney may synthesize DOPA and DA independently of tyrosine hydroxylase activity during salt loading. We find this an exciting possibility since it flies in the face of accepted dogma and suggests either a separate enzymatic pathway or deconjugation of the large pool of circulating conjugated DA. Results from a previous study, which indicated that there was a post-ganglionic source of cerebrospinal fluid norepinephine (NE), were confirmed in dogs and rats with superior cervical ganglionectomies. In addition, we demonstrated that juvenile, spontaneously hypertensive rats had marked sympathoadrenal hyperreactivity after administration of the alpha 2 adrenoreceptor antagonist, yohimbine. A similar response was demonstrated in hypertensive human beings and may demonstrate an important defect in central neurocontrol of blood pressure in hypertensive subjects.

In our clinical studies, we were able to show that there were similar proportionate physiological increases in antecubital venous NE levels and in directly recorded skeletal muscle sympathoneural activity during nitroprusside-induced hypotension in normal volunteers. These results confirmed that plasma NE levels validly indicate sympathetic activity in man. Dietary salt loading increased urinary DOPA and DA excretion in a parallel fashion. We are currently exploring whether salt-sensitive hypertensive humans have defective renal DOPA-DA responses to salt loading. We demonstrated that sympathoadrenal hyperactivity to yohimbine and to mental challenge occurred in young adult patients with essential hypertension in a fashion similar to that seen in spontaneously hypertensive rats. These studies will now be applied to normotensive offspring of both hypertensive and normotensive parents to determine if this yohimbine-induced response is an early marker for hypertension. Patients with

hypertrophic cardiomyopathy were shown to have evidence of decreased uptake-I activity by means of our tracer-labelled NE spillover studies. Since NE can induce cardiac hypertrophy and uptake-I blockade augments and prolongs the action of NE, decreased uptake-I activity could be a mechanism for development of cardiac and vascular hypertrophy. DOPA is the product of tyrosine hydroxylase, the rate limiting step in catecholamine biosynthesis. We have now shown in man that the heart and brain both release DOPA into the blood stream and that regional catecholamine turnover appears to be reflected by the rate of DOPA release.

Work continued on studies of the mechanisms of secretion, retention and recapture of NE by synaptic vesicles. According to the model that this work has developed, a new uptake and secretion mechanism is formed by the merger of the plasma membrane with the synaptic vesiclar membrane. A recent finding is that manganese, which blocks voltage dependent calcium entry into nerve terminals, also blocks the depletion of stored 3H-NE. In addition, control of the calcium effect seems to be a function of sodium ion. Thus, we find that the response to calcium is delayed by the presence of I2.5 mM sodium in the medium that contains ATP, but not if the sodium is replaced by either potassium or choline. Lithium, in a concentration of I mM, inhibits this response to ATP. Whether the response to sodium involves calcium excretion, sodium potassium ATPase, or binding of membrane to membrane, is not yet known. It will be important to show the applicability of this model to physiologic function and to compare it with the currently proposed models of exocytosis.

II. Vascular smooth muscle cell proliferation. The arteries from hypertensive patients and from spontaneously hypertensive rats have a greater mass of smooth muscle cells (SMC) than similar vessels from normotensive controls. Clearly, something has stimulated this vascular SMC proliferation. We have studied this activity by observing the rate of growth of vascular SMC in tissue culture. We have found that the rate of proliferation of SMC was greater for stroke prone spontaneously hypertensive rats (SHRSP) than for normotensive Wistar-Kyoto rats (WKR). We have found that some antihypertensive drugs such as hydralazine, nifedipine, and verapamil inhibit DNA synthesis of SMC from both WKR and SHRSP, while lower concentrations of both nifedipine and verapamil stimulated DNA synthesis slightly in the same strains of rats. Another antihypertensive agent, sodium nitroprusside (SNP), also inhibits cell proliferation in the GI to S phase. When SNP is added to SMC after stimulation with fetal calf serum, the inhibitory effect on DNA synthesis is greater in SMC from WKR than it is on SMC from SHRSP. SNP inhibited RNA synthesis of SMC from both SHRSP and WKR to almost the same degree. SNP inhibited overall protein synthesis in SMC when administered over the period of 9-I5 hours after stimulation of the cells with fetal calf serum. The inhibition of protein synthesis was significantly greater in WKR than in SHRSP. The SNP may have a cGMP independent mechanism in its inhibition of cellular proliferation and its mechanism of action is currently under study in our laboratory. Indeed, there appears to be more than one mechanism by which antihypertensive drugs inhibit DNA synthesis. These preliminary findings are exciting because they fit with our earlier observations that verapamil, an antihypertensive drug and calcium channel blocker, can reverse myocardial hypertrophy in SHR when given in doses that do not significantly reduce blood pressure.

III. Vasoactive hormones. Atrial natriuretic factor (ANF) is a potent vasodilator, diuretic and natriuretic peptide produced by atrial myocytes. We have studied the release and function of this peptide in a new experimental model of high output congestive heart failure (CHF) in rats. The CHF was produced by a large arteriovenous fistula produced between the aorta and the infrarenal vena cava of rats. This aortacaval fistula (ACF) leads to high plasma levels of ANF and reduced responsiveness to exogenous ANF. About one-third of rats develop uncompensated sodium retention and die from severe CHF. The remaining two-thirds are able to compensate and "escape" from the sodium retaining state. We have previously shown that the rats that cannot escape from the effects of CHF, can be successfully treated with an angiotensin converting enzyme inhibitor, which normalizes their renal function. We now have shown that closure of the ACF causes a similar diuretic and natriuretic effect accompanied by a rapid reduction in plasma renin activity. However, ANF levels remain high for 48 hours and then

decrease gradually. Prior bilateral renal denervation does not change the pattern of urine and sodium excretion, nor does a chronic infusion of angiotensin II in superphysiologic amounts. These findings suggest that ANF is not only a marker of volume overload in CHF, but that it is also an important compensatory mechanism and serves to promote natriuresis even after the primary cause of the CHF is removed.

Brain natriuretic peptide (BNP) is another vasodilator peptide with diuretic and natriuretic properties. The effects of this peptide are abolished in our ACF rats and thus the resistance to BNP in these rats is much more pronounced than that to ANF. It would thus appear that BNP is totally ineffective in our CHF model. The role of BNP in CHF is still unconfirmed, yet it may be that the blunted effects of both BNP and ANF may contribute to the sodium retention in CHF.

Endothelin (ET) is a powerful vasoconstrictor peptide derived from the endothelium. We are studying its properties in normal rats and show that it causes an initial selective vaso-relaxation which is associated with an increase in cardiac output. This reaction is followed within two minutes by a generalized marked vasoconstriction and a decrease in cardiac output that lasts for up to 20 minutes. The various vascular beds respond differently to ET. The renal artery is clearly most sensitive to the vasoconstrictor effects and renal function is severely impaired after ET. In our CHF model, the hemodynamic and renal effects of ET were significantly blunted. This blunted effect could not be reproduced in normal rats given high doses of ANF or angiotensin II along with the ET. Thus we do not have an explanation for the initial vasodilator action of ET, an action which is not found in in vivo muscle strip preparations. Further studies of the function of ET must proceed on the cellular level since this appears to be a pure autocrine action and normally ET does not circulate in the plasma.

IV. Role of proteins and amino acids in hypertension. We have continued our studies of SHRSP fed low, normal and high protein diets. As noted in the past, a low protein diet causes an earlier and more rapid development of more severe hypertension and leads to an earlier and more frequent development of stroke. These manifestations could be either delayed and/or reversed by protein supplementation of the diet, and specifically by the addition of the sulphur-containing amino acid, methionine, to the diet. We have now performed pathologic studies of the organs from these animals and found that, at 10 months of age, the degree of cardiac hypertrophy and fibrosis, the thickening of intramyocardial arterioles, and the presence of inflammatory cells were similar among the experimental SHRSP groups. However, the kidneys of SHRSP on low protein diet demonstrated marked thickening of the vascular walls, narrowing of the lumina of the arterioles, and glomerular sclerosis. Since the morphology of the kidneys from SHRSP fed methionine differed markedly from that of SHRSP fed a standard diet, but was close to that of WKR fed a standard diet, it would seem that it is the increased amount of methionine in the diet which provides protection against the renal complications of hypertension. However, further investigation is required to determine whether this protective action is primarily a result of the dietary effect on blood pressure, or whether the dietary changes influence some metabolic change that slows the rate and degree of tissue damage from prolonged hypertension.

V. Regulation of the dopamine reuptake system. In continuing studies, we have shown that high affinity binding sites for several potent dopamine uptake blockers are specifically located in dopaminergic nerve terminals of the caudate nucleus of the brain and appear to constitute a molecular component of the dopamine carrier. In addition, we now show that the recognition sites for ^{3}H-cocaine in dopaminergic nerve terminals appear to play a physiological role because they can be regulated by receptor-receptor interactions. The destruction of corticostriatal glutamatergic nerve fibers increased the density of ^{3}H-cocaine binding sites, while in washed striatal synaptosomal membrane preparations, the addition of L-glutamate and glycine decreased the number of ^{3}H-cocaine binding sites. In addition, L-glutamate receptor stimulation may also play a role in the depolarization-evoked release of dopamine. K^{+}-evoked dopamine release is facilitated by L-glutamate and is mediated through activity of N-type

calcium channels. In comparison, veratridine-evoked dopamine release requires extracellular calcium, but is not mediated through N- or L-type calcium channels. In future experiments, we will seek to determine whether glutamate receptors present at dopaminergic nigra-striatal nerve fibers can affect post-translational modification via phosphorylation of specific membrane proteins that modulate the dopamine carrier.

VI. Endogenous calcium channel modulator. We have previously extracted from rat brain homogenates and purified to homogeneity a ^{3}H-nitrendipine-displacing material (NDM) that appears to be an endogenous modulator of the calcium channel. We have now shown that NDM inhibits reversibly the depolarization-dependent calcium accumulation in primary cultures of neurons. In voltage-clamped neuronal cells, NDM inhibited the L- and T-type components of calcium channels. In contrast, in cardiac myocytes, NDM enhanced the L-type calcium current in a G protein-independent manner. This enhancing action was not inhibited by alpha- or beta-adrenergic receptor blockers nor by angiotensin II inhibitors. Since the activation of calcium channels elicited by this endogenous modulator in cardiac myocytes was relatively slow, it is suggested that it may interact at sites on the myoplasmic side. It is apparent that NDM modulates calcium channels differently in neurons and in cardiac cells, suggesting that the regulation and selectivity of calcium channels may differ in heart and in brain. The finding that in cardiac cells, when dialyzed with GDP-beta-S, a blocker of G protein, the effect of NDM was not blocked, suggesting that G proteins may not be involved in the signal transduction. Moreover, this suggests that NDM may function as a physiologic regulator of calcium channels by a novel mechanism.

VII. Interaction of prostaglandins and cytosolic calcium in platelet aggregation. Earlier reports that platelet calcium paralleled the blood pressure in man and our own interest in the possible antihypertensive role of prostaglandins led us to study calcium prostaglandin interactions in the platelet. We therefore studied the effects of U46619, a thromboxane mimic, on cytosolic calcium concentration and platelet aggregation in human platelets. Addition of U46619 (10^{-7} M) to the platelet suspension produced a sharp increase in cytosolic calcium, as well as platelet aggregation. Pre-treatment of the platelets with PGI_2, PGD_2, PGE_1, PGF_{2a}, db-cAMP, or forskolin prevented the increase in cytosolic calcium and the associated platelet aggregation induced by U46619. In platelets treated with PGE_2, TMB-8, or verapamil, U46619 produced a much slower increase in cytosolic calcium. Although calcium concentration eventually reached values equal to or greater than those of controls, no platelet aggregation was observed. These data suggest that U46619 induces platelet aggregation through an increase in cytosolic calcium, and that both calcium entry and its release from intracellular storage sites are necessary for the increase in cytosolic calcium. In addition, both the rate of the increase and the magnitude in cytosolic calcium concentration appear to be crucial in platelet aggregation induced by U46619. Our data also suggest that prostaglandins inhibit U46619-induced platelet aggregation by preventing the increase in cytosolic calcium, and that these effects are probably mediated by an increase in c-AMP. These data fit perfectly with results obtained previously in both platelets and chromaffin cells treated with angiotensin II. In these experiments, we demonstrated that not only was an increase in cytosolic calcium necessary for the response to angiotensin II, but that the response was directly dependent upon both the rate of increase as well as the maximum level of cytosolic calcium obtained for the release reaction to occur.

VIII. The biologic role of substance P. Over a number of years we have attempted to demonstrate the biosynthesis, distribution and biologic function of substance P (SP) and its receptors. Antibodies, idiotypic antibodies, and receptor antibodies were prepared by immunizing New Zealand white rabbits with SP, SP monoclonal antibody, and receptors from either olfactory bulb or intestinal mucosa. The total characterization of these antibodies and receptors is currently underway. The distribution of SP receptors in periaqueductal gray tissue in the brain of rats was observed by both autoradiographic techniques with labelled SP ligand as well as with immunocytochemical localization procedures with either idiotypic antibodies or anti-receptor antibodies. These studies have shown that blood vessels have an

unusually high density of SP receptors from the l3th day of gestation. The aortas of two strains of rats were therefore prepared for studies of both the distribution and chemical characteristics of SP receptors. A significantly higher B_{max} was observed in aortas of WKR than in SHR. Since SP is said to have a hypotensive effect in rats, these findings would raise the possibility that the hypotensive action of SP may be defective in this strain of SHR. Further attempts to characterize the SP receptor have been delayed by the observation that the N-terminal amino acid of the receptor protein is blocked. Thus enzymatic cleavage and analysis of the isolated and purified fragments will be necessary to characterize the binding site of this receptor protein. A collaborative project to screen SP receptor clones from a rat hypothalamus cDNA library was interrupted due to a shortage of funds at Genentech.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03559-03 HE

PERIOD COVERED

Oct. 1, 1988 to Sept. 30, 1989

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

Catechols and sympathoadrenal function in health and disease

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

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

Others: J. Bacher, VRB,DRS,NIH; P.C. Chang, Visiting Scientist,DIR,NINDS; B. Chidakel, BEIB,DRS,NIH; A. Deka-Starosta, Visiting Fellow, DIR,NINDS; G. Eisenhofer, Baker Med. Res. Inst., Victoria, Australia; R. Finn, Memorial-Sloan Kettering Hosp.,NewYork,NY; C.J. Folio, Clin.Nurse,HE,NHLBI; M. Garty, Chief,Dept.Med., Beilinson Med.Ctr.,Petach Tikve,Israel; J.R. Gill,Jr., Spec.Vol.,HE,NHLBI; P. Herscovitch, Chief,PET Section,NMD,CC,NIH; D. Hovevey-Sion, Visiting Fellow,DIR, NINDS; H.R. Keiser, Chief,HE,NHLBI; K.L. Kirk, LC,NIDDK; I.J. Kopin, Chief,CNB,DIR,

COOPERATING UNITS *(if any)*

NINDS; R. Miletich, Senior Staff Fellow,DIR,NINDS; R. Stull, Chemist,HE,NHLBI; K.Szemeredi, Visiting Fellow,DIR,NINDS; R. Zimlichman, Chief,Hypertension Unit, Soroka Med.Ctr.,Beer Sheva,Israel; A. Zukowska-Grojec, Guest Worker,HE,NHLBI

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Hypertension-Endocrine Branch

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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. We developed and applied techniques to measure activity of this homeostatic system in cardiovascular and neurologic diseases--especially hypertension. Our goals have been to understand better how this system is regulated, how its function is integrated with that of other homeostatic systems, and whether and how it plays a pathophysiologic role in hypertension, hypotension, and cardiovascular hypertrophy, by conducting appropriate clinical and preclinical research. Methodologic developments included in vivo estimation of regional turnover of catecholamines, including norepinephrine (NE), the neurotransmitter of the sympathoneural system, and Uptake-1, the main means of termination of the actions of andogenous NE; a preparation to assess pre-synaptic vs. central nervous system sites of action of anti-hypertensive drugs; simultaneous assays of levels of NE, epinephrine, dopa, dihydroxyphenylglycol (DHPG), dopamine, dihydroxyphenylacetic acid (DOPAC), and other catechols; and positron emission tomographic (PET) scanning to provide a noninvasive, in vivo means to examine cardiac sympathetic innervation and function in humans. Sympathoadrenal reactivity was excessive in young hypertensive humans and rats. The renal dopa-dopamine (DA) system participated in sodium homeostasis rats, dogs, and humans. Patients with hypertrophic cardiomyopathy (HCM) had neurochemical evidence of defective cardiac Uptake-1. Neurogenic orthostatic hypotension was associated with specific, abnormal patterns of plasma levels of catechols.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03563-03 HE

PERIOD COVERED
Oct. 1, 1988 to Sept. 30, 1989

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)*

PI:	Ingeborg Hanbauer	Pharmacologist	HE NHLBI
Others:	Mariagrazia Grilli	Visiting Fellow	HE NHLBI
	Gilbert Wright, Jr.	Research Chemist	HE NHLBI
	Angela Murphy	Research Chemist	HE NHLBI

COOPERATING UNITS *(if any)*
Martin Morad Professor, Dept. of Physiology, Univ. of Penna., Phila., Penna.
Donald Hunt Professor, Dept. of Chemistry, Univ. of VA, Charlottesville, VA

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☐ (a1) Minors
☐ (a2) Interviews

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

Calcium channels are widely distributed in excitable cells and mediate the generation of action potential, pace making activity, excitation-contraction coupling, and secretion. A low molecular weight material has been isolated and purified from rat brain that decreases the specific binding of dihydropyridines, known to be potent Ca2+ channel blockers. It also inhibits reversibly the depolarization-dependent calcium accumulation in primary cultures of neurons. In voltage-clamped neuronal cells it inhibited the L- and T-type components of calcium channels. In contrast, in cardiac myocytes the same substance enhanced the L-type calcium current in a G protein-independent manner. This enhancing action was not inhibited by alpha-1 or β-adrenergic receptor blockers. Since the activation of calcium channels elicited by this endogenous modulator in cardiac myocytes had a delayed onset, it is suggested that it may interact at sites close to the myoplasm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03565-03 HE

PERIOD COVERED
Oct. 1, 1988 to Sept. 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biosynthesis, distribution and biological action of substance P and its receptors

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

PI:	Mei-Lie Swenberg	Research Chemist	HE NHLBI
Others:	Rita Liu	Scientist	HE NHLBI
	Bernard Malfroy	Research Scientist	Genentech
	Brian M. Martin	Research Scientist	ADA MHA

COOPERATING UNITS (if any)
None

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☐ (a1) Minors
☐ (a2) Interviews

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

Antibody, preparation and application:

Idiotypic antibodies and receptor antibodies were successfully prepared by immunizing New Zealand white rabbits with purified SP monoclonal antibody (Ant-Mc) and receptors from olfactory bulb (Ant-Rc-olf) or intestinal mucosa (Ant-Rc-Int). The activities of the antibodies were verified by various methods: binding to prefixed brain tissue sections by immunocytochemical localization or to the membrane receptor protein by western Blot; inhibition of radioligand SP binding to the receptors on fresh tissue sections by autoradiography or to the specific SP antibodies in RIA.

A similar pattern of SP receptor distribution in periaqueductal gray (PAG) was observed by both autoradiography with labeled ligand SP and immunocytochemical localization with idiotypic antibodies or anti-receptor antibodies.

These results confirm that the receptor isolated possesses the binding activities of a SP receptor.

The idiotypic and SP receptor antibodies were also used to screen the SP receptor clones from the existing libraries of rat hypothalamus in collaboration with Genentech, Inc.

The structural studies of SP receptor protein has been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: **Z01 HL 03567-02 HE**

PERIOD COVERED

Oct. 1, 1988 to Sept. 30, 1989

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

Regulation of the dopamine reuptake system

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

PI:	Ingeborg Hanbauer	Pharmacologist	HE NHLBI
Other:	Mariagrazia Grilli	Visiting Fellow	HE NHLBI

COOPERATING UNITS *(if any)*

None

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1.0	1.0	

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☐ (a1) Minors

☐ (a2) Interviews

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

High affinity binding sites of several potent dopamine uptake blockers were shown to be specifically located in dopaminergic nerve terminals of caudate nuclei and appear to constitute a molecular component of the dopamine carrier. The recognition sites of 3H-cocaine in dopaminergic nerve terminals appear to play a physiologycal role because they can be regulated by receptor-receptor interaction. Destruction of cortico-striatal glutamatergic nerve fibers increased the density of 3H-cocaine binding sites, while in washed striatal synaptosomal membrane preparations the addition of L-glutamate and glycine decreased the number of 3H-cocaine binding sites. In addition, L-glutamate receptor stimulation may also play a role in the depolarization-evoked release of dopamine. K+-evoked dopamine release is facilitated by L-glutamate and is mediated through activation of N-type calcium channels. In comparison, vertatridine-evoked release of dopamine requires extracellular calcium, but is not mediated through N- or L-type calcium channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03574-02 HE

PERIOD COVERED

Oct. 1, 1988 to Sept. 30, 1989

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

Cellular mechanisms of hypertensionand atherosclerosis

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

PI:	Seiichi Shimizu	Visiting Associate	HE NHLBI
Others:	Margaret Hill	Medical Technician	HE NHLBI
	Reuben Brown	Phys. Sci. Technician	HE NHLBI
	Harry R. Keiser	Chief	HE NHLBI

COOPERATING UNITS *(if any)*

None

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Hypertension-Endocrine Branch

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2.0	2.0	

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☐ (a1) Minors

☐ (a2) Interviews

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

Some antihypertensive drugs (Hydralazine 10μM; Nifedipine 10μM; Verapamil 100μM) inhibited DNA synthesis of SMC from SHRSP and WKY. However, Nifedipine (0.1 μM) and Verapamil (10 μM, 1μM) stimulated slightly DNA synthesis of SMC from SHRSP and WKY.

Sodium nitroprusside (SNP) exerted its inhibitory action on cell proliferation in G1 to S phase. Inhibitory action of SNP on DNA synthesis by post-treatment is significantly greater on WKY SMC than SHRSP SMC. SNP inhibited RNA synthesis of both SMC to almost the same degree. Also, SNP inhibited protein synthesis in SMC for 9 to 12 hr and 12 to 15 hr after FCS stimulation. The inhibition of protein synthesis was significantly greater in WKY than in SHRSP.

Conditioned medium (DMEM without FCS for 24 hrs) prepared from SHRSP or WKY SMC stimulated DNA synthesis of SMC from SHRSP and WKY in the presence of 1% FCS. Both SMC were stimulated stronger by conditioned medium from SHRSP than that of WKY.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03577-01 HE

PERIOD COVERED: Oct. 1, 1988 to Sept. 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanisms of secretion and retention of NE in synaptic vesicles in axoplasm.

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

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1.0	1.0	

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☐ (a1) Minors
☐ (a2) Interviews

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

This laboratory has studied the uptake and retention of norepinephrine (NE) in synaptic vesicles in the axoplasm (in situ) of adrenergic nerve terminals in vitro. When slices of rat heart ventricle are incubated in a Na^+-deprived (12.5 to 25μM Na, choline) Krebs-bicarbonate medium (Ch+-Ca++), synaptic vesicles and axolemma appear to establish a new morphological unit which can secrete stored NE and recapture NE in the medium. This laboratory was among the first to recognize that electron transport could be involved in retention. Secretion is mediated by the amine pump in the axolemma and recapture is mediated by the amine pump in the vesicle membrane. In this preparation, the latter is dependent upon exogenous ATP. Retention as well as recapture are inhibited by Mg++-ATPase inhibitors, K+-H+ exchange ionophores, H+ ionophores and reserpine, which blocks the NE pump in the vesicle membrane. These inhibitors are all known to block uptake dependent upon H+ transport energized by the activity of Mg++-ATPase in the vesicle membrane. Furthermore, uptake and retention are prevented by ammonia. It was concluded that uptake and retention in vesicles in situ are energized by a pH gradient, Δ pH, across the vesicle membrane (Bogdanski, 1982,1983,1986,1988).

These findings are physiologically significant. The same inhibitors prevent the retention of NE in incubated terminals in KRB. Mg++ and Mn++ block the voltage dependent entry of Ca++ in nerve endings (Blaustein). Mg++ blocks Ca++ entry and NE depletion from vesicles in situ. Mn++ blocks amine secretion specifically. The excretion of deaminated metabolites is not inhibited. It would appear that the Ca++ serves a similar function in secretion in vivo and in vesicles in situ. Secretion is not mediated by exocytosis.

The fusion of vesicle to axollemma is controlled by Na+. Terminals in Ch+-Ca++ do not respond to ATP if Na+ is completely omitted from the medium. Lithium inhibits the Na^+ dependency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03578-01 HE

PERIOD COVERED

Oct. 1, 1988 to Sept. 30, 1989

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

Role of vasoactive hormones in congestive heart failure

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	Visting Associate	HE NHLBI

COOPERATING UNITS *(if any)*

None

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1.0	1.0	0

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☐ (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 investigated the effects of: 1) atrial natriuretic factor (ANF), 2) brain natriuretic peptide (BNP), and 3) endothelin (ET) on hemodynamics and renal function in an experimental model of high-output CHF in rats. CHF was produced by a large a-v fistula (ACF) between the aorta and vena cava of rats, confirmed by measuring daily urinary sodium excretion. These rats demonstrate high plasma levels of ANF and a reduced responsiveness to exogenous ANF. Some of the rats (approximately a third) display uncompensated sodium retention (RET) that leads to death from severe CHF. The rest of the rats were able to compensate and "escape" from the sodium retaining state (ESC). When the ACF was surgically closed, urine volume and sodium excretion increased abruptly and plasma renin activity decreased quickly but plasma ANF levels remained high for another 2 days and then gradually decreased. Prior bilateral renal denervation did not alter these findings. The pattern of changes in plasma ANF levels suggests that it is not only a marker of the volume overload but may also be important in the natriuresis after closure of the ACF. BNP had considerable hypotensive, diuretic and natriuretic effects in normal rats. These effects were completely abolished in CHF rats. Conversely, ET had marked hypertensive, antidiuretic and antinatriuretic effects in normal rats and these effects were markedly blunted in CHF rats. We conclude that: 1) ANF is an important compensatory mechanism both during and after an ACF. 2) The vascular and renal responses to vasoactive peptides, both vasoconstrictors and vasodilators, is markedly blunted in CHF. 3) Alterations in the levels of these peptides may contritute to the fluid and sodium retention in CHF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: **Z01 HL 03579-01 HE**

PERIOD COVERED

Oct. 1, 1988 to Sept. 30, 1989

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

U46619 causes platelet aggregation via increases in cytosolic Ca++

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

PI:	John Yun	Guest Worker	HE NHLBI
Others:	Peter Ohman	Visiting Scientist	HE NHLBI
	Harry Keiser	Chief	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

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☐ (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 effects of U46619, a thromboxane mimic, on cytosolic Ca++ concentration and platelet aggregation were determined in human platelets. Cytosolic Ca++ concentration was determined via Quin-2 fluorescence and platelet aggregation studied in an aggregometer. Addition of U46619 to the platelet suspension produced a sharp increase in cytosolic Ca++ and platelet aggregation. Pretreatment of platelets with prostaglandin I2, PGD2, PGE1, PGF2alpha, dibutyryl2 cyclic AMP or forskolin prevented the increase in cytosolic Ca++ and the associated platelet aggregation induced by U46619. In platelets treated with PGE2, 8-(diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8), or verapamil (V), U46619 produced a much slower increase in cytosolic Ca++. Although cytosolic Ca++ concentration eventually reached values equal to, or greater than, those of controls, no platelets aggregation was observed. These data suggest that U46619 induces platelet aggregation through an increase in cytosolic Ca++, and that both Ca++ entry and its release from intracellular storage sites probably contribute to the increase in cytosolic Ca++. Furthermore, the rate of the increase and the magnitude of the rise in cytosolic Ca++ concentration appear to be crucial in platelet aggregation induced by U46619. Our data also suggest that PGs inhibit U46619-induced platelet aggregation by preventing the increase in cytosolic Ca++, and these effects are probably mediated via an increase in cAMP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03580-01 HE

PERIOD COVERED
Oct. 1, 1988 to Sept. 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histopathology and dietary protein in hypertension

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

PI:	Martina Diolulu	Guest Researcher	HE NHLBI
Others:	Victor J. Ferrans	Section Chief	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:
1.0	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 were made of the morphology of the kidney and heart in 10-month old, stroke-prone spontaneously hypertensive rats (SHRSP) which had been maintained for nine months on one of three experimental diets: standard (STD; 25.3% crude protein and 0.5% methionine), low protein (LP: 19% crude protein and 0.39% methionine), or high methionine (MET: 25.3% crude protein and 2.0% methionine). Except for 24 and 40 weeks of age, SHRSP/LP had significantly lower blood pressure than those of SHRSP/STD and SHRSP/LP. Throughout the experiment, normotensive Wistar Kyoto rats fed standard diet (WKY/STD) maintained significantly lower BP than the SHRSP groups. From 4 to 10 months of age, SHRSP/STD and SHRSP/LP (25% - 63% and 46% - 80%, respectively) exhibited abnormal behavior which included excitement, repetitive lifting, hyperirritability and coarse tremor of the paws. At 10 months of age, the degree of cardiac hypertrophy and fibrosis, thickening of intramyocardial arterioles and the presence of inflammatory cells were similar among the three experimental SHRSP groups. The kidneys of SHRSP/LP demonstrated marked thickening of the vascular walls, narrowing of the lumina of arterioles, and glomerular sclerosis. Since the morphology of the kidneys from SHRSP/MET differed markedly from that of SHRSP/STD but appeared close to that of SKY/STD, it seems from these results that increasing the amount of methionine in the diet provides protection against the renal complications of hypertension in SHRSP; however, further investigation is required to determine whether this protective action is primarily a result of dietary effect on blood pressure or dietary influence on some metabolic changes that slow the rate and degree of tissue damage in prolonged hypertension.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03581-01 HE

PERIOD COVERED
Oct. 1, 1988 to Sept. 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neuropeptide Y: Sources and actions in the cardiovascular system

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

PI:	Zofia Zukowska-Grojec	Special Volunteer	HE NHLBI
Others:	Gregory H. Shen	Special Volunteer	HE NHLBI
	Anna Deka-Starosta	Visiting Fellow	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.)

Neuropeptide Y (NPY), a 36 amino acid peptide, coexists with norepinephrine (NE) in most sympathetic postganglionic neurons; in fact, the sympathetic innervation of the cardiovascular system may invariably contain both NE and NPY. The aim of our studies is to establish the regulation of the release of NPY into the circulation, and actions of the peptide in the cardiovascular system. At the sympathetic neuroeffector junction, NPY cooperates with NE via at least three principally different mechanisms. First, like NE, NPY is a vasoconstrictor; however, not all vascular beds are responsive to NPY. Second, NPY suppresses stimulated NE release and reciprocally, NE appears to regulate NPY release. Third, NPY and NE are postjunctionally synergetic; hence, small amounts of NPY potentiates NE-evoked vasoconstriction while vessels preexposed to NE increase their responsiveness to NPY.

Recently, we have provided evidence that in addition to the sympathoadrenomedullary system, platelets are a rich source of NPY which they release during secondary aggregation. Chromatographic (HPLC) characterization of platelet-derived NPY-immunoreactivity revealed two major peaks: one co-eluted with authentic NPY, and the other one remains unidentified. The peak corresponding to authentic NPY increased 11-fold during platelet stimulation with collagen; the unidentified peak increased 4-fold. To elucidate a possible functional role of NPY on platelets, rat platelet membranes were assayed for 125I-NPY binding. High-density, saturable, high affinity binding sites for NPY were found on rat platelets. Analogous to some vessels, platelets were unresponsive to direct effects of NPY. However, effects of threshold or subthreshold concentrations of collagen were potentiated by NPY at concentrations as low as 10 M. Studies are under way to better characterize platelet and neuronal NPY systems (in rats and in humans), and their role in regulation of vascular and platelet functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: **Z01 HL 03582-01 HE**

PERIOD COVERED
Oct. 1, 1988 to Sept. 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comparison of substance P receptors between hypertensive and normotensive rats

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

PI:	Mei-Lie Swenberg	Research Chemist	HE NHLBI
Others:	Seiichi Shimizu	Visiting Scientist	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.)*

On the basis of embryonic AP receptor localization with labeled ligand autoradiography, blood vessels are one group of peripheral tissue enriched in SP receptors initiated from gestation days 13-17. There are reports indicating that SP has hypotensive activity; therefore studies were conducted to examine the difference between the two strains of rats Wisto Kyoto (WKY) and stroke prone spontaneously hypertensive (SHRSP) in the distribution and chemical characteristic of SP receptors in their aorta. A significantly higher B_{max} was observed in aorta of WKY in our preliminary studies. The result corresponds well with the reported hypotensive activity of SP. Further studies of the differences in chemical and physical characteristics of SP receptors between the two strains have been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03583-01 HE

PERIOD COVERED

Oct. 1, 1988 to Sept. 30, 1989

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
Others:	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:
1.0	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.)*

We have studied dopa release and conversion of dopa to dopamine in Dahl salt-resistant (DR) and Dahl salt-sensitive (DS) rats on either low or high salt diet.

We found that both strains, DR and DS rats increase dramatically their urinary dopa and dopamine excretion in response to high salt diet. The increase in urinary dopa and dopamine was associated with only a mild increase in plasma dopa clearance and spillover. Thus, it seems that dopa and dopamine are synthesized locally in the kidney in response to high salt diet.

Molecular Disease Branch
National Heart, Lung, and Blood Institute
October 1, 1988 through September 30, 1989

The overall objective of the research program of the Molecular Disease Branch is the elucidation of the molecular and structural properties of the human plasma apolipoproteins (apo), the physiological role of the apolipoproteins and lipoproteins in lipid transport, the determination of the mechanisms involved in the regulation of cellular cholesterol metabolism and transport, the elucidation of the metabolic and molecular mechanisms involved in plasma lipoprotein synthesis, transport, and catabolism in normal individuals and patients with disorders of lipid metabolism and atherosclerosis, as well as the elucidation of the mechanisms involved in the development of atherosclerosis.

* Elevated plasma levels of Lp(a) are an important independent risk factor for the development of premature cardiovascular disease. Lp(a) is a cholesterol-rich lipoprotein that closely resembles LDL, and contains a unique apolipoprotein, apo(a), covalently linked to apoB-100. Approximately 20% of the population have Lp(a) levels above 30 mg/dl which is associated with a two-fold increase in relative risk of premature heart disease. With elevations of Lp(a) and LDL, the relative risk of vascular disease increases to approximately five-fold. The factors which modulate the biosynthesis and catabolism of Lp(a) are poorly understood. In order to gain insight into the role of the LDL receptor in the catabolism of Lp(a), kinetic studies were performed with radiolabeled Lp(a) in normal volunteers, and two patients with familial hypercholesterolemia (FH) with a defect in the LDL receptor. Analysis of the kinetic studies revealed that Lp(a) catabolism is delayed in FH as compared to normals. Lp(a) is bound and degraded by the LDL receptor on normal human fibroblasts, but not FH fibroblasts. It was concluded from these studies that the LDL receptor plays an important role in the <u>in vivo</u> catabolism of Lp(a), however, Lp(a) is a less efficient ligand for the LDL receptor than is LDL. These studies provide major insights into the metabolism of Lp(a), and will be important in developing new strategies for the treatment of patients with elevated plasma levels of Lp(a) and early heart disease.

* Patients with severe elevations of plasma triglycerides are at risk for the development of recurrent bouts of pancreatitis. Plasma triglycerides are hydrolysed by the enzyme lipoprotein lipase, which is activated by the plasma apolipoprotein, apoC-II. Genetic defects in apoC-II which lead to apoC-II deficiency are associated with markedly elevated plasma triglycerides and pancreatitis. Studies were undertaken to elucidate the molecular defect in the apoC-II gene in two kindreds with apoC-II deficiency. The defect in the first kindred from Padova, Italy, designated apoC-II$_{Padova}$ contained a premature termination mutation in exon 3 that generates a truncated 36 amino acid C-II protein. In the second kindred from Paris, France, the mutation in the apoC-II$_{Paris}$ gene changed the initiation (AUG) codon to GUG. Both of these mutations in the apoC-II gene result in defective synthesis of apoC-II, and elevated plasma triglycerides. The elucidation of the molecular defects in patients with severe hypertriglyceridemia will provide the basis for the development of new methods for the identification of patients at risk for pancreatitis, and more specific therapy of disorders of triglyceride

metabolism.

* Defects in lipoprotein lipase (LPL) results in defective metabolism of plasma triglycerides, and the development of severe hypertriglyceridemia and recurrent pancreatitis. The molecular defect in the lipoprotein lipase gene was investigated in a Bethesda kindred with familial type I hyperlipoproteinemia and recurrent pancreatitis. A single base substitution of a G to A was identified in the cDNA of the proband. This mutation resulted in a single amino acid substitution of a threonine for an alanine, and a diagnostic RFLP in the LPL gene. The single amino acid substitution resulted in an inactive enzyme which was unable to activate lipoprotein lipase for triglyceride hydrolysis. The analysis of the Bethesda kindred with LPL deficiency is the first established defect of a point mutation in the LPL gene which results in a deficiency of LPL and severe hypertriglyceridemia.

* High density lipoproteins (HDL) are important lipoproteins in modulating the risk of premature cardiovascular disease. Elevated and reduced levels of HDL are associated with a decreased and increased risk of early heart disease. The two major apolipoproteins in HDL are apoA-I and apoA-II. Lipoproteins containing apoA-I alone, LpA-I particles, appears to be particularly effective in protecting against cardiovascular disease. The liver and intestine have been proposed to be the major site of synthesis of both apoA-I and apoA-II. Recently the role of the liver and intestine in the synthesis of apoA-I and apoA-II was investigated by analysis of levels of apoA-I and apoA-II mRNA as well as the apoA-I and apoA-II isoproteins secreted by human intestinal organ cultures, and liver HepG-2 cells. Based on these studies it was concluded that the human liver secretes both apoA-I and apoA-II. In contrast, the intestine synthesizes only apoA-I, and no apoA-II mRNA or secreted A-II apolipoprotein could be detected. The results indicate that the human intestine may be an important site for the biosynthesis of LpA-I particles which protect against premature cardiovascular disease. The data from these studies provides a major change in our concepts regarding the sites of synthesis of apoA-I and apoA-II, and indicate that the intestine may be a primary site of synthesis of the anti-atherogenic LpA-I particle. The factors which modulates the biosynthesis of intestinal LpA-I particles will be an active area of research in the future.

* A detailed structural knowledge of the plasma apolipoproteins has permitted the identification of new diseases associated with apolipoproteins. A new disease, hereditary amyloidosis, is associated with a mutation in apoA-I, designated apoA-I_{Iowa}. The mutation is a single amino acid substitution of a glycine for arginine in apoA-I at residue 26. This substitution results in hypoalphalipoproteinemia and the amyloid protein which accumulates in the tissues of these patients is a fragment of apoA-I_{Iowa}. The metabolic reason for the hypoalphalipoproteinemia was investigated in two heterozygote apoA-I_{Iowa} patients and two control subjects. Kinetic analysis of simultaneously injected radiolabeled apoA-I_{Iowa} and normal apoA-I revealed markedly increased catabolism of apoA-I_{Iowa} compared to normal apoA-I in both control and apoA-I_{Iowa} subjects. These results were interpreted as indicating that apoA-I_{Iowa} is kinetically abnormal, and is rapidly catabolized from plasma. The abnormal metabolism of apoA-I_{Iowa} explains

the hypoalphalipoproteinemia as well as the accumulation of the apoA-I_{Iowa} protein in the amyloid deposits of this form of hereditary amyloidosis. In addition, the apoA-I_{Iowa} protein can now be used to identify those members of the kindred who are affected by the disease prior to the development of symptoms.

* Elevated levels of low density lipoproteins (LDL) are associated with an increased risk of premature cardiovascular disease. The major apolipoprotein on LDL is apoB-100. A second apoB isoprotein present in the plasma is apoB-48. Studies during the last two years have established the structural relationship between apoB-48 and apoB-100. A single gene for apoB-100 is located on chromosome 2. Liver and intestinal apoB cDNA clones were sequenced, and two types of apoB cDNA sequences were detected. One contained the 'CAA' codon coding for glutamine at amino acid residue 2153 in apoB-100, and the other contained a 'TAA' premature stop codon. The change in the 'CAA' codon for glutamine for the 'TAA' stop codon resulted in the synthesis of the apoB-48 isoprotein which terminated at amino acid 2152. Thus apoB-100 contains 4536 amino acids, and apoB-48 contains 2152 amino acids. Detailed analysis of genomic DNA and apoB mRNA established that the stop codon was introduced at the RNA level by a unique RNA editing mechanism.

* In man the site of synthesis of apoB-100 was proposed to be limited to the liver. Analysis of intestinal apoB mRNA and the apoB isoproteins secreted by intestinal human organ cultures revealed that the intestine synthesized both apoB-100 and apoB-48 containing lipoproteins. These studies were interpreted as indicating that potentially atherogenic apoB-100 containing lipoproteins may be synthesized by the intestine. These findings may ultimately explain the mechanisms involved in post-prandial atherosclerosis, and premature atherosclerosis in individuals with normal fasting lipid levels.

* Apolipoprotein E plays a major role in lipoprotein metabolism by facilitating the uptake of remnant lipoproteins into the liver by interacting with the apoE or remnant receptor and LDL receptor. Defects in the E apolipoprotein result in delayed clearance of remnant lipoproteins, the accumulation of atherogenic lipoproteins, and a type III hyperlipoproteinemia. The importance of apoE in lipoprotein metabolism has been further established by the identification of a patient with elevated plasma cholesterol and triglycerides as well as a type III hyperlipoproteinemia, and a deficiency of plasma apoE. The molecular defect in this kindred was established by sequencing the apoE gene in the proband. A single base substitution of a G → A which converted the tryptophan at amino acid 210 to a stop codon, thus leading to the production of a truncated apoE protein. The deficiency of apoE resulted in a type III phenotype, and the plasma accumulation of remnant lipoproteins. The identification of patients with a deficiency of a specific apolipoprotein provides the unique opportunity to clearing delineate the function of that apolipoprotein in lipoprotein metabolism.

* Structural defects in apolipoprotein E are associated with the development of type III hyperlipoproteinemia and premature cardiovascular disease. Homozygosity for apoE-2, an apoE variant with an arginine to cysteine substitution at residue 112, is associated with the type III

phenotype. Heterozygotes for apoE-2 have virtually normal lipoproteins. A new dominant form of type III hyperlipoproteinemia has been identified in a kindred with hyperlipidemia. A new apoE-1 variant, designated apoE-$1_{Harrisburg}$, has a single substitution of a glutamic acid for a lysine at amino acid residue 145. Kinetic analysis of radiolabeled apoE-$1_{Harrsburg}$ and normal apoE in normal subjects and the proband with apoE-$1_{Harrisburg}$ revealed that apoE-$1_{Harrisburg}$ had markedly delayed catabolism when compared to normal apoE. In vitro binding studies on human fibroblasts revealed that apoE-$1_{Harrisburg}$ had a decreased binding affinity for the LDL receptor when compared to normal apoE. These combined results established that apoE-$1_{Harrisburg}$ is a unique apoE variant that results in the development of type III hyperlipoproteinemia in the heterozygous state. The discovery of new apolipoprotein variants with unexpected clinical sequelae provide the opportunity to gain additional insights into the pathophysiology of the human dyslipoproteinemias.

* One of the major challenges in the field of lipoproteins and atherosclerosis is the pathophysiological mechanisms involved in the development of the atherosclerotic lesion. The study of cholesterol accumulation in early atherosclerotic lesions has been of particular interest. Two cholesterol-rich lipid particles, one rich in unesterified cholesterol and the other rich in esterified cholesterol have been identified in the extracellular space of human atherosclerotic lesions. Studies are underway to establish the origin of the particles using cholesterol-fed rabbits. We found that lipid particles accumulating in blood vessels induced by one week of cholesterol feeding, had an average density of 1.09 g/ml, contained cholesterol in a predominantly unesterified form (95%), and had an unesterified cholesterol to phospholipid molar (C/P) ratio of 0.6. By one month of cholesterol feeding, lipid particles had densities ranging between 1.03 to 1.09 g/ml, contained 89% of their cholesterol in an unesterified form and had a C/P ratio of 2.0. By three months of cholesterol feeding, mature lipid particles accumulated having an average density of 1.04, 77% of their cholesterol unesterified, and a C/P ratio of 2.5. Our findings suggest there may be a precursor cholesterol-poor lipid particle in early lesions which matures to cholesterol-rich lipid particles in advanced lesions. These extracellular cholesterol-rich lipid particles may represent components of a metabolic pathway by which cells excrete excess cholesterol.

* The role of activated platelets which induce cholesterol accumulation in cultured rat smooth muscle cells, is being investigated. We have observed that activation of rat platelets results in significant platelet phospholipid hydrolysis that accompanies loss of platelet viability. The decrease in phospholipid content and resulting elevated C/P ratio in activated rat platelets may represent a mechanism to promote cholesterol transfer from degenerating cells to viable cells and acceptor plasma lipoproteins such as HDL. These findings may help elucidate the mechanism of cholesterol removal from degenerating cells such as platelets within thrombi or smooth muscle cells within atherosclerotic lesions.

* A major cell type in the development of atherosclerosis is the monocyte-macrophage. These cells play a central role in the development

of the early vascular lesion in atherosclerosis. The lipoproteins and apolipoproteins which modulate lipid accumulation in normal human macrophages and macrophages isolated from patients with dyslipoproteinemias are being actively studied. Human monocyte-macrophages accumulate cholesterol when incubated with nonlipoprotein cholesterol, lipid particles isolated from the vessel wall, and lipid particles released from activated platelets. When normal monocyte-macrophages were incubated with native LDL or HDL, macrophages did not accumulate cholesterol. We have identified one human cholesterol storage disease in which monocyte-macrophages accumulated cholesterol when incubated with native LDL or HDL. Also, if LDL was previously degraded by exposure to cholesterol esterase, normal monocyte-macrophages then accumulated cholesterol. Interestingly, when monocyte-macrophages were incubated with nonlipoprotein cholesterol only a subpopulation of these cells accumulated cholesterol. These studies will provide additional insights into the mechanisms involved in cholesterol accumulation in atherosclerotic lesions, and may provide new approaches to the treatment of patients with premature cardiovascular disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02010-18 MDB

PERIOD COVERED
October 1, 1988 and September 30, 1989

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)*

PI: H. Bryan Brewer, Jr., M.D., Chief, MDB, NHLBI
Others: F. Thomas, Ph.D., Research Chemist, MDB, NHLBI
J. Hoeg, M.D., 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 Medizin, Medizinische Hochschule Hannover, Hannover, West Germany

LAB/BRANCH
Molecular Disease Branch

SECTION
Peptide Chemistry

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 5.9	PROFESSIONAL: 2.9	OTHER: 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 apoB isoproteins secreted by the human adult intestine have been evaluated by analysis of the B apolipoproteins secreted by intestinal organ cultures in vitro. The apoB isoproteins were analyzed by ^{35}S methionine labelling and immunoblot analysis. The results of these studies established that apoB-100 and apoB-48 were synthesized and secreted by the adult human intestine. The major newly synthesized apoB isoprotein secreted by the adult human intestine was apoB-48.

The truncated apoC-II variant peptides, apoC-II$_{Padova}$ and apoC-II$_{Nijmegen}$ identified in the Padova and Nijmegen kindreds with apoC-II deficiency have been synthesized by solid phase methodology. The synthetic 17 amino acid apoC-II$_{Padova}$ and 36 amino acid apoC-II$_{Nijmegen}$ peptides were not able to activate lipoprotein lipase, and the hypertriglyceridemia present in the apoC-II deficient kindreds was due to the failure of the truncated apoC-II peptides to activate lipoprotein lipase.

The intestine has been previously considered to be a major site of synthesis of apoA-I and apoA-II, the two major apolipoproteins of high density lipoproteins. Analysis of apoA-I and apoA-II mRNA, and the apoA isoproteins secreted by intestinal organ cultures have established that the human intestine synthesized apoA-I, but not apoA-II. These studies indicate that the intestine may be an important site of synthesis of apoA-I containing HDL particles which protect against the development of premature cardiovascular disease.

The molecular defect in hereditary amyloidosis is a structural variant of apoA-I designated apoA-I$_{Iowa}$. ApoA-I$_{Iowa}$ has been isolated and sequenced, and contains an arginine replacing glycine at positive 26. The apoA-I$_{Iowa}$ variant protein accumulation in the amyloid deposit in hereditary amyloidosis and is the first human apolipoprotein variant associates with ths hereditary disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HL-02012-14 MDB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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)

Peptide Chemistry

LAB/BRANCH

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 ☒ (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 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. We have also demonstrated that the enzymic activity of HMG-CoA reductase is also modulated in vitro by a protein kinase C-mediated phosphorylation. Recently we have demonstrated that HMG-CoA reductase activity is modulated by a third kinase system, a Ca^{2+} calmodulin dependent kinase, involving covalent phosphorylation. In order to understand the coordinate regulation of HMG-CoA reductase, cholesterol synthesis, and role of apolipoproteins such as apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) 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-translational modification of human plasma apoA-I involving reversible phosphorylation. We have also demonstrated that secreted and intracellular apoA-I from Hep G2 cells were phosphorylated. The phosphorylation of apoA-I may play an important role in lipoprotein assembly, intracellular transport, as well as processing and lipoprotein secretion. Plasma low-density lipoproteins (LDL) have been correlated directly with the development of premature cardiovascular disease. During the past year we have established that the post-translational modification of human apoB (apoB-100) involves reversible phosphorylation. We have also demonstrated that secreted and intracellular apoB from Hep G-2 were phosphorylated. 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

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 HL 02019-11 MDB

PERIOD COVERED

October 1, 1988 and September 30,1989

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

Metabolism of lipoprotein and apolipoproteins in humans

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

PI: Richard E. Gregg, M.D., Senior Investigator MDB, NHLBI

Others: Daniel Rader, M.D., Medical Staff Fellow, MDB, NHLBI
Juergen Schaefer, M.D., Visiting Fellow, MDB, NHLBI
Alexander Mann, M.D., Visiting Fellow, MDB, NHLBI
Marie Kindt, Chemist, MDB, NHLBI
Yashiko Dogherty, Chemist, MDB, NHLBI
Martha Meng, Chemist, MDB, NHLBI
H. Bryan Brewer, Jr., M.D., MDB, NHLBI

COOPERATING UNITS *(if any)*

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Merril Benson, M.D., Professor, Indiana University

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, 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.)*

A kindred with a structural defect in apoA-I, designated apoA-I_{Iowa} has been identified with familial amyloidosis and hypoalphalipoproteinemia. ApoA-I_{Iowa} contains a glycine to arginine substitution at amino acid residue 26. The metabolic explanation for the hypoalphalipoproteinemia was determined by kinetic analysis of radiolabeled normal apoA-I and apoA-I_{Iowa}. ApoA-I_{Iowa} was rapidly catabolizes and may explain the hypoalphalipoproteinemia as well as the accumulation of apoA-I_{Iowa} in the amyloid deposits in the various organs in patients with hereditary amyloidosis. The elucidation of the molecular defect in apoA-I_{Iowa} also provides a method for the identification of individuals with the disease prior to the development of symptoms.

A new dominant form of type III hyperlipoproteinemia has been identified. The variant form of apoE designated apoE-$1_{Harrisburg}$ contains a lysine to glutamine acid substitution in normal apoE-3. Kinetic analysis of normal apoE-3 and apoE-$1_{Harrisburg}$ established that apoE-$1_{Harrisburg}$ and normal apoE were catabolized slower in the proband with apoE-$1_{Harrisburg}$. Based on these data it is proposed that the apoE-$1_{Harrisburg}$ variant results either in a significant decrease in the multimeric binding of apoE to the apoE or remnant receptor or that there is a protein-protein interaction between the normal apoE and apoE-$1_{Harrisburg}$ which results in a net decrease in binding of both apoE isoproteins to the apoE receptor.

Kinetic studies have been initiated utilizing stable isotopes to gain new insights into lipoprotein metabolism. Kinetic analyses of VLDL apoE and apoB-100 were performed in patients with familial hypercholesterolemia. The synthesis of apoE was normal, however, there was a newly recognized fast turning over component of VLDL-apoB which has not been previously identified, and may have been attributed to direct synthesis of LDL. These studies illustrate the power of this new method for the study of lipoprotein metabolism in normal subjects and patients with premature coronary artery disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 02022-09 MDB

PERIOD COVERED

October 1, 1988 and September 30,1989

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)*

PI: Jeffrey M. Hoeg, M.D., Senior Investigator MDB, NHLBI

Others: Thomas Eggerman, M.D., Ph.D., Medical Staff Fellow, MDB, NHLBI
Robert S. Ross, M.D., Medical Staff Fellow, MDB, NHLBI
Stephen J. Demosky, Jr., Chemist, MDB, NHLBI
Uwe K. Schumacher, Biologist, MDB, NHLBI
Barbara Winterrowd, Medical Technician, MDB, NHLBI

COOPERATING UNITS *(if any)*

Drs. Repin, Sviridov, Kosych, and Smirnov, Cardiocenter, Moscow, USSR

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Betheda, Maryland

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
3.0	3.0	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.)*

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-02028-05 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
Cathy Parrott	Chemist	MDB	NHLBI
Obaidullah Beg	Visiting Fellow	MDB	NHLBI

COOPERATING UNITS (if any): Washington Adventist Hospital, Washington, D. C.

LAB/BRANCH: Molecular Disease Branch

SECTION: Molecular Disease Branch

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.2	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 genetic defects that lead to deficiency of apoC-II in 2 apoC-II deficient patients from independent kindreds have been identified by sequence analysis. In the apoC-II$_{padova}$ gene a single base substitution results in the introduction of a premature termination codon and the synthesis of an inactive, truncated C-II$_{padova}$ apolipoprotein. In the apoC-II$_{paris}$ gene,a single base transition changes the initiation methionine codon to GUG which prevents the normal translation initiation of apoC-II. These studies have allowed us to identify non-obligate heterozygote for the gene defect and have established the molecular heterogeneity that underlies this syndrome.

Analysis of the lipoprotein lipase gene of a patient from the bethesda kindred at the cDNA level has revealed the presence of a point mutation that results in the substitution of Ala to Thr in the patient's lipoprotein lipase and may lead to the synthesis of a non-functional enzyme. These studies suggest that the region of the mutation is critical for lipoprotein lipase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02826-08 MDB

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of 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:	Ya-Jun Chen	Guest Worker	MDB, NHLBI
	Howard S. Kruth	Chief, Sect. of Exp. Athero.	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)

LAB/BRANCH
Molecular Disease Branch

SECTION
Section of Experimental Atherosclerosis

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 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 have recently described cholesterol-rich lipid particles located in advanced atherosclerotic lesions of rabbit and human aortas. These particles constitute a pathologic form of cholesterol that accumulates in the vessel wall during atherogenesis. The purpose of this project is to study the early development of these cholesterol-rich lipid particles in lesions.

No cholesterol-rich lipid particles were present in non-atherosclerotic aortas. In rabbits fed a cholesterol diet for 1 week, vesicular cholesterol-containing particles appeared in lesions. These lipid particles had a density of 1.09 g/ml. They contained cholesterol in a predominantly unesterified form (>95%) and had an unesterified cholesterol to phospholipid molar ratio (C/P) of only 0.6. In rabbits fed cholesterol for 1 month, cholesterol-rich lipid particles accumulated at densities ranging between 1.03 to 1.09 g/ml. In these particles, 89% of their cholesterol was unesterified and they had a C/P ratio of 2.0:1. These lipid particles were comprised of vesicles and multilamellar structures. In rabbits fed cholesterol for greater than 3 months, particles with similar structures accumulated in aortas. However, in these mature particles 77% of their cholesterol was unesterified and their C/P molar ratio was 2.5:1. They had an average density of 1.04 g/ml.

Our findings suggest there might be a precursor cholesterol-poor lipid particle in early lesions which matures to cholesterol-rich lipid particles in advanced lesions. These extracellular cholesterol-rich lipid particles may represent components of a metabolic pathway by which cells excrete excess cholesterol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02828-04 MDB

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Platelet-mediated Cellular Cholesterol Accumulation

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:	Howard S. Kruth	Chief, Sect. of Exp. Athero.	MDB, NHLBI

COOPERATING UNITS *(if any)*
Department of Transfusion Medicine, CC
Section of Laboratory Animal Medicine and Surgery, NHLBI
B.F. Dickens, Div. of Exp. Med., George Washington University

LAB/BRANCH
Molecular Disease Branch

SECTION
Section of Experimental Atherosclerosis

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	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.)*

We wondered what happens to cholesterol and phospholipid in dying cells such as platelets that make up thrombi, often associated with atherosclerotic lesions. Specifically, because cholesterol and phospholipid are associated with one another in membranes of cells, we wondered if changes occurred in phospholipids upon cell death that might affect the ability of cholesterol in these membranes to be mobilized. Therefore, we have examined the long term changes in phospholipids in rat and human platelets activated with thrombin and incubated for various times, up to three days.

Phospholipid content decreased significantly (80% decrease) in thrombin-activated rat platelets. Even though phospholipid (P) content decreased, cholesterol (C) content remained constant, thus, the C/P molar ratio increased. By contrast, phospholipid content of thrombin-activated human platelets did not change and the C/P molar ratio remained constant.

Because of the substantial loss of phospholipid observed in activated rat platelets, we next determined whether this was associated with lysis of platelets as monitored by release of lactate dehydrogenase (LDH), a cytoplasmic marker. Indeed, by 24 hours, essentially all platelet LDH had been released into the medium of activated rat platelets, whereas, only 13% of LDH was released into the medium of activated human platelets.

We have observed that activation of rat platelets results in significant platelet phospholipid hydrolysis while activation of human platelets does not result in significant phospholipid hydrolysis. The decrease in phospholipid content and resulting elevated C/P molar ratio in activated rat platelets may represent a mechanism to promote cholesterol transfer from degenerating cells to viable cells and acceptor plasma lipoproteins such as HDL. These findings may help elucidate the mechanism of cholesterol removal from degenerating cells such as platelets within thrombi or smooth muscle cells within atherosclerotic lesions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02030-02 MDB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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)*

PI: Gregory E. Tennyson, Senior Staff Fellow, MDB:NHLBI
Others: Thomas L. Eggerman, 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 Chemsitry

INSTITUTE AND LOCATION
NHLB, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	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.)*

Two B apolipoproteins (apo) circulate in man and represent isoprotein products from a single copy apoB gene, and are generated by an RNA editing mechanism which alters a single encoded nucleotide (CAA in apoB-100) to an edited one (UAA in apoB-48) thereby truncating the genomically encoded apoB-100 to apoB-48 size. We have evaluated the rat apoB gene and its transcripts for similar RNA editing. The rat apoB gene was found to exist as a single copy, and using the polymerase chain reaction (PCR) we identified an encoded CAA codon homologous to that in humans. By adapting the PCR to amplification of RNA, rat apoB transcripts from both liver and intestine were found to contain predominantly edited apoB mRNA. A survey of RNA editing in mammalian species subsequently demonstrated that human and rabbit liver and intestine appear remarkably similar containing predominantly encoded hepatic and edited intestinal apoB transcripts. Initial studies aimed at determining the primary apoB sequence required for RNA editing showed truncated apoB transcripts to be unstable in rat liver extracts, yielding nonspecific results in RNA mobility shift and UV - crosslinking assays. For simplified analysis of both modulation and the biochemical mechanism of RNA editing, in vitro systems were examined. Five rat hepatocyte cell lines were studied and two of these, cell lines Fao and H4IIEC3, were found to contain significant amounts of edited apoB mRNAs, as well as to secrete the corresponding edited isoprotein. Neither intact animal nor in vivo organ system are therefore required for apoB editing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02831-02 MDB

PERIOD COVERED
October 1, 1988 through September 30, 1989

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:	Daniel Rader	Med. Staff Fellow	MDB, NHLBI
	H. Bryan Brewer	Chief	MDB, NHLBI
	Howard S. Kruth	Chief, Sect. of Exp. Athero.	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-derived macrophages, a major cell that accumulates cholesterol in atherosclerotic lesions. Cholesterol also accumulates predominantly in macrophages in many genetically determined cholesterol storage diseases. We are, therefore, also using human monocyte-derived macrophages from patients with cholesterol storage diseases to investigate possible cellular abnormalities in lipoprotein and cholesterol processing in these diseases.

To characterize cholesterol metabolism in human monocyte-derived macrophages, we incubated these cells with lipoprotein and nonlipoprotein forms of cholesterol. Human monocyte-derived macrophages accumulate cholesterol when incubated with nonlipoprotein cholesterol, lipid particles isolated from the vessel wall, and lipid particles released from thrombin-activated human platelets. When human monocyte-derived macrophages were incubated with native low density lipoprotein or high density lipoprotein, macrophages do not accumulate cholesterol. However, if low density lipoprotein is previously degraded by exposure to cholesterol esterase, macrophages then accumulate cholesterol. Interestingly, when human monocyte-derived macrophages are incubated with nonlipoprotein cholesterol only a subpopulation of these cells accumulate cholesterol.

Because macrophages are one of the major types of cells which accumulate cholesterol in atherosclerotic lesions, it is of great significance to develop an in vitro model to study their cholesterol metabolism. By incubating macrophages with lipoprotein and nonlipoprotein cholesterol forms, we are able to examine various pathways of cholesterol metabolism in normal macrophages as well as in macrophages from patients with various cholesterol storage diseases.

Annual Report of the Pathology Branch
Division of Intramural Research
National Heart, Lung, and Blood Institute
October 1, 1988, to September 30, 1989

As in the past years, studies focused on morphologic aspects of coronary, myocardial, and valvular, and miscellaneous cardiovascular conditions.

CORONARY ARTERY DISEASE

During recent years, several studies have emanated from the Pathology Branch having to do with determining *the amount of cross-sectional area narrowing of each 5-mm segment of the 4 major* (left main, left anterior descending, left circumflex, and right) *epicardial coronary arteries in patients with fatal coronary artery disease.* A study compared findings in 4 subsets of coronary patients. Of the 129 patients studied at necropsy, an average of 2.7 of the 4 arteries were narrowed >75% in cross-sectional area at some point (controls = 0.7/4.0), and the group with unstable angina pectoris (3.2/4.0) had more narrowing than did the groups with sudden coronary death (2.8/4.0), acute myocardial infarction (2.7/4.0) and healed myocardial infarction (2.3/4.0). In the 129 patients, 35% of the 5-mm segments were narrowed 75-100% in cross-sectional area (controls = 3%) and the group with unstable angina had the highest percent (48%) of segments severely narrowed compared to the groups with sudden coronary death (36%), acute myocardial infarction (34%), and healed myocardial infarction (31%). Thus, of the 4 subsets of patients with fatal coronary artery disease, those with unstable angina pectoris had the most severe and extensive coronary atherosclerosis.

A new endeavor focused on the *composition of atherosclerotic plaques in the various subsets of coronary patients.* Each 5-mm segment of the 4 major coronary arteries was examined in 15 patients who died of consequences of acute myocardial infarction (AMI) and in 12 patients dying suddenly outside the hospital without acute myocardial infarction (sudden coronary death [SCD]). Within the AMI group and within the SCD group, there were no differences in plaque composition. Within both groups plaque morphology varied as a function of cross-sectional-area narrowing of the segments. In both groups, the amount of dense relatively acellular fibrous tissue, calcified tissue, and pultaceous debris increased in a linear fashion with increasing degrees of cross-sectional-area narrowing of the segments and the amount of cellular fibrous tissue decreased linearly. In the AMI group, the percent of plaque consisting of pultaceous debris and of cellular fibrous tissue separated significantly narrowed (>75% cross sectional area) segments from less narrowed (<75%) segments. A comparison of the AMI group to the SCD group showed signficant differences. The percent of plaque consisting of pultaceous debris (16% in the AMI group and 7% in the SCD group), of cellular fibrous tissue (11% -vs- 18%) and of heavily calcified tissue (8% -vs- 16%) were significantly different in the severely narrowed segments in the AMI and SCD groups. Thus, plaque composition differs in patients with AMI and in those with SCD without AMI.

Modes of death, frequency of a healed or an acute myocardial infarct or both, numbers of major epicardial coronary arteries severely narrowed by atherosclerotic plaque, and heart weight were studied at necropsy in 889 patients aged 30 years of age or older with fatal atherosclerotic coronary artery disease. No patient had had a coronary bypass operation or coronary angioplasty. The 889 patients were divided into 4 major groups and each major group was divided into 2 subgroups: 1) *acute myocardial infarct* without (306 patients) and with (119 patients) a healed myocardial infarct; 2) *sudden out-of-hospital death* without (121 patients) and with (118 patients) a healed myocardial infarct; 3) *chronic congestive heart failure with a healed myocardial infarct* without (137 patients) and with (33 patients) a left ventricular aneurysm; and 4) *sudden in-hospital death* without (20 patients) or with (35 patients) unstable angina pectoris. The mean age of the 687 men (77%) was 60 $\pm$ 11 years, and of the 202 women (23%), 68 $\pm$ 13 years ($p=.0001$). Although men included 77% of all patients, they made up approximately 90% of the out-of-hospital sudden death group, the chronic congestive heart failure group, and the in-hospital (non-angina) sudden death group. The frequency of systemic hypertension and angina pectoris was similar in each of the 4 major groups. The frequency of diabetes mellitus was least in the sudden-out-of-hospital death group and similar in the other 3 major groups. The mean heart weight and the percent of patients with hearts of increased weight was highest in the chronic congestive heart failure group and similar in the other 3 major groups. All patients in the chronic congestive heart failure group (by definition) had healed left ventricular infarcts, which were similar in frequency in the other 3 major groups. The percent of patients in whom 3 or 4 of the 4 major coronary arteries were severely narrowed (>75% in cross-sectional area) by atherosclerotic plaque was highest in the unstable angina subgroup and similar in all other major groups.

Review of 18 publications *before* the widespread use of cardiac care units disclosed that the frequency of rupture of the left ventricular free wall or ventricular septum among necropsy cases of acute myocardial infarction ranged from 4 to 24% (mean 8%) (619 of 7905 cases). We analyzed the frequency of rupture of the left ventricular free wall or ventricular septum among patients studied at necropsy in our laboratory since 1968 with fatal acute myocardial infarction. Of 648 such patients, 204 (31% had rupture of the left ventricular free wall or ventricular septum. Rupture occurred in 171 (40%) of 431 patients without healed myocardial infarcts (grossly visible left ventricular scars), and in 29 (13%) of 217 patients with a healed myocardial infarct ($p<.01$). Thus, the frequency of rupture of the left ventricular free wall or ventricular septum during acute myocardial infarction appears to have increased substantially since the widespread use of coronary care units, and the frequency of rupture is nearly 3 times greater in those in whom rupture occurred during the first acute myocardial infarction compared to those with a previous infarct which healed.

VALVULAR HEART DISEASE

We studied at necropsy 80 *opiate addicts with anatomic evidence of active* (59 patients) or *healed* (11 patients) *infective endocarditis (IE) or both* (10 patients). Of the 80 patients, the first episode of IE involved

a single right-sided cardiac valve in 24 patients (30%); both a right- and a left-sided valve in 13 patients (16%); a single left-sided valve in 33 patients (41%), and both left-sided valves in 10 patients (13%). Of the 320 cardiac valves in the 80 patients, 103 were sites of vegetations, an average of 1.3 of the 4 valves. Of the 80 patients, the tricuspid valve was infected in 35 (44%), mitral in 34 (43%), aortic in 32 (40%), and pulmonic in 2 (3%). Of the 103 infected cardiac valves, the infection caused sufficient damage to cause dysfunction in 70 (68%): in 28 (88%) of 32 infected aortic valves; in 22 (53%) of 35 infected tricuspid valves; in 19 (56%) of the 34 infected mitral valves; and in 1 of the 2 infected pulmonic valves. Of the 80 patients, 57 (71%) had sufficient valvular damage to cause valvular dysfunction. Of the 80 patients, gross examination of the valves at necropsy indicated that the infected valve almost certainly had been anatomically normal in 65 patients (81%) and abnormal in 25 patients (19%) before the onset of IE. Of the 65 patients with previously anatomically normal valves, 86 (33%) of their 260 cardiac valves were sites of infection (average 1.3 valves per patient); of the 15 patients with infection superimposed on a previously abnormal valve, the infection in each involved previously abnormal valves (21 in the 15 patients) or 17 (28%) of their 60 cardiac valves were sites of infection (average 1.1 valve per patient). Of the 15 patients with abnormal cardiac valves before the infection, 7 had congenitally bicuspid aortic valves and 8 had diffuse fibrous thickening of the mitral valve typical of rheumatic heart disease with (6 patients) or without (2 patients) diffuse fibrous thickening of 3-cuspid aortic valves.

Opiate addicts may get heart diseases other than infective endocarditis. We studied at necropsy 168 opiate addicts whose hearts were submitted for study with prime focus on modes of death and types of cardiac abnormalities. In the 168 patients, 20 various modes of death were identified; active infective endocarditis or its consequences in 67 (40%); drug overdose in 39 (24%), coronary artery disease in 14 (8%), pulmonary granulomatosis in 7 (4%), and 15 various diseases (7 cardiac and 8 non-cardiac) in the remaining 41 (24%) patients. Of the 168 hearts examined, only 7 (4%) were normal. Although infective endocarditis (active, healed or both) was most common [80 (48%) patients], there were a broad range of other cardiac abnormalities present: cardiomegaly in 114 (68%) (including 22 patients without another cardiac abnormality), coronary artery disease in 35 (21%), acquired valvular heart disease in 16 (10%), myocardial heart disease in 14 (8%), and congenital cardiac anomaly in 19 (11%).

MYOCARDIAL HEART DISEASE

The *morphologic features of 220 patients with hypertrophic cardiomyopathy (HC)* were described. The major gross morphologic features of HC, in order of decreasing frequency, were as follows: 1) dilated atria (100%); 2) increased heart weight (95%); 3) nondilated left ventricle (79%); 4) thickened mitral valve (75%); 5) fibrous endocardial mural plaque, left ventricular outflow tract (72%); and 6) a ventricular septum thicker than the left ventricular free wall (60%). Gross left ventricular fibrosis occurred in 84% of the patients and rarely was a consequence of

associated narrowing of an epicardial coronary artery disease. HC may cause death in any decade of life. The diagnosis of HC in the very young (age ≤10 years) and in the very old (age >70) is more difficult clinically and at necropsy than in the intermediate age groups. Patients with the obstructive type of HC usually have larger hearts, a higher frequency of a fibrous plaque in the left ventricular outflow tract, and a higher frequency of a thickened mitral valve than those without obstruction. Left ventricular cavity dilation was present in about 20% of patients with HC and was the result of either operation (with survival >43 months) or extensive left ventricular wall scarring.

The *sensitivity of the total 12-lead QRS amplitude was compared to certain standard electrocardiographic criteria for left ventricular hypertrophy in 57 necropsy patients with hypertrophic cardiomyopathy (HC).* The total 12-lead QRS amplitude ranged from 66 to 339 mm (mean 179) (10 mm = 1 mV). Using 175 mm as the upper limit of normal, this technique yielded a sensitivity of 53% which was the highest sensitivity of any criteria tested. The Sokolow-Lyon index had a sensitivity of 39%; the Romhilt-Estes voltage criteria, 37%; the Romhilt-Estes point score system, 49%, and the criterion of RV6 > RV5, 39%. No correlation was found between total 12-lead QRS voltage and heart weight, left ventricular free wall thickness, left ventricular peak systolic and end-diastolic pressures or left ventricular outflow tract peak systolic pressure gradient. The 10 patients (18%) with transmural left ventricular scars had significantly lower total 12-lead QRS voltage than did the 48 patients (78%) without such scars (155 mm - vs. - 205 mm, p=0.02). Total 12-lead QRS amplitude >175 mm is a useful indicator of left ventricular hypertrophy, and among patients with HC it is more sensitive than other more commonly employed criteria.

To evaluate possible relations between clinical and histopathologic cardiac findings in patients with the *acquired immune deficiency syndrome (AIDS)*, 58 consecutively autopsied AIDS patients were reviewed retrospectively. Twenty-six (45%) had histopathologic myocarditis. Fifteen of these 26 (58%) had ≥1 clinical cardiac abnormalities: 6 had congestive heart failure or left ventricular dysfunction, or both, 4 had ventricular tachycardia, 10 had electrocardiographic abnormalities, and 4 had pericardial abnormalities. Of the 32 patients without myocarditis, 6 (19%) had pericardial or electrocardiographic abnormalities, or both, but none had congestive heart failure, left ventricular dysfunction or ventricular tachycardia. Overall, clinical cardiac abnormalities were found in 21 patients (36%). Patients with myocarditis had a significantly higher incidence of clinical cardiac abnormalities than patients without myocarditis (58% vs 19%, p <0.01). All patients with congestive heart failure, left ventricular dysfunction or ventricular tachycardia had myocarditis. Thus, serious clinical cardiac abnormalities were common in patients with AIDS and were associated with myocarditis.

MISCELLANEOUS

The use of *interleukin 2 (IL-2)*, either alone or in combination with lymphokine activated killer-cells, tumor infiltrating lymphocytes, or other immunotherapeutic agents has added a new list of alternatives to conventional

antineoplastic regimens. Little information is available about the pathologic changes occurring in patients treated with these agents. In this study, we reviewed the necropsy materials from 19 patients, 12 men and 7 women with a variety of malignancies including melanoma, renal cell carcinoma, gastrointestinal and pulmonary adenocarcinoma, and metastatic gastrinoma who died after receiving IL-2 based immunotherapy. Death occured at intervals ranging from less than 1 hour to 143 days following the last dose of therapy. All patients dying at or less than 43 days following cessation of therapy had lymphoid infiltrates of varying intensity in residual tumor. At necropsy, the major cause of death, unrelated to the presence of metastatic tumor, was bacterial sepsis. In addition, we found evidence of significant cardiac and pulmonary toxicity: 2 patients with acute myocardial infarction, one with and one without significant coronary artery disease; 2 cases of unexplained lymphocytic myocarditis; and 1 case of fatal pulmonary capillary plugging following an infusion of LAK cells. Thus, not unlike other forms of therapy for cancer, IL-2 based immunotherapy does not appear to be without significant toxicity.

Connective tissue changes in myocardial interstitium were studied in patients with *chronic Chagasic cardiomyopathy*. It is known that the heart in such patients becomes extremely fibrotic; however the components of this fibrous tissue have not been characterized. A recent study reported accumulations of laminin in association with cardiac myocytes. Therefore, the present study was undertaken to investigate the morphology of basement membranes in chronic Chagasic cardiomyopathy. Morphologic examination of myocardium, including 5 right ventricular endomyocardial biopsies and 2 operatively resected left ventricular aneurysms, from 7 patients with chronic Chagas' disease disclosed widespread foci of marked thickening of the basement membranes of cardiac myocytes, endothelial cells and vascular smooth muscle cells. The thickened basement membranes had a homogeneous, finely fibrillar appearance, were not multilayered, and measured up to 1 um in thickness, compared with 500 A in unaffected areas. In addition to these alterations, nonspecific changes of myocyte hypertrophy and degeneration, interstitial fibrosis and lymphocytic infiltration were also found to a variable extent. The basement membrane changes observed in this study differ from those observed in other types of cardiomyopathies and may be helpful in distinguishing between idiopathic dilated cardiomyopathy and the cardiomyopathy associated with chronic Chagas' disease.

Summary of the Ultrastructure Section
Pathology Branch, National Heart, Lung, and Blood Institute
October 1, 1988, to September 30, 1989

Research interests of the ultrastructural sections have consentrated on the following areas:

Myocardial toxicology; the cardiomyopathies; metabolic diseases; bioprosthetic cardiac valves, and pulmonary diseases.

Myocardial toxicology. This unit has continued to explore various aspects of the chronic myocardial toxicity produced by the administration of anti-neoplastic agents of the anthracycline family, particularly doxorubicin. The latter agent has been shown to produce, as a dose-dependent phenomenon, a syndrome of dilated cardiomyopathy characterized clinically by congestive heart failure and anatomically by myofibrillar loss and dilatation of the sarcoplasmic reticulum of cardiac myocytes. We have previously shown that the chronic toxicity of doxorubicin can be considerably decreased by their concomitant administration of ICRF-187, a bis-dioxoketopiperazine. It has the mechanism of the cardiac toxicity of doxorubicin is very complex, it has been suggested that the mechanism of cardiotoxity of anthracyclines involves the formation of oxygen free radicals, and that the protective effect of ICRF-187 is due to its chelation of iron, which is necessary for the formation of these radicals.

A new animal model of chronic myocardial toxicity of doxorubicin was developed this year, using spontaneously hypertensive rats. These animals were found to be much more sensitive than were their genetically closely match normatensive Wistar-Kyoto rats to the development of the chronic cardiotoxicity produced by the administration of up to 12 weekly doses of 1 mg/kg of doxorubicin. ICRF-187 provided protection against doxorubicin cardiomyopathy in both types of rats. These observations are in accord with evidence suggesting that systemic hypertension is a risk factor in the development of doxorubicin cardiomyopathy in humans. We have used the hypertensive rat model to study the cardioprotective effects of a number of analogues of ICRF-187 on doxorubicin toxicity.

The beagle dog model was used to explore the possibility that pretreatment with ICRF-187 would allow an increase in the total cumulative dose of doxorubicin that could be given without inducing chronic cardiomyopathy. Such a study was necessary because the cardiac toxicity of doxorubucin previously limited the total cumulative dose of this agent that could be used safely clinically, and because simular dose limitations had been used in animal experiments. To test this hypothesis, studies were made of the influence of ICRF-187 on the functional and morphological effects of very large cumulative doses of doxorubicin given for up to two years. Adult beagles of either sex (6.2-11.6 kg) were given doxorubicin (1.75 mg/kg i.v.) either alone or 15 min after ICRF-187 (25 mg/kg i.v.) at 3-week intervals. Control dogs received ICRF-187 (25 mg/kg, i.v.) or 0.9% saline without doxorubicin. Each animal receiving doxorubicin alone (up to 14 mg/kg total cumulative dose) had severe myocardial lesions (lesion score 3+). Of the animals given ICRF-187 and doxorubicin, one that received 35 mg/kg doxorubicin had no lesions; of four given 43.75 mg/kg, three had no lesions and one had minimal lesions (lesion score 1+); of three given 52.5 mg/kg, one had minimal (lesion score 1+), and two had moderate (lesion score 2+) lesions. Control animals had no myocardial lesions. Thus ICRF-187 provided significant protection when administered with doxorubicin over a period of 90 weeks, and made it possible to give doses of doxorubicin which otherwise would have been lethal: these tolerated doses were at least 3 times larger than those which produce severe cardiomyopathy when given without ICRF-187. After giving such large doses of doxorubicin and

ICRF-187, gastrointestinal lesions became an important cause of death. Efforts are now being made to provide other types of protection for the gastrointestinal tract.

Based on animal studies such as those summarized above, a therapeutic trial of the effect of ICRF-187 on doxorubicin cardiotoxicity was undertaken in 92 human patients with metastatic breast cancer by Dr. J. Speyer and colleagues at New York University, and the Ultrastructure Section, NHLBI participated in this trial by evaluating myocardial biopsy specimens from patients in the study. The patients were divided into 2 groups, which received doxorubicin, cyclophosphamide and 5-fluorouracil, with or without ICRF-187, respectively. These 2 groups of patients did not differ significantly from each other in terms of tumor response or hematological alterations; however they did differ significantly in terms of their cardiovascular manifestations: Cardiac toxicity was evaluated by clinical examination, determination of the left ventricular ejection fraction by multigated nuclear scans, and endomyocardial biopsy. In comparison of the control group with the ICRF-187 group, congestive heart failure as observed in 11 as compared with 2 patients; the mean decrease in the left ventricular ejection fraction was 7 vs. 1 percent when the cumulative dose of doxorubicin was 250 to 399 mg per square meter (P=0.02), 16 vs. 1 percent at 400 to 499 mg (P=0.001), and 16 vs. 3 percent at 500 to 599 mg (P=0.003); and the Billingham biopsy score was 2 or more in 5 of 13 patients undergoing biopsy vs. none of 13 (P=0.03). Thus, ICRF-187 also provided cardioprotection in the context of cancer chemotherapy in human patients.

Familial and Metabolic Diseases. Based on the experience of the Pathology Branch, NHLBI, an extensive review was made of the pathology of the coronary arteries, including both the large, extramural and the small, intramural coronary arteries, in familial and metabolic diseases. Coronary artery disease in metabolic disorders may be a manifestation of one or more of the following processes, many of which result in lesions that have characteristic appearances: a) deposition of specific substances that accumulate in vascular walls as a consequence of the metabolic defect (the mucopolysacchaidosis, Fabry's disease, type II hyperlipoproteinema); b) accentuation of fibromuscular intimal proliferation (the mucopolysacchaidosis, homoystinui); c) acceleration of the "usual" atherogenic process, in which the metabolic disease acts as a risk factor, either directly by increased deposition of one or more of the components of atheroma or indirectly as a result of the coexistence of other processes, such as systemic hypertension (the hyperlipoproteinema, gout); d) calcification of coronary arteries; and e) lesions in the sinuses of Valsalva leading to compression of, or embolization to, coronary arteries (Ehlers-Danlos Syndrome.

The pathology of the cardiomyopathies. The pathology of the Cardiomyopathies continues to be a major area of interest of the Ultrastructure Section. An investigation of myocardial ultrastructure in Chagas' disease was made, based on the availability of myocardial biopsy specimens from Argentine patients with the chronic form of Chagas' disease. These studies, which were undertaken in collaboration with Dr. J. Milei, National Institute of Cardiology, Buenos Aires, Argentina, showed that the basement membranes of cardiac myocytes and cardiac capillaries are greatly thickened in patients with chronic Chagasic cardiomyopathy. It is most unusual to find such a degree of basement membrane thickening in cardiomyopathies of other causes. Immunohistochemical studies are now being made to try to identify the components responsible for this thickening.

A new type of cardiomyopathy was described. This cardiomyopathy is

associated with light chain disease, an amyloid-like syndrome in which immunoglobulin light chains are produced in large quantities. Such chains, however, are not capable of aggregating to form amyloid fibrils; instead, they form amorphous deposits. In myocardium, these deposits become localized along the basement membranes of cardiac myocytes and within the walls of myocardial small arteries and arterials. These deposits are composed of immunoglobulin light chains; they react with the appropriate immunohistochemical antisera, and they do not give the usual staining reactions of amyloid, but clinically they produce a syndrome of restrictive cardiomyopathy similar to that caused by amyloid deposits.

Bioprosthetic cardiac valves. As part of our continuing program of evaluation of structural changes that develop in bioprosthetic cardiac valves, attention was focused this year on collagen as the major component of tissue valves. Collagen in bioprosthetic valves exist in a crimped form, and the degree of crimping can be marketly influenced by the conditions of fixation at the time when the bioprosthesis is prepared. The degree of crimping influences the distribution of the mechanical forces to which the valve is subjected during opening enclosure, and for this reason it is considered desirable to retain the crimping as much as possible by using low pressure fixation. However, this issue has not completely resolved. We have made extensive studies of collagen crimping in porcine aortic valvular bioprostheses and in bovine pericardial bioprostheses. We have compared the results obtained by transmission electron microscopy, scanning electron microscopy and polarized light microscopy, and we have been able to devise a method by which the crimping can be evaluated by polarized light microscopy of intact leaflets using this technique we also have been able to study various other pathological changes that develop in collagen in bioprostheses. In other studies we have been able to determine that a solution of 1% benzy alcohol in Dulbecco's buffered saline was a satisfactory storage and transport medium for explanted bioprosthetic valves that need to undergo both morphological analysis and functional testing. Aldehyde-containing solutions can not be used for these dual purposes, because they interfer with functional testing by producing considerable stiffening of valvular leaflets. Treatment with benzyl alcohol resulted in acceptable preservation of tissue morphology.

Pulmonary diseases. Langerhans' cells are a defined subpopulation of the mononuclear phagocyte system known to accumulate in the lung in histiocytosis X, an interstitial lung disorder strongly linked to cigarette smoking. To evaluate the hypothesis that cigarette smoking itself may be associated with the accumulation of Langerhans' cells in the lung, normal nonsmokers (n=5) and normal smokers (n=10) were evaluated by bronchoalveolar lavage for the presence of Langerhans' cells as identified by the OKT6 monoclonal antibody and by transmission electron microscopy (Birbeck granules). While the OKT6 antibody identified 0.1 0.1% of the cells recovered from nonsmokers, it labeled 1.1 0.3% of those recovered from smokers ($p < 0.01$). Furthermore, while electron microscopy demonstrated no Langerhans' cells among the lavage cells from nonsmokers, 0.4 0.1% of the cells recovered from normal smokers contained Birbeck granules, identifying them as Langerhans' cells. Thus, the study demonstrates that Langerhans' cells are present in lower respiratory tract of smokers who do not have pulmonary histiocytosis X.

The Ultrastructure Section has undertaken a number of studies on in situ hybridization of nucleic acids to investigate various aspects of the molecular biology of the lung. These studies have been made in collaboration with, and under the guidance of, Dr. Ronald G. Crystal, Pulmonary Branch, NHLBI, and are summarized in the report of the Pulmonary Branch.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03949-01

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.)*
Qualitative & Quantitative Comparison of Amounts of Narrowing by Atherosclerotic

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
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.03	0.03	

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 amounts of narrowing of the 4 major (left main, left anterior descending, left circumflex, and right) epicardial coronary arteries by atherosclerotic plaques were compared in 4 subsets of coronary patients. Of the 129 patients studied at necropsy, an average of 2.7 of the 4 arteries were narrowed >75% in cross-sectional area at some point (controls = 0.7/4.0), and the group with unstable angina pectoris (3.2/4.0) had more narrowing than did the groups with sudden coronary death (2.8/4.0), acute myocardial infarction (2.7/4.0) and healed myocardial infarction (2.3/4.0). Each of the 4 major epicardial coronary arteries were divided into 5-mm long segments and a histologic section was prepared and stained by the Movat method of each of the 6,461 segments in the 129 patients and in the 1,849 segments in the 40 control subjects. In the 129 patients, 35% of the 5-mm segments were narrowed 75-100% in cross-sectional area (controls = 3%) and the group with unstable angina had the highest percent (48%) of segments severely narrowed compared to the groups with sudden coronary death (36%), acute myocardial infarctoin (34%), and healed myocardial infarction (31%). Thus, of the 4 subsets of patients with fatal coronary artery disease studied at necropsy, those with unstable angina pectoris have the most severe and extensive coronary artherosclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03950-01 PA

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.)*

Frequency of Myocarditis, Left Ventricular Dysfunction and Ventricular

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: Joseph M. Reilly, MD, Armed Forces Institute of Pathology, Washington, D.C, Robert E. Cunnion, MD, Clinical Center, David W. Anderson, MD, PhD, Timothy J. O'Leary, MD, PhD, J. Thayer Simmons, MD, H. Clifford Lane, MD, I, Laboratory of Immunoregulation, Anthony S. Fauci, MD, I, Laboratory of Immunoregulation, Renue Virmani, MD, Joseph E. Parrillo, MD, CC, Critical Care Medicine

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology, Washington, D.C.

LAB/BRANCH

Pathology Branch

SECTION

Cardiac Pathology Branch

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.09	0.09	

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 evaluate possible relations between clinical and histopathologic cardiac findings in patients with the acquired immune deficiency syndrome (AIDS), 58 consecutively autopsied AIDS patients were reviewed retrospectively. Twenty-six (45%) had histopathologic myocarditis. Fifteen of these 26 (58%) had ≥1 clinical cardiac abnormalities: 6 had congestive heart failure or left ventricular (LV) dysfunction, or both, 4 had ventricular tachycardia (VT), 10 had electrocardiographic abnormalities, and 4 had pericardial abnormalities. Of the 32 patients without myocarditis, 6 (19%) had pericardial or electrocardiographic abnormalities, or both, but none had congestive heart failure, LV dysfunction or VT. Overall, clinical cardiac abnormalities were found in 21 patients (36%). Patients with myocarditis had a significantly higher incidence of clinical cardiac abnormalities than patients without myocarditis (58% vs 19%, $p < 0.01$). All patients with congestive heart failure, LV dysfunction or VT had myocarditis. Thus, serious clinical cardiac abnormalities were common in patients with AIDS and were associated with myocarditis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03951-01 PA

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.)*
Recent Studies on the Effects of Beta Blockers on Blood Lipid

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
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.03	0.03	

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 effects of beta blockers on blood lipid levels were reviewed. This review was based on publications from other investigations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03952-01 PA

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.)*
Safety of Fenofibrate as Gained From Studies Worldwide

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
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.03	0.03	

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.)*

All data collected and published to date indicate that fenofibrate is a safe and well-tolerated lipid-lowering drug. Since its introduction in 1975, fenofibrate has been evaluated in 131 short- and long-term non-U.S. clinical studies involving 8,836 patients. Additionally, data has been collected by a postmarketing surveillance program initiated in France in 1979. Two recently completed double-blind, placebo-controlled, randomized U.S. studies conducted in 374 patients with types IIa and IIb or types IV or V hyperlipoproteinemia provide further evidence of the safety of fenofibrate.

In the U.S. studies, clinical adverse events occurred in 25% of fenofibrate-treated patients and 18% of placebo-treated patients. The 8% higher frequency of adverse effects with fenofibrate corresponds to the 6 to 11% frequency observed in European clinical trials. About 3% of patients receiving fenofibrate discontinued treatment because of clinical adverse reactions. The most common adverse reactions in all studies involved gastrointestinal, dermatologic, central nervous system or muscloskeletal systems.

Laboratory abnormalities in the U.S. studies occurred in 16% of fenofibrate-treated patients and in 7% of placebo-treated patients resulting in a discontinuation of treatment in 2% and 1% of patients, respectively. The difference between the 2 U.S. groups (9%) is similar to the frequency observed in the short-term European studies (6%). The most frequently reported (3-7%) laboratory adverse reaction has been an increase in liver enzymes. These elevations were not associated with clinical or functional disturbances of hepatic function and they usually normalized with continued treatment.

The safety profile of fenofibrate is superior to that of clofibrate and is at least equal to gemfibrozil. Fenofibrate is a safe drug for patients with hyperlipidemia based on the uniformity and consistency of its safety record in the European studies, U.S. studies, and European post-marketing surveillance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03953-01 PA

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.)*

Frequency of Rupture of the Left Ventricular Free Wall or Ventricular Septum

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.G. Reddy, MD, Cleveland Memorial Hospital, Cleveland, Ohio

COOPERATING UNITS *(if any)*

Cleveland Memorial Hospital, Cleveland, Ohio

LAB/BRANCH

Pathology Branch

SECTION

Cardiac Pathology Branch

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	

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.)*

Review of 18 publications *before* the widespread use of cardiac care units disclosed that the freqeuncy of rupture of the left ventricular free wall or ventricular septum among necropsy cases of acute myocardial infarction ranged from 4 to 24% (mean 8%) (619 of 7905 cases). We analyzed the frequency of rupture of the left ventricular free wall or ventricular septum among patients studied at necropsy in our laboratory since 1968 with fatal acute myocardial infarction. Of 648 such patients, 204 (31% had rupture of the left ventricular free wall or ventricular septum. Rupture occurred in 171 (40%) of 431 patients without healed myocardial infarcts (grossly visible left ventricular scars), and in 29 (13%) of 217 patients with a healed myocardial infarct ($p<.01$). Thus, the frequency of rupture of the left ventricular free wall of ventricular septum during acute myocardial infarction appears to have increased substantially since the widespread use of coronary care units, and the frequency of rupture is nearly 3 times greater in those in whom rupture occurred during the first acute myocardial infarction compared to those with a previous infarct which healed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03954-01 PA

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.)*
Usefulness of Total 12-Lead QRS Voltage Compared to Other Criteria

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: A.L. Dollars, MD , NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pathology Branch

SECTION
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	

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 sensitivity of the total 12-lead QRS amplitude was compared to certain standard electrocardiographic criteria for left ventricular (LV) hypertrophy in 57 necropsy patients with hypertrophic cardiomyopathy (HC). The total 12-lead QRS amplitude ranged from 66 to 339 mm (mean 179) (10 mm = 1 mV). Using 175 mm as the upper limit of normal, this technique yielded a sensitivity of 53% which was the highest sensitivity of any criteria tested. The Sokolow-Lyon index had a sensitivity of 39%; the Romhilt-Estes voltage criteria, 37%; the Romhilt-Estes point score system, 49%, and the criterion of RV6 > RV5, 39%. No correlation was found between total 12-lead QRS voltage and heart weight, LV free wall thickness, LV peak systolic and end-diastolic pressures or LV outflow tract peak systolic pressure gradient. The 10 patients (18%) with transmural LV scars had significantly lower total 12-lead QRS voltage than did the 48 patients (78%) without such scars (155 mm - vs. - 205 mm, p=0.02). Total 12-lead QRS amplitude >175 mm is a useful indicator of LV hypertrophy, and among patients with HC it is more sensitive than other more commonly employed criteria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03955-01 PA

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.)*
Infective Endocarditis in Opiate Addicts: Analysis of 80 Cases Studied

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: F.A. Dressler, MD, University Hospital-St. Louis University Medical Center, St. Louis, MO.

COOPERATING UNITS *(if any)*

University Hospital-St. Louis University Medical Center, St. Louis, MO

LAB/BRANCH
Pathology Branch

SECTION
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.03	0.03	

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 studied at necropsy 80 opiate addicts with anatomic evidence of active (59 patients) or healed (11 patients) infective endocarditis (IE) or both (10 patients). Of the 80 patients, the first episode of IE involved a single right-sided cardiac valve in 24 patients (30%); both a right- and a left-sided valve in 13 patients (16%); a single left-sided valve in 33 patients (41%), and both left-sided valves in 10 patients (13%). Of the 320 cardiac valves in the 80 patients, 103 were sites of vegetations, an average of 1.3 of the 4 valves. Of the 80 patients, the tricuspid valve was infected in 35 (44%), mitral in 34 (43%), aortic in 32 (40%), and pulmonic in 2 (3%). Of the 103 infected cardiac valves, the infection caused sufficient damage to cause dysfunction in 70 (68%): in 28 (88%) of 32 infected aortic valves; in 22 (53%) of 35 infected tricuspid valves; in 19 (56%) of the 34 infected mitral valves; and in 1 of the 2 infected pulmonic valves. Of the 80 patients, 57 (71%) had sufficient valvular damage to cause valvular dysfunction. Of the 80 patients, gross examination of the valves at necropsy indicated that the infected valve almost certainly had been anatomically normal in 65 patients (81%) and abnormal in 25 patients (19%) before the onset of IE. Of the 65 patients with previously anatomically normal valves, 86 (33%) of their 260 cardiac valves were sites of infection (average 1.3 valves per patient); of the 15 patients with infection superimposed on a previously abnormal valve, the infection in each involved previously abnormal valves (21 in the 15 patients) or 17 (28%) of their 60 cardiac valves were sites of infection (average 1.1 valve per patient). Of the 15 patients with abnormal cardiac valves before the infection, 7 had congenitally bicuspid aortic valves and 8 had diffuse fibrous thickening of the mitral valve typical of rheumatic heart disease with (6 patients) or without (2 patients) diffuse fibrous thickening of 3-cuspid aortic valves.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03956-01 PA

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.)*
Modes of Death, Frequency of Healed and Acute Myocardial Infarcts

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: B.N. Potkin, MD, Cedars-Sinai Medical Center, Los Angeles, CA
D.E. Solus, Undergraduate student, University of California at Davis, Davis, CA,
S.G. Reddy, MD, Cleveland Memorial Hospital, Cleveland, Ohio

COOPERATING UNITS *(if any)*
Cedars-Sinai Medical Center, Los Angeles, CA, University of California at Davis, Davis, CA, Cleveland Memorial Hospital, Cleveland, Ohio

LAB/BRANCH
Pathology Branch

SECTION
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.04	0.04	

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.)*

Modes of death, frequency of a healed or an acute myocardial infarct (MI) or both, numbers of major epicardial coronary arteries severely narrowed by atherosclerotic plaque, and heart weight were studied at necropsy in 889 patients aged 30 years of age or older with fatal atherosclerotic coronary artery disease (CAD). No patient had had a coronary bypass operation or coronary angioplasty. The 889 patients were divided into 4 major groups and each major group was divided into 2 subgroups: 1) *acute MI* without (306 patients) and with (119 patients) a healed MI; 2) *sudden out-of-hospital death* without (121 patients) and with (118 patients) a healed MI; 3) *chronic congestive heart failure (CHF) with a healed MI* without (137 patients) and with (33 patients) a left ventricular aneurysm; and 4) *sudden in-hospital death* without (20 patients) or with (35 patients) unstable angina pectoris. The mean age of the 687 men (77%) was 60 ± 11 years, and of the 202 women (23%), 68 ± 13 years (p=.0001). Of the patients studied, 53% had had systemic hypertension, 39% angina pectoris, and 33% diabetes mellitus. Of the 889 patients, 415 (48%) had 1 or more grossly visible left ventricular scars. Over half (51%) of the 889 patients had 3 or 4 (includes left main) major epicardial coronary arteries narrowed >75% in cross-sectional area by atherosclerotic plaque. The mean heart weight in the men was 505 ± 110 g, and in the 202 women, 427 ± 94 g (normal in men ≤400 g; in women, ≤350 g) (p=.0001), and only 20% of the patients had a heart of normal weight.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03957-01 PA

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.)*
Morphologic Features of Hypertrophic Cardiomyopathy

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: Charles Steward Roberts, MD, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pathology Branch

SECTION
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Betheda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	

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 morphologic features of 220 patients with hypertrophic cardiomyopathy (HC) were described. The major gross morphologic features of HC, in order of decreasing frequency, are as follows: 1) dilated atria (100%); 2) increased heart weight (95%); 3) nondilated left ventricle (79%); 4) thickened mitral valve (75%); 5) fibrous endocardial mural plaque, left ventricular outflow tract (72%); and 6) a ventricular septum thicker than the left ventricular free wall (60%). Gross left ventricular fibrosis occurred in 84% of the patients and rarely was a consequence of associated narrowing of an epicardial coronary artery disease. HC may cause death in any decade of life. The diagnosis of HC in the very young (age $\leq$10 years) and in the very old (age >70) is more difficult clinically and at necropsy than in the intermediate age groups. Patients with the obstructive type of HC usually have larger hearts, a higher frequency of a fibrous plaque in the left ventricular outflow tract, and a higher frequency of a thickened mitral valve than those without obstruction. Left ventricular cavity dilation is present in about 20% of patients with HC and is a result of either operation (with survival >43 months) or extensive left ventricular wall scarring.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03958-01 PA

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.)*
Modes of Death and Types of Cardiac Diseases in Opiate 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: F.D. Dressler, MD, University Hospital-St. Louis University Medical Center, St. Louis, MO

COOPERATING UNITS *(if any)*

University Hospital-St. Louis University Medical Center, St: Louis, MO

LAB/BRANCH
Pathology Branch

SECTION
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	

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 studied at necropsy 168 opiate addicts whose hearts were submitted for study with prime focus on modes of death and types of cardiac abnormalities. In the 168 patients, 20 various modes of death were identified; active infective endocarditis or its consequences in 67 (40%); drug overdose in 39 (24%), coronary artery disease in 14 (8%), pulmonary granulomatosis in 7 (4%), and 15 various diseases (7 cardiac and 8 non-cardiac) in the remaining 41 (24%) patients. Of the 168 hearts examined, only 7 (4%) were normal. Although infective endocarditis (active, healed or both) was most common [80 (48%) patients], there were a broad range of other cardiac abnormalities present: cardiomegaly in 114 (68%) (including 22 patients without another cardiac abnormality), coronary artery disease in 35 (21%), acquired valvular heart disease in 16 (10%), myocardial heart disease in 14 (8%), and congenital cardiac anomaly in 19 (11%).

Of the 35 hearts with various coronary artery disease, 28 had significant (>75%) narrowing of the cross-sectional area of 1 or more of the 4 major (left main, left anterior descending, left circumflex, and right) epicardial coronary arteries by atherosclerotic plaque. Of 112 coronary arteries in these 28 hearts, 52 (46%) were significantly narrowed (a mean of 1.9 of the 4 major coronary arteries per patient). In 27 of these 28 cases, each 5-mm segment of the 4 major coronary arteries were examined histologically. Of the 1435 five-mm segments examined, 189 (13%) were narrowed 76 to 100% in cross-sectional area by plaque; 347 (24%), 51 to 75%; 336 (23%), 26 to 50%; and 563 segments (39%) were narrowed 0 to 25% in cross-sectional area by plaque.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03959-01 PA

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.)*
Pathologic Findings Associated With Interleukin 2 Based Immunotherapy for Cancer

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:
A.H. Kragel, MD, NHLBI, W.D. Travis, MD, Laboratory of Pathology, NCI, L. Feinberg, MD, Mayo Medical School, Mayo Clinic, Rochester, MN, S. Pittalugia, MD, Laboratory of Pathology, NCI, L.M. Striker, MD, Metabolic Disease Branch, NIDDK, M.T. Lotze, MD, Surgery Branch, NCI, J.J. Yang, MD, Metabolic Disease Branch, NIDDK, S.A. Rosenberg, MD, Metabolic Disease Branch, NIDDK

COOPERATING UNITS *(if any)*
Mayo Medical School, Mayo Clinic, Rochester, MN

LAB/BRANCH
Pathology Branch

SECTION
Cardiac Pathology Branch

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.09	0.09	

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 use of interleukin 2 (IL-2), either alone or in combination with lymphokine activated killer-cells, tumor infiltrating lymphocytes, or other immunotherapeutic agents has added a new list of alternatives to conventional antineoplastic regimens. Little information is available about the pathologic changes occurring in patients treated with these agents. In this study, we reviewed the necropsy materials from 19 patients, 12 men and 7 women with a variety of malignancies including melanoma, renal cell carcinoma, gastrointestinal and pulmonary adenocarcinoma, and metastatic gastrinoma who died after receiving IL-2 based immunotherapy. Death occured at intervals ranging from less than 1 hour to 143 days following the last dose of therapy. All patients dying at or less than 43 days following cessation of therapy had lymphoid infiltrates of varying intensity in residual tumor. At necropsy, the major cause of death, unrelated to the presence of metastatic tumor, was bacterial sepsis. In addition, we found evidence of significant cardiac and pulmonary toxicity: 2 patients with acute myocardial infarction, one with and one without significant coronary artery disease; 2 cases of unexplained lymphocytic myocarditis; and 1 case of fatal pulmonary capillary plugging following an infusion of LAK cells. Thus, not unlike other forms of therapy for cancer, IL-2 based immunotherapy does not appear to be without significant toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03960-01 PA

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.)*

Morphometric Analysis of the Composition of Atherosclerotic Plaques

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:
A.H. Kragel, MD, NHLBI
S.G Reddy, MD, Cleveland Memorial Hospital, Cleveland, Ohio
J.T. Wittes, PhD, Biostatistics Research Branch, NHLBI

COOPERATING UNITS *(if any)*

Cleveland Memorial Hospital, Cleveland, Ohio

LAB/BRANCH

Pathology Branch

SECTION

Cardiac Pathology Branch

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.03	0.03	

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 studied at necropsy atherosclerotic plaque composition in the 4 major (right, left main, left anterior descending and left circumflex) epicardial coronary arteries in 15 patients who died of consequences of an acute myocardial infarction (AMI) and in 12 patients with sudden coronary death (SCD) without AMI. The coronary epicardial arteries were sectioned at 5-mm intervals, and a Movat stained section of each segment of artery was prepared and analyzed using a computerized morphometry system. Within the AMI group and within the SCD group, there were no differences in plaque composition among any of the 4 major epicardial coronary arteries. Within both groups plaque morphology varied as a function of cross-sectional-area narrowing of the segments. In both groups, the amount of dense relatively acellular fibrous tissue, calcified tissue, and pultaceous debris increased in a linear fashion with increasing degrees of cross-sectional-area narrowing of the segments and the amount of cellular fibrous tissue decreased linearly. In the AMI group, the percent of plaque consisting of pultaceous debris and of cellular fibrous tissue separated significantly narrowed (>75% cross sectional area) segments from less narrowed (<75%) segments. A comparison of the AMI group to the SCD group showed signficant differences. The percent of plaque consisting of pultaceous debris (16% in the AMI group and 7% in the SCD group), of cellular fibrous tissue (11% -vs- 18%) and of heavily calcified tissue (8% -vs- 16%) were significantly different in the severely narrowed segments in the AMI and SCD groups. Occlusive coronary thrombi were present in 13 of the 15 AMI cases and in 1 of the 12 SCD cases. Thus, the frequency of coronary thrombi and plaque composition differ in patients with AMI and in those with SCD without AMI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03961-01 PA

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.)*

Studies of the morphology of the bioprosthetic 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:
W.C. Roberts, Pathology Branch, NHLBI
M. Jones, Surgery Branch, NHLBI
Y. Tomita, Surgery Branch, NHLBI
S.L. Hilbert, Center for Devices and Radiological Health, FDA, Washington, D.C.

COOPERATING UNITS *(if any)*

Surgery Branch, NHLBI
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:
0.05	0.05	

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 studies were made of different aspects of the morphology of bioprosthetic cardiac valves: 1) in one of these studies 1% benzyl alcohol in Dulbecco's solution was found to be a satisfactory storage medium in which to keep explanted bioprosthetic heart valves that need to undergo both functional testing and morphological study. Valves kept in this solution retain adequate morphology and do not become excessively stiff (as do valves kept in aldehyde-containing fixatives); 2) Extensive reviews were made of the morphology of collagen in unimplanted and explanted bioprosthetic heart valves, of the anatomic changes that bioprosthetic valves undergo as the result of preimplantation processing, and of the pathological changes that develop after these valves are implanted as substitute cardiac valves in patients with valvular heart disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03962-01 PA

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.)*

Thickened cardiac basement membranes in Chagasic cardiomyopathy.

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

PI: Victor J. Ferrans, Chief, Ultreastructure Section, Pathology Branch, NHLBI
Others:
J. Milei, Instituto de Cardiologia, Academia Nacional de Medicina, Buenos Aires, Argentina
Y. Tomita, Surgery Branch, NHLBI
R.A. Storino, Instituto de Cardiologia, Academia Nacional de Medicina, Buenos Aires, Argentina

COOPERATING UNITS *(if any)*

Academia Nacinal de Medicina, Buenos Aires, Argentina
Surgery Branch, NHLBI

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

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.)*

Electron microscopic studies disclosed marked thickening of basement membranes in myocytes, capillary endothelial cells and vascular smooth muscle cells in myocardium of patients with chronic Chagasic cardiomyopathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03963-01 PA

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.)*

Langerhans cells accumulate in lower respiratory tract of smokers

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

PI: J. F. Bernaudin, Ultrastructure Section, Pathology Branch, NHLBI
Others:
M. Anthony Casolaro, Pulmonary Branch, NHLBI
V.J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Cesare Saltini, Pulmonary Branch, NHLBI
Ronald 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:
0.05	0.05	

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.)*

Immunohistochemical (OKT6 monoclonal antibody) and electron microscopic studies (presence of Birbeck granules) of cells recovered by bronchoalveolar lavage disclosed accumulation of small numbers of Langerhans' cells in lower respiratory tract of smoking individuals. Cells with these characteristics are extremely infrequent in bronchoalveolar lavage fluid of normal, nonsmoking individuals. Because of these findings it appears necessary to evaluate very carefully the number and the morphologic characteristics of Langerhans' cells in bronchoalveolar lavage fluid of patients suspected of having pulmonary histocytosis X.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03964-01 PA

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.)*

ICRF-187: Effect on doxorubicin cardiomyopathy in cancer patients

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:
J.L. Speyer, M.D. Green, E. Kramer, M. Rey, J. Sanger, C. Ward, N. Dubin, P. Stecy, A. Zeleniuchi-Jacquotte, J. Wernez, F. Feit, W. Slater, R. Blum, F. Muggia, New York University Medical Center, New York

COOPERATING UNITS *(If any)*

New York University Medical Center, New York

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

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 a randomized clinical trail ICRF-187 was found to provide cardiac protection (as shown by changes in echocardiographic measurements, radionuclide studies and endomyocardial biopsies) against the development of doxorubicin-induced cardiomyopathy in patients with metastatic carcinoma of the breast. This protection was exerted without significant clinical side effects and without interfering with the effectiveness of the anti-neoplastic therapy. Thus, ICRF-187 is useful in extending the therapeutic effectiveness of doxorubicin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03965-01 PA

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.)*
Coronary artery disease in metabolic disorders

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, Ultrastructure Section, Pathology, NHLBI
W.C. Roberts, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*
NONE

LAB/BRANCH
Pathology Branch

SECTION
Ultrastructure Section

INSTITUTE AND LOCATION
NHLBI/NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

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 extensive review was made, using a search of the literature and study of the files of the Pathology Branch, NHLBI of the pathologic anatomy of coronary arterial lesions that occur in various types of familial and metabolic diseases. Different patterns of morphologic alterations in the coronary arteries were recognized as occurring in association with certain types of disorders. The pathogenesis of these changes was discussed in detail in terms of the associated metabolic lesion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03966-01 PA

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.)*

Effect of ICRF-187 on the doxorubicin dose tolerated by dogs.

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:
Eugene H. Herman, Division of Drug Research Testing, FDA, Washington, D.C.
Robert S. K. Young, Division of Drug Research Testing, FDA, Washington, D.C.
R.L. Hamlin, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

COOPERATING UNITS *(if any)*

FDA, Washington, D.C.
Ohio State University, Columbus, Ohio

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

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.)*

ICRF-187 protected against the chronic cardiomyopathy induced by the administration of doxorubicin to beagle dogs. This protection continued when the total dose of doxorubicin was increased up to 3 times the usual dose that would produce cardiomyopathy in animals not pretreated with ICRF-187. ICRF-187 allowed full doses of doxorubicin to be given to beagle dogs for up to two years without producing cardiomyopathy, thus raising the possibility of using a similar mode of treatment in patients with cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03967-01 PA

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.)*

Effect of ICRF-187 doxorubicin cardiomyopathy in hypertensive rats

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

PI: Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Others:
E.H. Herman, Division of Drug and Research Testing, FDA, Washington, D.C.
A. El-Hage, Division of Drug and Research Testing, FDA, Washington, D.C.

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:
0.05	0.05	

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 were found to be more sensitive than the genetically closely related normotensive Wistar-Kyoto rats to the chronic cardiotoxic and nephrotoxic effects produced by the administration of 12 weekly doses of 1 mg/kg of doxorubicin; in both strains of rats these toxicities were attenuated by pretreatment with ICRF-187.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03968-01 PA

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.)*

The pathology of the cardiomyopathies

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:
Julian Sanchez, Ultrastructure Section, Pathology Branch, NHLBI
E. Rene' Rodriguez, Ultrastructure Section, Pathology Branch, NHLBI
Hugh A. McAllister, St. Luke's Episcopal Hospital, Houston, Texas
Jun Zhang, Ultrastructure Section, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

St. Lukes' Episcopal Hospital, Houston, Texas

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI/NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

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 extensive review was made of the pathologic anatomy of the cardiomyopathies, with emphasis on the following: 1) the different s btypes of hypertrophic cardiomyopathy; 2) the relationship of inflammatory myocarditis to idiopathic dilated cardiomyopathy; 3) the distinction between different types of endomyocardial diseases, and 4) various secondary cardiomyopathies. A new type of cardiomyopathy was described as being characterized by myocardial deposition of Kappa Light Chains of immunoglobulins.

Annual Report of the Pulmonary Branch
National Heart, Lung, and Blood Institute
October 1, 1988 through September 30, 1989

The research of the Pulmonary Branch centers on diseases of the alveolar structures, the site in the body where gases are exchanged between air and blood. All of the disorders under investigation are chronic, progressive, and often fatal. Together they affect more than two million individuals in the USA. Three categories of disease are investigated: destructive disorders, fibrotic disorders, and granulomatous disorders. All are characterized by chronic inflammatory processes in the lower respiratory tract. This local inflammation is central to the pathogenesis of each disorder as it is the inflammation that causes the changes in the lung parenchyma that results in lung dysfunction and eventual failure of the lung as an organ of gas exchange. In this context, the research of the Pulmonary Branch utilizes the tools of cellular biology, immunology, and molecular biology to understand the pathogenesis of these disorders and to develop new therapeutic strategies to prevent the progressive loss of functioning alveolar-capillary units.

Inflammation in the lower respiratory tract can be evaluated by bronchoalveolar lavage, a technique that permits direct access to the epithelial surface of the lower respiratory tract where inflammatory cells and relevant molecules in the epithelial lining fluid can be easily and repetitively sampled. To accomplish bronchoalveolar lavage, local anesthesia is administered to the upper respiratory tract and a fiberoptic bronchoscope is gently wedged into a distal bronchus. Sterile saline is infused into the bronchoscope and then suctioned back, thus recovering lower respiratory tract epithelial lining fluid and its contents. Over the past decade, this procedure has been used by the Pulmonary Branch to evaluate several thousand individuals. Using available cellular and molecular biologic methods, the numbers of inflammatory cells recovered by this procedure are sufficient to evaluate the <u>in situ</u> expression of the host of genes used by inflammatory cells participating in the pathogenesis of these disorders.

In addition to direct evaluation of the inflammatory processes ongoing in the lower respiratory tract, a significant amount of work by the Pulmonary Branch has been directed toward the structure and expression of genes coding for molecules that are capable of injuring the lung or protecting the lung from such injury. These studies relate to both basic mechanisms and hereditary disorders in which mutations in such genes have a profound influence on susceptibility to lung disease.

I. Disorders characterized by destruction of the alveolar walls.

This group of disorders, referred to as "emphysema," is associated with progressive dissolution of the lung parenchyma. The destruction of the alveolar walls is caused by a combination of events: a chronic, mild inflammatory process and a faulty protective screen insufficient to defend the alveolar walls from proteolytic enzymes released by the

inflammatory cells.

The most dramatic clinical example of this process is the hereditary disorder "α1-antitrypsin (α1AT) deficiency". α1AT is a 52 kDa, 394 amino acid, single chain glycoprotein normally present in serum at 20 to 48 μM. α1AT provides the major defense to the lower respiratory tract against the ravages of neutrophil elastase, a powerful serine protease. A variety of mutations in the coding exons of the α1AT gene result in "α1AT deficiency", permitting chronic neutrophil elastase attack of the lung, culminating in emphysema at an early age. The α1AT gene, composed of 7 exons dispersed over 12 kb of chromosomal segment 14q31-32.3, is expressed in hepatocytes and mononuclear phagocytes. The α1AT protein, a member of the class of protease inhibitors proteins known as Serpins (Serine Protease Inhibitors), is a globular molecule composed of 9 alpha-helices and 3 beta-pleated sheets. The major function of α1AT is to inhibit neutrophil elastase; α1AT does so through an active site centered around Met^{358} contained within an external stressed loop on the surface of the molecule. α1AT is a highly pleomorphic protein with greater than 75 variants determined at the protein and/or gene level. These variants can be categorized into four groups according to their serum α1AT level and function; normal, deficient, dysfunctional, and absent. Clinically relevant variants can be distinguished by a combination of isoelectric focusing of serum, restriction fragment length analysis of genomic DNA, oligonucleotide probes, allele specific amplification using the polymerase chain reaction (PCR), and direct sequencing of the variant α1AT genes.

The most common deficiency allele in the Caucasian population associated with a high risk for disease is the Z-type variant (allelic frequency 0.01-0.02), caused by a G to A nucleotide substitution in exon V, leading to a change in amino acid 342 of the mature protein (Glu^{342} GAG→ Lys^{342} AAG). This modification causes abnormal processing of the newly synthesized protein, resulting in accumulation within α1AT synthesizing cells, leading to deficient levels in the serum and hence the high risk for emphysema. From a clinical viewpoint, definitive identification of the Z homozygous state is important to estimate risk for disease, to make recommendations for augmentation therapy, and in some cases, prenatal diagnosis. Conventionally, the Z homozygous state is identified by an analysis of serum by combining a measure of the level of α1AT and analysis of the pattern of banding obtained by isoelectric focusing (IEF) at pH 4 to 5. We have expanded the methodology available to analyze the α1AT genome by adopting DNA amplification using the PCR together with ribonuclease A (RNase A) cleavage methodology to detect the single base substitution in exon V of the α1AT gene that defines the Z mutation. Taking advantage of the concept that RNase A will cleave at points of mismatch of RNA-DNA hybrids, a 0.79 kb antisense RNA probe was designed with complementarity to the sense strand of exon V of the α1AT gene (the site of the Z mutation) along with small regions of the 5′ and 3′ flanking sequences. After amplification of exon V of the α1AT gene from genomic DNA by PCR, the amplified DNA was analyzed by hybridization to a ^{32}P-labeled exon V antisense RNA probe followed by digestion with

RNase A. Any substitution mutations resulting in DNA-RNA mismatch were detected by evaluation with polyacrylamide gel electrophoresis under denaturing conditions followed by autoradiography (expected fragment lengths: 0.33 kb when the exon V probe hybridized to the normal amplified genomic DNA, 0.25 and 0.08 kb fragments when the exon V probe hybridized to the amplified genomic DNA with the Z mutation). Double-blinded evaluation of genomic DNA of 36 individuals (phenotypes MM n=14, MZ n=5, ZZ n=16, Znull n=1; included among the "M" alleles were representatives of all the major normal M alleles) demonstrated definitive diagnosis of the Z mutation with absolute specificity for all 36 specimens, i.e. ZZ homozygotes, MZ heterozygotes and normals were all detected accurately. This approach should be useful not only for screening for the Z mutation of the αlAT gene, but by this type of analysis, mutational alterations of the αlAT gene can be screened for without prior knowledge of the sequence changes and without complex cloning and sequencing methods.

A simple, rapid, nonradioactive method has been developed to facilitate the direct detection of point mutations causing genetic disease. The method is based upon the specific amplification of a target allele by PCR using extension primers designed such that their 3′ end is placed at the mutation site. When this base is complementary to that of the specific allele, the DNA segment is amplified; when it is not complementary, PCR cannot proceed. Utilizing αlAT deficiency as a model, the technique of allele specific amplification was capable of selective detection of five different mutations causing the αlAT deficiency state, including three different naturally occurring single base substitution mutations (alleles Z, S, and $Null_{bellingham}$), an insertion mutation ($Null_{mattawa}$) and a deletion mutation ($Null_{granite\ falls}$). Double blind evaluation of 47 samples of genomic DNA demonstrated 100% accuracy of the method. The technique of allele specific amplification is rapid, simple, and does not require the existence of a convenient restriction endonuclease site or the use of radioactive materials, and thus should have broad applicability for the detection of known genetic diseases in a highly sensitive and specific fashion.

The $M_{mineral\ springs}$ αlAT allele, causing αlAT deficiency and emphysema, is unique among the αlAT deficiency alleles in that it was observed in a Black family, while most mutations causing αlAT deficiency are confined to Caucasian populations of European descent. Immobilized pH gradient analysis of serum demonstrated that αlAT $M_{mineral\ springs}$ migrated cathodal to the normal M2 allele. Characterization of the αlAT $M_{mineral\ springs}$ gene demonstrated it differed from the common normal $M1(Ala^{213})$ allele by a single base substitution causing the amino acid substitution $Gly^{67}G\underline{G}G \rightarrow Glu^{67}G\underline{A}G$. Capitalizing on the fact that this mutation creates a polymorphism for the restriction endonuclease AvaII, family analysis demonstrated that the $M_{mineral\ springs}$ αlAT allele was transmitted as expected. Evaluation of genomic DNA showed that the index case was homozygous for the αlAT $M_{mineral\ springs}$ allele. Cytoplasmic blot analysis of blood monocytes of the $M_{mineral\ springs}$ homozygote demonstrated levels of αlAT mRNA transcripts comparable to cells of a

normal M1(Val^{213}) homozygote control. Evaluation of *in vitro* translation of $M_{mineral\ springs}$ αlAT mRNA transcripts demonstrated a normal capacity to direct the translation of αlAT. Evaluation of secretion of αlAT by the blood monocytes by pulse-chase labeling with [^{35}S]methionine, however, demonstrated reduced secretion by the $M_{mineral\ springs}$ cells compared to normals. To characterize the post-translational events causing the αlAT secretory defect associated with the αlAT $M_{mineral\ springs}$ gene, retroviral gene transfer was used to establish polyclonal populations of murine fibroblasts containing either a normal human M1 αlAT cDNA or an $M_{mineral\ springs}$ αlAT cDNA and expressing comparable levels of human αlAT mRNA transcripts. Pulse-chase labeling of these cells with [^{35}S]methionine demonstrated reduced secretion of human αlAT from the $M_{mineral\ springs}$ cells compared to the M1 cells, and evaluation of cell lysates also demonstrated reduced amounts of intracellular human αlAT in the $M_{mineral\ springs}$ cells compared to the normal M1 control cells. Thus, the Gly^{67}→Glu mutation that characterizes $M_{mineral\ springs}$ causes reduced αlAT secretion and hence "αlAT deficiency" and its associated risk for emphysema because the mutation likely renders the protein less stable, resulting in intracellular degradation prior to secretion.

The αlAT "Null" alleles are those for which no αlAT can be detected in the serum attributable to the gene. The intracellular consequences of the various substitution, deletion, and insertion mutations causing the Null state can be categorized into two groups: those associated with detectable αlAT mRNA transcripts and those with no detectable αlAT mRNA transcripts. To classify the intracellular mechanism associated with the $Null_{granite\ falls}$ allele (Tyr^{160} TA**C**, C deletion, 5′ frameshift → Stop^{160} TA**G**), a $Null_{granite\ falls}$ homozygote was evaluated. Genotypic diagnosis of the $Null_{granite\ falls}$ homozygous state was determined using PCR and $Null_{granite\ falls}$ specific primers. Total cellular RNA extracted from alveolar macrophages of the index case was compared to that from a normal M1 homozygote for the presence of αlAT mRNA transcripts using Northern blot analysis and hybridization to either a ^{32}P-labeled full length αlAT cDNA probe or (as a control) a ^{32}P-labeled γ-actin cDNA probe. Although the macrophages of both the $Null_{granite\ falls}$ homozygote and the normal showed γ-actin mRNA transcripts in comparable amounts, $Null_{granite\ falls}$ macrophages contained no detectable αlAT mRNA transcripts while the normal had the expected 1.8 kb αlAT mRNA transcripts. Thus, the $Null_{granite\ falls}$ allele can be classified along with $Null_{bellingham}$ as a Null allele associated with no detectable αlAT mRNA. These observations highlight the marked heterogeneity in the molecular processes causing the Null state, despite an identical phenotype at the clinical level.

The P-family of αlAT alleles is defined by the position of migration of the αlAT protein on isoelectric focusing of serum with immobilized pH gradient in the pH range 4.45-4.75, between the N and S variants. To evaluate the DNA sequence heterogeneity among this family of αlAT variants, genomic DNA of two unrelated individuals identified by isoelectric focusing as carrying the P allele was evaluated using direct sequencing of single stranded DNA generated from genomic DNA using the PCR and

unequal concentrations of primers flanking each of the 5′ coding exons of the αlAT gene. The most anodal variant on isoelectric focusing, P_{lowell}, differed from the normal M1(Val^{213}) allele by a single nucleotide substitution A→T causing an amino acid change Asp^{256} GAT→Val^{256} GTT. In contrast, $P_{saint\ albans}$, a slightly more cathodal variant, differed from the normal M1(Val^{213}) allele by 2 mutations, a G→A substitution causing the amino acid change Asp^{341} GAC→ Asn^{341} AAC and the other, a T→C substitution in the same codon as the P_{lowell} variant, but without an amino acid change, Asp^{256} GAT→ Asp^{256} GAC. Interestingly, among these variants, the $P_{saint\ albans}$ type is associated with normal serum levels of αlAT while the P_{lowell} variant is associated with levels approximately 35% of normal i.e., P_{lowell} is a moderately severe deficiency allele which, if inherited in a heterozygous combination with the Z-type or a Null αlAT allele, would be associated with an increased risk of emphysema. This is illustrated by the index case of the family with the P_{lowell} variant who, having the genotype $P_{lowell}Z$ has an αlAT level of 9 μM (normal 20-48 μM) which is below the threshold for protection from lung damage by neutrophil elastase (10 μM), and had severe emphysema. Thus, although these P variants have a similar pattern of migration on isoelectric focusing, they are heterogeneous at the nucleotide and amino acid level and the consequences of the amino acid substitutions to the αlAT proteins stability and/or conformation are dramatically different as evidenced by the deficiency state associated with the P_{lowell} variant and its attendant clinical sequelae.

Of the known αlAT variants, >95% in the U.S.A. Caucasian population are those of the "normal" M family, including M1(Ala^{213}), M1(Val^{213}), M2, and M3, with M3 the least common of the group. Quantification of the functional capacity of the M3 protein as an inhibitor of neutrophil elastase demonstrated a $K_{association}$ for neutrophil elastase of $10.1 \pm 1.5 \times 10^6\ M^{-1}\ sec^{-1}$, a value comparable to the common normal M1(Val^{213}) αlAT. To define the nucleotide sequence of the M3 gene, the five coding exons of the αlAT gene of an M3 homozygote were amplified by the PCR and cloned into the plasmid vector pUC19. Sequence analysis demonstrated that the αlAT M3 gene differs from the αlAT M1(Val^{213}) gene by a single base substitution (Glu^{376} GAA → Asp^{376} GAC) and from the αlAT M2 gene by a single base substitution (Arg^{101} CGT → His^{101} CAT). To establish the consistency of the αlAT M3 genotype among individuals identified by isoelectric focusing of serum to have the M3 phenotype, analysis of genomic DNA of 16 individuals by means of allele specific amplification revealed that residues 101 and 376 were Arg and Asp, respectively, in all M3 alleles, while residue 101 was His in all M2 alleles and residue 376 was Glu in all M1 alleles. Since the M1(Val^{213}) and M2 variants each differ from the M3 variant by single nucleotide substitutions at residues 376 and 101, respectively, it is likely that the M3 variant represents an intermediate in the evolution of the αlAT genes, occurring between the M1(Val^{213}) variant (the oldest of these 3 variants) and the M2 variant (the most recent).

In addition to emphysema, a subset of individuals with αlAT deficiency are at risk for liver disease. However, whereas there is an increased

risk for emphysema associated with at least 10 αlAT deficiency and null alleles, the hepatic disease is observed only in a subset of these alleles, suggesting it is not the reduced serum levels of αlAT, per se, that cause the liver disease. One example of this subset is the αlAT deficiency allele M_{malton}, an allele that like the common Z deficiency mutation ($Glu^{342} \rightarrow Lys$), is associated with both αlAT deficiency and hepatic disease. Capitalizing on the identification of the homozygous inheritance of the rare M_{malton} αlAT deficiency allele, it was demonstrated that, although caused by a very different mutation, the M_{malton} allele shares with the Z allele the association of liver disease with the same type of abnormalities of αlAT biosynthesis. Cloning of the M_{malton} gene and sequence analysis demonstrated that it differs from the normal αlAT M2 allele by deletion of the entire codon (TTC) for residue Phe^{52}. Liver biopsy of the M_{malton} homozygote revealed inflammation, mild fibrosis, and intrahepatocyte accumulation of αlAT. Evaluation of de novo αlAT biosynthesis in αlAT synthesizing cells of this individual demonstrated normal levels of αlAT mRNA transcripts but abnormal intracellular accumulation of newly synthesized αlAT at the level of the rough endoplasmic reticulum with consequent reduced αlAT secretion. Finally, retroviral gene transfer of a normal αlAT cDNA and an αlAT cDNA with the M_{malton} Phe^{52} deletion into murine cells demonstrated that the M_{malton} cells reproduced the abnormal accumulation of newly synthesized αlAT, thus directly demonstrating that the deletion mutation is responsible for the intracellular accumulation of the newly synthesized αlAT. Thus, not only is the liver disease associated with αlAT deficiency restricted to a subset of αlAT deficiency alleles, it appears to be restricted to those alleles associated with intracellular accumulation of newly synthesized αlAT, suggesting that it is the abnormal intrahepatocyte αlAT accumulation which incites the liver injury.

$M_{heerlen}$ is a rare αlAT mutation causing the deficiency state. The restriction fragment patterns obtained by probes covering the whole $M_{heerlen}$ gene and flanking sequences were normal, suggesting no major rearrangements. The nucleotide sequence of the exons, intron/exon junctions, and a part of the promoter region is similar to that of a PI M1(Ala^{213}) gene except for an C→T mutation in codon 369, causing a Pro→Leu substitution. Haplotype analysis and oligonucleotide hybridization studies demonstrated the homozygous state of the mutation in the index case. It is most likely that the $Pro^{369} \rightarrow Leu$ substitution is responsible for the low serum αlAT concentration of the patient because this mutation is solely confined to the $M_{heerlen}$ allele and no other relevant mutations could be revealed. As proline is important for the secondary and tertiary structure of proteins, the mutation may cause an abnormal processing of the nascent polypeptide.

Of the five known representatives of the "null" group of αlAT deficiency alleles evaluated at the gene level, all result from mutations causing the formation of stop codons in coding exons of the αlAT gene. We have identified a unique αlAT allele (referred to as $Null_{procida}$) defines a new subclass of αlAT deficiency null alleles caused by complete deletion of the αlAT coding exons. The $Null_{procida}$ allele was identified in a

individual with heterozygous inheritance of $M_{procida}$ (an allele associated with α1AT deficiency) and a null allele. Although karyotypic analysis was normal, quantification of the copies of α1AT genes in this individual revealed that the index case had only half the normal copies of α1AT genes. Cloning and mapping of the $Null_{procida}$ gene demonstrated a deletion of a 17 kb fragment that included exons II-V of the α1AT structural gene. As a consequence of the deletion, the normal noncoding exons (I_A-I_C) were followed by exons II-V of the downstream α1AT-like gene. Sequence analysis of the deletion demonstrated a 7 bp repeat sequence (GAGGACA) both 5′ to the deletion and at the 3′ end of the deletion, a 4 bp palindromic sequence (ACAG vs CTGT) bracketing the deletion, and a novel inserted 4 bp sequence (CCTG) at the breakpoint, suggesting the mechanism of the deletion may have been by "slipped mispairing". Interestingly, the structure of the $Null_{procida}$ gene approximates that of a fusion gene of exons I_A-I_C of the normal α1AT gene and exons II-V of the α1AT-like gene, providing further evidence that the α1AT-like gene is a pseudogene.

Although most α1AT in plasma is produced by liver hepatocytes, it is also known that the α1AT gene is expressed in mononuclear phagocytes. The single copy, 7 exon α1AT gene contains four protein coding exons (II-V) and three, 5′ untranslated exons (I_A, I_B, and I_C). Evaluation of liver and mononuclear phagocyte transcripts reveals two major α1AT transcript start sites: exon I_A - "macrophage" transcripts; and exon I_C - "liver" transcripts. In this regard, we have asked the questions: (1) Is there absolute segregation of 5′ untranslated exon usage among different cell types expressing the α1AT gene? and (2) Is there modulation of usage of different 5′ untranslated exons by resting or surface stimulated cells expressing the α1AT gene? To answer these questions, relative usage of "liver"- and "macrophage"-type transcripts by RNase protection of exon-specific ^{32}P-labeled cRNA probes. The data demonstrated that while resting monocytes use exon I_A "macrophage"-type transcripts 4-5 times more commonly than exon I_C "liver"-type transcripts, LPS stimulates monocytes to only upregulate exon I_C "liver"-type transcripts. With this background, we then asked: Are there regulatory sequences 5′ to exon I_C that might modulate enhanced expression of this exon in mononuclear phagocytes? In this regard, analysis of the 5′ region of α1AT gene reveals 2 binding sites for the c-jun proto-oncogene transcription factor 5′ to α1AT exon I_C. Following surface stimulation, monocytes exhibit increased mRNA levels for the c-jun proto-oncogene, with PMA upregulating c-jun far more than LPS. When stimulated by LPS, monocytes upregulate α1AT mRNA levels by increasing relative numbers of "liver"-type transcripts, but when stimulated by PMA, monocytes down regulate all α1AT transcript levels. Thus, modulation of α1AT gene expression in mononuclear phagocytes is complex, involving at least 2 different major transcription start sites. The transcription start site favored by resting monocytes is not upregulated by surface stimuli but the transcription start site favored by liver can be upregulated in monocytes. Further, if the c-jun response elements 5′ to exon I_C play a role in the expression of "liver"-type α1AT transcription in monocytes, the mechanisms involved are not simply a direct correlation of relative

regulation of c-jun and αlAT mRNA levels.

Neutrophil elastase, the potent serine protease that causes the lung destruction in αlAT deficiency, is carried and released by activated neutrophils. However, neutrophil elastase is not synthesized by neutrophils, but only by their bone marrow precursor cells. Using in situ hybridization with [^{35}S]-labeled antisense and sense neutrophil elastase cRNA probes, we have demonstrated that expression of the neutrophil elastase gene is tightly controlled in bone marrow precursors and occurs during a very limited stage of differentiation of the neutrophil myeloid series, almost entirely at the promyelocyte stage. Neutrophil elastase mRNA transcript levels are detectable to a limited extent in blasts, increase markedly in the promyelocyte stage, and then disappear as promyelocytes further differentiate. Control probes specific for myeloperoxidase, lactoferrin, and β-globin mRNA transcripts, respectively, demonstrated contrasting gene expression. Myeloperoxidase mRNA transcripts were also found almost exclusively at the promyelocyte stage, but myeloperoxidase mRNA levels disappeared earlier than do neutrophil elastase mRNA levels, suggesting that expression of these genes may be differently controlled. In comparison, lactoferrin mRNA transcripts were detected late in the neutrophil lineage, while β-globin mRNA was detected only in cells of the erythroid lineage. Together these observations suggest that the expression of the neutrophil elastase gene is likely under very tight control, and is likely different than that for other constituents of the neutrophil granules.

Because mature neutrophils cannot synthesize NE, the amount of NE activity in the region surrounding a neutrophil can only be modulated by varying the amount of NE released by the cell or by inhibiting the function of NE with antiproteases such as αlAT. In the context of the danger posed by uninhibited NE in the extracellular milieu, we evaluated the hypothesis that the neutrophil may be capable of modulating at least some of its own extracellular NE activity by synthesizing and secreting αlAT. This hypothesis was based on the knowledge that: (1) despite the fact that neutrophils are regarded as terminally differentiated cells and cannot synthesize NE, they can synthesize a variety of proteins; and (2) immunohistochemical studies have demonstrated αlAT in the neutrophil. Northern analysis and in situ hybridization with αlAT-specific probes demonstrated the presence of αlAT mRNA transcripts within neutrophils. [^{35}S]methionine-labeling of neutrophils followed by immunoprecipitation of the supernatant with an anti-αlAT antibody and SDS-acrylamide gel analysis demonstrated that neutrophils can synthesize αlAT de novo and secrete the synthesized molecule. In the presence of major neutrophil degranulation, the antiprotease effect of neutrophil αlAT is overwhelmed, allowing the NE to act unopposed in the extracellular microenvironment. However, in conditions where small amounts of NE are released by neutrophils, at least some of the secreted newly synthesized αlAT was capable of complexing with NE. Thus, despite the fact that the neutrophil cannot synthesize NE, it can synthesize and secrete αlAT, the inhibitor of NE i.e., the neutrophil is capable, to some extent, of modulating NE activity in the local milieu, without the help

of antiproteases produced by other cells.

In a series of studies of the past decade, the Pulmonary Branch, NHLBI developed a specific treatment for αlAT deficiency in the form of weekly intravenous infusions of 60 mg/kg of purified human plasma αlAT. This therapy adequately augments serum and lung αlAT levels and effectively reconstitute the anti-elastase screen of the lung in these individuals. On the basis of these studies, in December, 1987, the FDA approved αlAT augmentation therapy for general use. In an attempt to reduce the frequency of therapy, studies in 20 individuals with αlAT deficiency have shown that monthly intravenous infusions of 250 mg/kg of purified αlAT is also fully capable of adequately augmenting serum and lung αlAT levels and anti-elastase capacity, and is therefore a rational alternative to weekly therapy. In the past year, we have attempted to reduce the frequency of administration of αlAT even further by evaluating the concept that isovolumetic continuous flow centrifugation with αlAT (52 kDa) substituting for albumin (66 kDa) as the major oncotic force in plasma would permit even larger amounts of αlAT to be delivered to the circulation. To evaluate this, "plasma exchange" therapy of αlAT deficiency was carried out in 4 Z homozygotes using up to 3 liter exchange permitting delivery of as much as 550 mg/kg in less than 2 hrs. With this approach peak serum levels of up to 3300 mg/dl can be achieved and the time of administration is markedly reduced compared to the conventional intravenous route. However, despite dramatic augmentation of peak serum αlAT levels, the duration of time serum αlAT levels remained above the threshold protective level apparently cannot be lengthened i.e., after 1 month, the serum levels become insufficient to protect the lung. Thus, while plasma exchange therapy markedly shortens the time required to achieve adequate therapy for 1 month, apparently the body will not allow serum levels to persist at markedly exaggerated levels for very long i.e., once monthly therapy with 250 mg/kg seems to be the maximum limit that can be effectively administered.

With a normal human αlAT cDNA and molecular genetic manipulations of yeast, a recombinant form of α1-antitrypsin (rAAT) can be produced in large quantities. Although the rAAT functions normally as an inhibitor of human neutrophil elastase, it differs from the normal plasma molecule (pAAT) in that: (1) rAAT lacks the 3 carbohydrate side chains on pAAT; (2) rAAT has a N-acetyl-methionine at the N-terminus preceding the normal Glu^1 residue; and (3) rAAT has a markedly shortened half-life after being infused intravenously. Since one explanation for its rapid clearance is that the rAAT is less stable than pAAT, we evaluated the stability of rAAT to physical stress. Resistance to denaturation by urea was assessed using a denaturing gradient gel in which a gradient of urea from 0 to 8 M was formed across a polyacrylamide gel perpendicular to the direction of electrophoresis of the protein. Since protein denaturation increases the size of the protein, migration would be slowed as the protein unfolds. However, the curves formed by recombinant and normal αlAT in this assay were superimposable demonstrating that the stability to urea denaturation of the recombinant protein is identical to normal. Evaluation of the thermal stability at 37°, 12 hr for rAAT and pAAT

demonstrated the 2 molecules retained anti-neutrophil elastase activity, but at higher temperature (56°) rAAT lost activity more rapidly than pAAT. Finally, resistance to oxidation was assessed by subjecting recombinant and plasma αlAT to oxidation with $HOCl^-$. Following oxidant exposure to 10 to 80 μM for 10 min, the rAAT and pAAT exhibited similar neutrophil elastase inhibitory activity. These observations demonstrate that rAAT resists physical stress in a manner sufficient to function as an effective inhibitor of neutrophil elastase under physiologic conditions and that the carbohydrate side chains play a limited role in maintaining the stability of the molecule. In terms of its response to harsh environments, rAAT is a good candidate for use in therapy of disorders such as αlAT deficiency.

Although yeast produced rAAT has normal function in vitro, its lack of carbohydrate side chains is associated with high renal clearance and very short half-life, thus obviating its chronic intravenous administration to humans. To circumvent this problem, we evaluated administration of rAAT to αlAT deficient individuals by the aerosol route i.e., directly targeting the rAAT to the site of disease. Following aerosol administration of single doses of 10 mg to 200 mg of rAAT, utilizing an aerosol generating system which optimally targeted rAAT directly to the lower respiratory tract epithelial lining fluid (ELF) αlAT levels and anti-neutrophil elastase capacity increased in proportion to the dose of rAAT administered. ELF αlAT levels and anti-neutrophil elastase capacity 4 hr post 200 mg rAAT aerosol were increased 40-fold over pre-aerosol levels, and were 5-fold increased over baseline at 24 hr post-aerosol. Importantly, rAAT was detectable in serum following aerosol, suggesting that the lower respiratory tract epithelium may be permeable to rAAT, and that aerosolized rAAT may be capable of gaining access to lung interstitial tissues. No adverse clinical effects were noted as a result of rAAT aerosol administration. These observations demonstrate that aerosol administration of rAAT is safe and results in significant augmentation of lung anti-neutrophil elastase defenses, demonstrating that this method is a feasible approach to therapy for αlAT deficiency. Since this approach to therapy is clinically unproven, further studies will be necessary to establish the long-term clinical efficacy of aerosol rAAT therapy in αlAT deficiency.

To determine if aerosolization of purified human plasma αlAT is effective as a means of increasing lower respiratory anti-neutrophil elastase defenses in αlAT deficiency, we have carried out a non-randomized, before-after trial with a 7 day treatment period. Twelve individuals with homozygous Z-type αlAT deficiency and mild to moderate emphysema were administered an aerosol of human plasma αlAT, 100 mg every 12 hours for 7 days. Single 100 mg aerosol dose to anesthetized sheep with indwelling thoracic lymph duct catheters was also carried out to directly assess lung permeability. In the patients, treatment resulted in increase in lung epithelial lining fluid αlAT levels in the lung epithelial lining fluid and increased antineutrophil elastase capacity. Aerosolized αlAT diffused across the respiratory epithelium and entered lung interstitial lymph (in sheep) and reached the systemic circulation (in sheep and

man). No side effects were noted. We conclude that short-term aerosol administration of human plasma αlAT in αlAT deficient individuals is safe and feasible, resulting in the normalization of lower respiratory tract anti-neutrophil elastase defenses.

To evaluate the role of the carbohydrate side chains on the in vivo behavior of αlAT after it enters the circulation, oligonucleotide directed mutagenesis and retroviral gene transfer were used to direct murine fibroblasts to produce human αlAT molecules in which one or more asparaginyl attachment sites had been eliminated. αlAT molecules were produced with all three carbohydrate side chains, αlAT molecules missing the carbohydrate side chains attached at Asn^{46}, Asn^{83}, or Asn^{247}, and αlAT molecules missing all three carbohydrates. All of these variants functioned as inhibitors of human neutrophil elastase in a fashion similar to that of the mouse fibroblast produced human αlAT with all three carbohydrate side chains. In contrast, the lack of even a single carbohydrate side chain markedly modified the in vivo behavior of the αlAT molecule such that its half-life following intravenous infusion into a mouse was significantly reduced. While the serum half-life of the fully glycosylated molecule was 2.0 days, the half-life of the variant missing the Asn^{46} side chain was 1.6 days, the 83^{-} variant was 1.0 days, the 247^{-} variant was 1.6 days, and the variant missing all side chains was 0.5 days. Thus, for this serum glycoprotein, while the carbohydrate side chains do not influence the function of the molecule, they do play a major role in modulating the availability of the molecule after it has been secreted into the vascular space.

Gene therapy represents a future approach to preventing the emphysema associated with αlAT deficiency. Prior studies from our laboratory (Science 237:762, 1987) demonstrated that the ecotropic retroviral vector N2-FAT stably integrates the human αlAT cDNA under control of the constitutive early SV40 promoter into the genome of mouse fibroblasts, resulting in the production and secretion of glycosylated, functional human αlAT. With this background, over the past year, we have evaluated the concept that T-lymphocytes could be used as a cellular vehicle for gene therapy of αlAT deficiency. There are several theoretical advantages to this approach. T-cells do not normally produce αlAT, but can secrete proteins; they can be readily maintained in vitro with interleukin-2; they can be easily characterized for safety evaluation; and they are differentiated (and thus have less potential for modulation of transferred gene). Further, the methodology for autologous T-cell transplantation has been established, and the antigen specificity of the T-cell permits specific selection and potential for in vivo homing and expansion. Using a retroviral vector containing the human αlAT cDNA, we have demonstrated that T-lymphocytes can be modified to synthesize and secrete human αlAT and transplantation of these cells to mice can augment serum and lung epithelial lining fluid levels of αlAT in a physiologic manner. Importantly, modification of the genome of T-cells to include an αlAT cDNA does not alter the exquisite sensitivity of T-cells to specific antigen. Capitalizing on this, T-cells modified to secrete αlAT can be directed to accumulate preferentially in a specific loca-

tion. Further, using retroviral gene transfer, human T-cells can be modified to secrete αlAT, suggesting it should be possible to adopt these techniques for gene therapy of αlAT deficiency.

As another approach to gene therapy of αlAT deficiency, we capitalized on the knowledge that the αlAT-synthesizing cells of individuals with the Z deficiency gene have normal αlAT messenger RNA levels, but αlAT secretion is markedly reduced secondary to accumulation of newly synthesized αlAT in the rough endoplasmic reticulum. Crystallographic analysis of αlAT predicts that in normal αlAT, a negatively charged Glu^{342} is adjacent to positively charged Lys^{290}. Thus the $Glu^{342} \rightarrow Lys^{342}$ Z mutation causes the loss of a normal salt bridge, resulting in the intracellular aggregation of the Z molecule. The prediction was made that a second mutation in the αlAT gene that changed the positively charged Lys^{290} to a negatively charged Glu^{290} would correct the secretion defect. When the second mutation was added to the Z-type complementary DNA, the resulting gene directed the synthesis and secretion of amounts of αlAT similar to that directed by the normal αlAT complementary DNA in an in vitro eukaryotic expression system. This suggests the possibility that a human hereditary disease can be corrected by inserting an additional mutation in the same gene.

With availability of augmentation therapy to prevent lung destruction in αlAT deficiency, it is rational to initiate therapy once there is clinical evidence of disease. Conventional clinical evaluation includes chest x-ray, lung volumes, flow rates, diffusing capacity, and arterial blood gases. In a search for methods that will lead to earlier diagnosis, we have evaluated the concept that scintigraphic assessment of ventilation and perfusion and chest thin section computed tomography may be more sensitive than conventional approaches in assessing lung destruction in αlAT deficiency. Assessment of 25 Z homozygotes with αlAT deficiency demonstrated that 18 had lung abnormalities as detected by conventional x-ray and lung function methods. All those with abnormal lung function had abnormal V-Q and CT scans. However, of the 7 with normal lung function, 5 had lung destruction detected by both V-Q scanning and CT scanning. We conclude that both V-Q and CT scanning are more sensitive than conventional methods in detecting lung destruction in αlAT deficiency. In this regard, individuals with αlAT deficiency who are judged normal by conventional methods should be further investigated with V-Q and/or chest CT scans. In the context of the availability of αlAT augmentation therapy for αlAT deficiency, it is rational to seriously consider initiation of therapy in those individuals in whom lung destruction is clearly documented.

The emphysema of αlAT deficiency is conceptualized to result from insufficient αlAT allowing neutrophil elastase to destroy lung parenchyma. In addition to the deficiency of αlAT in these individuals resulting from mutations in the αlAT gene, it is recognized that, for unknown reasons, there are also increased numbers of neutrophils in their lungs compared to normal individuals. With the knowledge that alveolar macrophages have surface receptors for neutrophil elastase, we hypothesized that the neu-

trophil accumulation in the lower respiratory tract in α1AT deficiency may result, in part, from release of neutrophil chemotactic activity by alveolar macrophages as they bind uninhibited neutrophil elastase. Consistent with this hypothesis, α1AT deficient alveolar macrophages spontaneously released nearly 3-fold more neutrophil chemotactic activity than normal alveolar macrophages. Analysis of α1AT deficient macrophage supernates by reverse phase, high pressure liquid chromatography, molecular sieve chromatography, and radioimmunoassay revealed that the majority of the chemotactic activity was leukotriene B_4 (LTB_4), a mediator absent from normal macrophage supernates. Consistent with this hypothesis, incubation of normal macrophages with human neutrophil elastase resulted in the release of the same neutrophil chemotactic mediator. Furthermore, purified human α1AT was able to prevent the neutrophil elastase from stimulating the macrophages to release the chemotactic factor. Together, these findings suggest that the absence of a normal anti-neutrophil elastase screen in the lower respiratory tract permits free neutrophil elastase to bind to alveolar macrophages, resulting in the release of LTB_4, a process which attracts neutrophils to the alveoli of α1AT deficient individuals, thus accelerating the lung destruction that characterizes this disorder.

In the context of the broad substrate range of neutrophil elastase, we have evaluated the context that it might be used as a therapeutic strategy in attacking the envelope protein GP120 of the human immunodeficiency virus (HIV). GP120, the major extracellular envelope protein of HIV, serves to target the virus to CD4+ cells and initiates membrane fusion during viral infection. Since these functions are dependent on the unique characteristics of the highly glycosylated 483 amino acid GP120 structure, intra- or extracellular cleavage of GP120 may provide a means of suppressing HIV infection. In this context, we evaluated the susceptibility of GP120 to a human protease present in inflammatory cells, human neutrophil elastase, an enzyme which has the capacity to function at neutral pH as an omnivorous protease. As assessed by denaturing SDS-polyacrylamide gels, recombinant glycosylated GP120 was readily cleaved by equimolar concentrations of purified NE in a time dependent manner (1 to 20 min., 22°), initially producing a 50 kDa peptide and several smaller fragments. With increasing time of digestion all fragments, including the 50 kDa fragment, was degraded to peptides smaller than 20 kDa. Western blot analysis with a monoclonal antibody directed toward an epitope defined by residues 482-493, demonstrated that the 50 kDa peptide occupies the major portion of the C-terminus of GP120, including the CD4 binding region (residues 414-460). Preincubation of the NE with α1AT, or the specific synthetic inhibitor phenylmethylsulfonyl fluoride prevented the cleavage of GP120, demonstrating the specificity of the reaction and that it can be modulated. Functional studies demonstrated that CD4 binding by GP120 is eliminated by neutrophil elastase proteolysis. We conclude that, if appropriately shielded from antiproteases and targeted to infected or potentially infected cells, neutrophil elastase may be capable of preventing HIV assembly and/or infectivity.

Secretory leukoprotease inhibitor (SLPI), a 12 kD nonglycosylated serine antiprotease capable of inhibiting neutrophil elastase, is produced by cells of mucosal fluids and surfaces, including the human lung. Immunohistologic studies have demonstrated SLPI in the serous cells of submucosal bronchial glands and in non-ciliated cells of the bronchial and bronchiolar epithelium, suggesting it is produced throughout the bronchial tree, but in greater concentrations in the upper airways. To evaluate the consequences of this skewed distribution of SLPI producing cells, we measured the levels of SLPI in each of 5, 20 ml sequential bronchoalveolar lavage specimens in healthy normal nonsmokers using an enzyme linked immunoassay and anti-SLPI antibody. In upper airways fluid, the concentrations of SLPI are 3-fold greater than α1AT, consistent with the concept that SLPI provides the major anti-neutrophil elastase screen of the upper airways. In the lower respiratory tract fluid, there is more α1AT than SLPI, but concentrations of SLPI are 50% of α1AT, suggesting SLPI may be more important to the lower respiratory tract than previously thought. However, evaluation of the functional capacity of the SLPI in the lower respiratory tract suggests it is only 25-39% functional. The mechanisms causing the SLPI to be nonfunctional are not, as yet, known, but may result from prior interaction with proteases or inactivation by local processes i.e., SLPI may be the first line defense against neutrophil elastase. Independent of the mechanisms inactivating the SLPI, these observations are consistent with the concept that, in the steady state, α1AT is, by far, the major anti-neutrophil elastase protective molecule in the lower respiratory tract.

II. Disorders characterized by extensive fibrosis of the lung parenchyma

These disorders represent a large subgroup of the interstitial lung disorders, chronic disorders in which derangements of the alveolar wall are accompanied by "fibrosis," a process in which the normal parenchyma is replaced by mesenchymal cells and the collagenous matrix produced by these cells. In general, the inflammation of these disorders is dominated by alveolar macrophages, and to a lesser extent, neutrophils and/or eosinophils. Examples of these fibrotic disorders include idiopathic pulmonary fibrosis and asbestosis. The current concepts of the mechanisms of pulmonary fibrosis hold that the accumulation of fibroblasts and fibroblast products result from two general processes. First, there must be damage to the alveolar wall; in most cases this is mediated by products of inflammatory cells, including macrophages, neutrophils and eosinophils. Second, there is enhanced proliferation of mesenchymal cells in the alveolar walls driven by growth signals released in the local milieu.

A major mechanism of damage to the alveolar walls in the fibrotic disorders is the release of oxidants by the inflammatory cells, particularly alveolar macrophages. Like other phagocytes, when alveolar macrophages are stimulated, they generate oxidants such as superoxide anion ($O_2^{\cdot-}$), a mechanism used in antimicrobial defense. In the inflammatory lung disorders, alveolar macrophages are chronically stimulated and are spontaneously releasing $O_2^{\cdot-}$, a process thought to play a role in the paren-

chymal injury associated with these disorders. It is known that phagocytes generate $O_2^{\cdot-}$ via the membrane bound NADPH-oxidase system that catalyzes the transfer of an electron to O_2. NADPH-oxidase consists of at least 5 components coupled to NADPH. The membrane components (cytochrome b245) include a 90 kDa heavy chain, and a 22 kDa light chain. The cytoplasmic components include proteins that are 65 kDa and 45 kDa, plus "others", not well defined. Surface stimulation, such as with phorbol esters, activate the NADPH-oxidase system to produce $O_2^{\cdot-}$. With this background, we hypothesized that exposure of alveolar macrophages to the milieu of chronic inflammation causes upregulation of components of the NADPH-oxidase system such that local stimuli cause alveolar macrophages to release oxidants in an exaggerated fashion. To evaluate this concept, we compared the maximum capacity of alveolar macrophage NADPH-oxidase system to generate $O_2^{\cdot-}$ to levels of mRNA transcripts coding for the 90 kDa heavy chain of the membrane bound component of the NADPH-oxidase system. Several "models" of chronic inflammation were evaluated including: (1) in vitro - exposure of normal alveolar macrophages to interferon-γ; and (2) in vivo - two inflammatory lung disorders - idiopathic pulmonary fibrosis (IPF) and chronic cigarette smoking. The data demonstrated that human alveolar macrophages exposed to a chronic inflammatory milieu have an exaggerated capacity to release $O_2^{\cdot-}$ in response to in vitro inflammation, IFN-γ, and to in vivo inflammation (IPF and cigarette smoking). Importantly, the same cells demonstrate increased mRNA transcript levels for the 90 kDa heavy chain membrane bound component of the NADPH-oxidase system. From these studies, we conclude that mRNA transcript levels for the heavy chain of cytochrome b245, the terminal component of the electron transport chain of the NADPH-oxdiase, correlate with the maximal capacity of alveolar macrophages to produce oxidants such as $O_2^{\cdot-}$. In this regard, while increased b245 transcript levels and $O_2^{\cdot-}$ production may simply represent generalized macrophage activation, it is a reasonable working hypothesis that in the chronic inflammatory milieu of the lower respiratory tract the levels the levels of cytochrome b245 heavy chain transcripts in alveolar macrophages modulate, at least in part, the capacity of these cells to release exaggerated amounts of $O_2^{\cdot-}$. If so, in the context of the central role of these oxidants in the injury to the lung in chronic inflammation, understanding the basis of regulating biosynthesis of components of the NADPH-oxidase system may reveal vulnerable molecular targets for future therapy of these disorders.

One example of an interstitial lung disease in which there is chronic injury caused by alveolar macrophages and eosinophils together is tropical pulmonary eosinophilia (TPE), a filarial disorder found throughout the tropical world but especially in the Indian subcontinent, resulting from an exaggerated immune response to the human filarial parasites Wuchereria bancrofti and Brugia malayi. Over the past several years, in collaboration with the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases; and the Tuberculosis Research Center, Indian Council of Medical Research, Madras, India, we have evaluated individuals with TPE in Madras, India. The syndrome presents initially as an acute inflammatory interstitial lung disorder with markedly

elevated numbers of eosinophils in the lower respiratory tract. Most symptoms of acute TPE are referable to the lungs, including cough, dyspnea, nocturnal wheezing and chest discomfort, occasionally accompanied by constitutional symptoms such as weight loss, anorexia, and fever. Typically, laboratory studies are characterized by a chest radiograph with diffuse parenchymal infiltrates, lung function changes consistent with an interstitial process, and high levels of blood eosinophilia with elevated serum levels of IgE and specific anti-filarial antibodies. Following a standard three-week course of diethylcarbamazine (DEC), patients respond rapidly with reduced symptoms, clearing of the chest radiograph, improved pulmonary function, and a fall in both blood eosinophils and anti-filarial antibodies. Despite the general concept that DEC treatment is "definitive" therapy for most cases of acute TPE, there are reports of progression of the pulmonary disease to a mild, chronic form of interstitial lung disease. Although the biologic processes underlying such disease progression are unknown, with the knowledge that the acute disease is characterized by an intense eosinophilic alveolitis, it is reasonable to hypothesize that DEC therapy does not in all instances completely suppress the lung inflammation that characterizes acute TPE, resulting in a mild, chronic inflammatory process in the lower respiratory tract that slowly injures the fragile alveolar walls. To evaluate the concept that DEC therapy is not completely "curative" for TPE, but rather leaves most individuals with a mild, chronic form of TPE defined by persistent inflammation of the lower respiratory tract, we evaluated 23 individuals an average of 12 months following a standard 3-week course of diethylcarbamazine for acute TPE. In the majority, there were mild, persistent symptoms referable to the lung, chest x-ray abnormalities, blood eosinophilia, and elevated serum IgE and filarial specific IgG. On the average, lung function was consistent with the presence of chronic, mild interstitial lung disease. When the inflammatory cells from the lower respiratory tract were examined, there was a persistent eosinophilic alveolitis. Evaluation of the lower respiratory tract inflammatory cells recovered from the TPE/post-DEC treated individuals demonstrated release of exaggerated amounts of O_2^- and H_2O_2 compared to normals. Thus, following a standard 3-week course of DEC therapy, most patients show improvement, but not complete resolution of TPE, and many are left with chronic respiratory tract inflammation and a mild form of interstitial lung disease.

Under normal conditions, the lower respiratory tract is adequately protected against oxidants by an antioxidant screen that includes the glutathione system, catalase, superoxide dismutase, ceruloplasmin, methionine and vitamins. However, in disorders such as idiopathic pulmonary fibrosis and adult respiratory distress syndrome, the oxidant burden outweighs the antioxidant screen, resulting in progressive epithelial damage. In this regard, we have strategized that it should be possible to reestablish an adequate antioxidant defense of the lower respiratory tract with glutathione [L-γ-glutamyl-L-cysteinyl-glycine (GSH)], a naturally occurring antioxidant produced by all cells, including alveolar macrophages. Glutathione levels in normal human lower respiratory tract epithelial lining fluid (ELF) are 350-500 μM. It is known that glutath-

ione provides antioxidant protection as a scavenger of H_2O_2, a key oxidant produced by inflammatory cells. Since GSH is a naturally occurring molecule, it should be possible to use it as a therapeutic agent to safely augment the antioxidant screen of the epithelial surface of the lower respiratory tract. As a first approach to this concept, we administered GSH to normal sheep and compared GSH levels in epithelial lining fluid, lung lymph, venous plasma and urine. Two routes of administration were used: intravenous and aerosol. The data demonstrated that GSH given intravenously to sheep only transiently increases venous plasma, lymph and urine GSH levels, while lung epithelial lining fluid levels do not change significantly. However, in vitro, 50-60% of a GSH solution can be placed in droplets 0.2-3 μm mass median diameter while still remaining reduced, i.e., in a form where functional GSH can be targeted directly to the lower respiratory tract. Strikingly, aerosol administration of GSH to sheep markedly increases the GSH levels in the epithelial lining fluid of the lower respiratory tract for up to 2 hours. From these studies we conclude that aerosolization of GSH should significantly augment the antioxidant screen of the lung epithelial surface, making this a feasible therapeutic approach for lung disorders associated with an enhanced oxidant burden.

Tissue macrophages like alveolar macrophages play a central role in normal wound healing and pathologic tissue fibrosis by virtue of their ability to release a variety of polypeptide mediators that serve as growth factors for mesenchymal cells. Human alveolar macrophages are known to be capable of expressing the genes for several defined growth factors including the c-sis gene [the B-chain of platelet-derived growth factor (PDGF)], fibronectin, insulin-like growth factor-1 (IGF-I), interleukin 1-β and tumor necrosis factor. In the past year we have concentrated on alveolar macrophage IGF-I.

IGF-I functions as a growth factor for mesenchymal cells by interacting through a specific surface receptor to provide a "progression" signal acting late in G1 to stimulate the cell to complete G1, synthesize DNA and proliferate. As presently understood, the human IGF-I gene contains 5 coding exons [the 5' end of exon I has not been defined, and it is not known if there are separate non-coding exons 5' to what is now defined as exon I). Insulin-like growth factor-I, the common designator for the 7.6 kDa protein found in plasma, represents coding sequences in the 3' portion of exon II and the 5' portion of exon III. IGF-I is presumably formed from a precursor protein, but the mechanisms of this process are not defined. While the plasma form of IGF-I is 7.6 kDa, it is now recognized that some cells are capable of secreting "tissue" forms of IGF-I, representing more of the coding sequences in the IGF-I gene. Examples of human IGF-I-type molecules that are clearly larger than the 70 residue plasma form of IGF-I include molecules produced by human skin fibroblasts, rat Sertoli cells, and human alveolar macrophages. The knowledge that human alveolar macrophages are capable of expressing the IGF-I gene and releasing an IGF-I-type protein evolved from studies demonstrating that alveolar macrophages contained mRNA transcripts coded by the IGF-I gene and were capable of releasing a protein that: (1) functioned as a

"progression" type growth signal for fibroblasts; (2) was detected by an anti-IGF-I antibody; (3) displaced IGF-I from its receptor; and (4) activated the IGF-I receptor to phosphorylate a tyrosine substrate. Because the alveolar macrophage IGF-I molecule comigrated on chromatographic separation procedures with the previously described alveolar macrophage progression-type growth signal activity referred to as "alveolar macrophage derived growth factor (AMDGF)", it is likely that the alveolar macrophage IGF-I and AMDGF are the same molecule(s). Consistent with the concept that the alveolar macrophage IGF-I represents a "tissue form" of IGF-I, the molecular mass of the alveolar macrophage IGF-I is identical under neutral and acidic conditions, suggesting that alveolar macrophage IGF-I is not associated with other proteins, such as an IGF-I binding protein. Thus, it is likely that alveolar macrophage IGF-I contains sequences in addition to those found in human plasma IGF-I. In this regard, IGF-I transcripts have been observed in human liver to contain sequences of the IGF-I gene that predict IGF-I-type proteins much larger than that of plasma IGF-I. While no proteins corresponding to these liver IGF-I transcripts have been identified, their sequences predict IGF-I-type proteins containing 153 to 195 residues, forms of IGF-I much closer in mass to alveolar macrophage IGF-I than plasma IGF-I. Consistent with this concept, in vitro translation of RNA produced from cloned IGF-I liver cDNAs results in polypeptides with molecular weights of 17.5 and 22 kDa. In the context of the importance of IGF-I as a mesenchymal growth factor, and with the knowledge that alveolar macrophages are capable of expressing the IGF-I gene and releasing functional IGF-I molecules, we have compared IGF-I release by normal alveolar macrophages to that of alveolar macrophages recovered from the lower respiratory tract of individuals with chronic inflammation.

The data demonstrated that the normal, resting human alveolar macrophages recovered from the lower respiratory tract do not spontaneously release IGF-I molecules but only release IGF-I when activated. Consistent with the observation that activated alveolar macrophages release IGF-I, evaluation of alveolar macrophages recovered from the lungs of individuals with idiopathic pulmonary fibrosis and chronic asbestos exposure spontaneously release an IGF-I-type molecule as detected by an IGF-I enzyme-linked immunoassay. Thus, alveolar macrophages release an IGF-I protein in inflammatory states, circumstances in which the IGF-I likely plays a role in the localized proliferation of mesenchymal cells.

To examine the control of IGF-I gene expression in mononuclear phagocytes, the U937 human macrophage-like cell line was evaluated at rest and following surface activation with phorbol myristate acetate (PMA) or Ca^{2+} ionophore. Northern analysis and RNase protection analysis with ^{32}P-labeled IGF-I-specific probes demonstrated the IGF-I mRNA transcripts of resting U937 cells were similar in size and amount to resting human alveolar macrophages, mononuclear phagocytes known to express the IGF-I gene. Nuclear run-off assays demonstrated that surface activation of U937 cells increased the transcription rate of the IGF-I gene 4 to 5 fold, a process that was inhibited by cycloheximide, suggesting active

protein synthesis was involved in the activation signal. Despite this, cytoplasmic IGF-I mRNA levels following surface activation declined markedly, a process blocked by a protein kinase C inhibitor (for PMA activation) or a calmodulin antagonist (for Ca^{2+} ionophore activation). Like the increased transcription of the IGF-I gene, modulation of IGF-I mRNA transcript levels required active protein synthesis; in the presence of cycloheximide, constitutive IGF-I mRNA levels increased and surface activation no longer caused a decrease in transcript numbers. Interestingly, surface activation caused a rapid release of IGF-I, even in the presence of a protein synthesis inhibitor, suggesting mononuclear phagocytes have a pre-formed, stored, releasable pool of IGF-I. Together, these observations demonstrate that IGF-I gene expression is complex and likely involves control of transcription rate, cytoplasmic mRNA levels possibly mediated through protein kinase C, calcium influx and calmodulin, and finally, release of preformed IGF-I from a storage pool.

As a natural extension of the focus of the Pulmonary Branch, NHLBI, on the importance of the alveolar macrophage in the pathogenesis of chronic inflammatory diseases of the lower respiratory tract, we have begun the examine the major role of the alveolar macrophage in host defenses. In this regard, over the past year, we have initiated studies to evaluate interferon-γ and mononuclear phagocyte activation. Interferon-γ is a 17 kDa T-cell produced mediator capable of interacting with a specific surface receptor on mononuclear phagocytes. As a result, the mononuclear phagocyte is activated as demonstrated by enhanced oxidant production and enhanced ability to defend against intracellular parasites. Further, and relevant to human disease, if there is a relative "deficiency" of T-cell produced interferon-γ, as occurs with human immunodeficiency virus infection, there is likely a relative "deficiency" of normal mononuclear phagocyte activation. In this regard, if alveolar macrophages are activated by interferon-γ, and if sufficient amounts of interferon-γ could be delivered to alveolar macrophages, it should be possible to use interferon-γ to help the lung defend against infections in disorders such as AIDS. In this context, our initial studies have asked 3 questions: (1) What is the amount of recombinant interferon-γ (rIFN-γ) necessary to activate normal alveolar macrophages *in vitro*?; (2) Will the same amount of rIFN-γ *in vitro* activate alveolar macrophages of individuals with HIV infection?; and (3) Can these amounts of rIFN-γ be safely delivered to the human lower respiratory tract of individuals with HIV infection? The data demonstrates that 250 units of recombinant interferon-γ (rIFN-γ) *in vitro* is capable of activating normal human alveolar macrophages to: enhance capacity to release O_2^-; enhance surface HLA-DR expression; and enhance suppression of intracellular Toxoplasma growth. The same amount of rIFN-γ will suppress intracellular Toxoplasma growth in alveolar macrophages of individuals that are HIV seropositive, no AIDS, and individuals with AIDS. Further, it is possible to aerosolize 7×10^6 units of rIFN-γ daily for 1 week to individuals that are HIV positive with no side effects. Assuming 10% of the rIFN-γ reaches the lower respiratory tract, the epithelial lining fluid levels should be >7000 units/ml levels sufficient to activate alveolar macrophages. From these

studies we conclude that it is rational to evaluate direct delivery of rIFN-γ to the human lower respiratory tract as a means to augment alveolar macrophage defenses against opportunistic infections.

As another aspect of the host defense role of the alveolar macrophage, in collaboration with the Pathology Branch, NHLBI, we have carried out studies to morphologically characterize alveolar macrophages from individuals with occupational exposure to inorganic particles. Alveolar macrophages recovered by bronchoalveolar lavage from 43 non-smoking or >5 year ex-smoking individuals with occupational exposure to inorganic particles (asbestos, n=19; silica, n=10; and coal, n=14) were evaluated by light microscopy and transmission and scanning electron microscopy to determine the morphologic changes resulting in these cells from chronic inorganic particulate inhalation. Alveolar macrophages from dust-exposed individuals, including those who had been free of exposure to particles for more than one year, contained particles in higher proportion than did those of normal unexposed individuals. Most of these particles were located within phagolysosomes. The frequency of multinucleated alveolar macrophages was significantly higher in the dust-exposed groups. Ultrastructural studies showed alterations of the morphology of the surfaces of alveolar macrophages from the dust-exposed individuals, including increased numbers of rufflings, filopodia, pinocytotic vesicles, subplasmalemmal linear densities, and increased frequency of macrophage-macrophage, and macrophage-lymphocyte interactions. Furthermore, the numbers of lysosomes were significantly increased in alveolar macrophages from the dust-exposed individuals. Together, these morphological changes are consistent with the sequelae of phagocytosis and emphasize both the role of alveolar macrophages in eliminating inorganic particles from the alveolar spaces, and the consequences this role has in the alveolar macrophage population.

III. Disorders characterized by granulomata in the alveolar walls.

Disorders characterized by granulomata in the alveolar walls are a subgroup of the interstitial lung disorders. Although occasionally associated with progressive fibrosis of the lung parenchyma late in their course, these disorders are usually more benign and are universally characterized by large numbers of T-lymphocytes that dominate the inflammation and direct the formation of granulomata. Together, the T-cells and granulomata cause dysfunction by their presence which distorts the alveolar, bronchial, and vascular walls, thus modifying the intimate relationship between air and blood. Examples of these disorders include sarcoidosis and berylliosis.

Sarcoidosis is a non-malignant, chronic, systemic disorder characterized by a T-lymphocyte and mononuclear phagocyte inflammatory process associated with granuloma formation in affected organs. The etiology of sarcoidosis is unknown, but it is recognized that the T-cell plays a critical role in the pathogenesis of the disease. In this regard, evaluation of the T-lymphocytes in the lower respiratory tract of individuals with active sarcoidosis has shown that they are dominated by active

helper/inducer T-lymphocytes that are releasing lymphokines, processes fundamental to formation of immune granulomata. These T-cells accumulate in the lung, at least in part, because of processes that stimulate the activation and proliferation of helper/inducer T-lymphocytes in the local milieu. In the context of these observations, a central question in understanding the pathogenesis of sarcoidosis is to understand why T-lymphocytes accumulate at sites of disease such as the lower respiratory tract. Three general hypotheses can be proposed to explain the pathogenesis of these T-lymphocyte infiltrations in sarcoidosis. First, the accumulated T-cells may represent a polyclonal T-cell response, perhaps secondary to a generalized enhancement of T-helper cell processes or ineffective T-cell suppressor networks that are not antigen-specific. Second, the T-cells may accumulate secondary to a monoclonal or oligoclonal process such as that observed in malignancies in which a "transformed" cell with a growth advantage accumulates in tissues. Third, the T-cells may accumulate secondary to antigen-driven processes, in which the clonal expansion of antigen-specific T-cells together with the secondary expansion of populations of immunoregulatory and/or bystander T-cells results in a skewed distribution of T-cells. Since the mechanisms responsible for T-cell accumulation in these three categories are different, a characterization of the "clonality" of sarcoid T-cells is an important step in understanding the pathogenesis of this disorder.

One approach to separating these possibilities is to analyze the T-cell antigen receptors of T-cells that have accumulated in an organ in active sarcoid. In this regard, antigens presented to T-cells in the context of major histocompatibility complex molecules are recognized by the T-cell antigen receptor (TCR). Most T-cells utilize the $\alpha\beta$ TCR, a heterodimer comprised of α- and β-chains. The enormous diversity of the $\alpha\beta$ TCR is based on the rearrangement of their variable, junctional, diversity and constant regions during T-cell ontogeny. When an $\alpha\beta$ TCR recognizes an antigen, the adjacent CD3 complex is stimulated, resulting in initiation of a series of events culminating in T-cell activation and proliferation. As part of this process, the $\alpha\beta$ TCR-CD3 complex is down-regulated, probably by internalization, and there is a concomitant increase in the number of TCR α-and β-chain mRNA transcripts. In the context of sarcoid, if the T-cells accumulated in affected organs because of an ordered immune process, the T-cells at sites of disease should demonstrate evidence of recent triggering of the $\alpha\beta$ TCR (i.e., down-regulation of surface TCR and up-regulation of TCR mRNA transcript numbers) whereas the TCR components of blood T-cells in the same individuals should be similar to that of resting T-cells. In contrast, if the T-cell accumulation is random or results from a monoclonal expansion, then the activation state of the $\alpha\beta$ TCR of the T-cells in the affected organs and in the blood should be similar. To evaluate this concept, we capitalized on the knowledge that individuals with pulmonary sarcoid have large numbers of T-lymphocytes on the epithelial surface of the lower respiratory tract, a site easily sampled by bronchoalveolar lavage. Lung and blood T-cells of individuals with active pulmonary sarcoid were compared for the density of $\alpha\beta$ surface receptors and the number of β-chain mRNA transcripts. As a control, normal T-cells were eval-

uated at rest and following triggering of the TCR-CD3 complex with an anti-CD3 monoclonal antibody. The surface density of T-cell surface $\alpha\beta$ TCR expression was evaluated by flow cytometry with an anti-$\alpha\beta$ antibody and TCR β-chain mRNA transcript number quantified by in situ hybridization with ^{35}S-labeled antisense and sense cRNA probes. Control studies utilizing normal blood T-lymphocytes stimulated with the anti-CD3 monoclonal antibody, OKT3, in the presence of autologous mononuclear monocytes, demonstrated the expected down-regulation of surface $\alpha\beta$ TCR expression and increased β-chain mRNA transcript number. When lung and blood T-cells of individuals with pulmonary sarcoidosis were compared immediately upon recovery (i.e., without in vitro stimulation), the lung T-cells of 10 of 10 individuals demonstrated a decreased surface density of $\alpha\beta$ TCR compared to their autologous blood T-cells. Furthermore, lung T-cells of 8 of 9 of these individuals exhibited an increase in β-chain mRNA transcripts compared to their autologous blood T-lymphocytes. Together, these observations are consistent with the concept that lung, but not blood, T-cells in active pulmonary sarcoidosis have recently been stimulated through the T-cell surface antigen receptor, giving support to the hypothesis that the T-cells accumulate in the lung in pulmonary sarcoid in response to persistent, specific antigenic stimulation.

To further evaluate the concept that the bias in the types of T-cells accumulating at sites of disease in sarcoid extends to the accumulation of T-cells with the preferential use of certain T-cell antigen receptor β-chain constant region elements, we evaluated the lung and blood T-cells of normal individuals (n=19) and individuals with active sarcoidosis (n=12) for T-cell antigen receptor β-chain mRNA transcripts containing constant region β1 elements or constant region β2 elements i.e., assessing whether their use is random or whether there is bias in the β-chain constant region elements being used by the accumulated T-cells. Quantitative evaluation of Cβ1 and Cβ2 mRNA transcripts were made using a ^{32}P-labeled Cβ1 cRNA probe in which mRNA-probe hybrids were exposed to RNase and subsequently fractionated by polyacrylamide gel electrophoresis to differentiate the Cβ1 versus Cβ2 transcripts secondary to their sequence differences and hence differential protection from RNase by the probe. Densitometric scans of the resulting autoradiograms demonstrated a Cβ1/Cβ2 usage in normal blood of 0.63 ± 0.02, similar to that of normal lung (0.64 ± 0.06, $p>0.7$). Likewise, T-cells from sarcoid blood showed a ratio of 0.58 ± 0.04, similar to blood and lung of normal individuals ($p>0.2$). Strikingly, the lung T-lymphocytes of individuals with active sarcoidosis reflected a marked bias in the usage of Cβ1 elements (Cβ1/Cβ2 0.92 ± 0.05, $p<0.001$ compared to sarcoid blood, normal lung, and/or normal blood). These observations provide further evidence that the T-lymphocytes that accumulate in sarcoidosis preferentially use specific T-cell antigen receptor elements, suggesting that there is selection for T-lymphocytes with specific T-cell antigen receptors in association with sarcoid inflammation.

To directly examine the concept that lung T-cell accumulation in pulmonary sarcoidosis is based on an exaggerated, but ordered immune response, we have compared the sequences of lung T-cell antigen β-chain

mRNA transcripts of individuals with sarcoidosis with those of normal individuals. To do so, we capitalized on the knowledge that studies using an anti-$V_\beta 8$ specific monoclonal antibody has identified a subgroup of individuals with active sarcoidosis with lung T-cells exhibiting a bias for the usage of $V_\beta 8$ elements. To accomplish this, we capitalized on our recent observation of a subgroup of sarcoid patients demonstrating the biased accumulation of T-cells with antigen receptor β-chains using the specific variable region $V_\beta 8$ and evaluated whether the lung T-cell β-chains of these individuals had similar sequences of the highly variable V-D-J junctional region of the $V_\beta 8$ mRNA transcripts. The sequence data strikingly demonstrates that, in this subgroup of individuals with active pulmonary sarcoidosis demonstrating a bias for $V_\beta 8^+$ T-cells in the lung, there are increased proportions of T-cells with identical $V_\beta 8^+$ T-cell receptors, providing direct evidence of clonal expansion and accumulation of $V_\beta 8^+$ T-cells at local sites of disease. Furthermore, even among T-cells with differences in the nucleotide sequences of the hypervariable V_β-D_β-J_β junctional regions, apparent conserved amino acid sequences are present in some of these $V_\beta 8^+$ T-cell antigen receptors both on an individual basis and between different individuals. Although the function of these $V_\beta 8^+$ T-cells is unknown, it is likely they accumulate in response to specific stimulus due to their T-cell antigen receptor specificity, i.e., T-cell accumulation in sarcoidosis results from an exaggerated, but ordered immune response.

There are two types of TCR: one comprised of α and β chains and another comprised of γ and δ chains. The $\alpha\beta$+ and $\gamma\delta$+ TCR are expressed independently on different T-cell subsets. In this regard, if the TCR among the T-cell populations were broadly diverse (as is observed in normals), it would support the concept that the activation of the T-cells was non-specific. In the context that there are clear biases in the use of the β-chain (and thus likely the $\alpha\beta$ TCR) among subgroups of individuals with sarcoidosis, we have expanded these concepts by asking whether there might also be some sarcoid individuals who have expanded numbers of T-cells expressing the $\gamma\delta$ TCR. To accomplish this, T-cells of sarcoid individuals were compared to those of normals for the presence and the characteristics of T-lymphocytes that are TCR $\alpha\beta$- $\gamma\delta$+. Interestingly, using anti-$\alpha\beta$ TCR and anti-$\gamma\delta$ TCR monoclonal antibodies, we evaluated blood and lung T-cells of sarcoid individuals for the presence of the $\gamma\delta$ TCR. Blood and lung T-cells of normal individuals (n=19) and individuals with pulmonary sarcoidosis (n=20) were stained using monoclonal antibodies reacting with the CD3 complex (Leu4), the $\alpha\beta$+ heterodimer (TCR1), all $\gamma\delta$+ T-cells (TCRδ1), and two subsets of $\gamma\delta$+ T-cells (TiγA and δTCS1). Compared to normals, the group with sarcoidosis had elevated proportions of blood CD3+ $\alpha\beta$- T-cells (normals 5 ± 1%, sarcoid 17 ± 4%, $p<0.01$), increased numbers of CD3+ $\alpha\beta$- T-cells in the blood (normals 58 ± 12 cells/μl, sarcoid 192 ± 45 cells/μl, $p<0.05$) and in the epithelial lining fluid of the lung (normals 78 ± 14 cells/μl, sarcoid 240 ± 60 cells/μl, $p<0.04$) and a concomitant elevated number of blood and lung CD3+ TCRδ1+ T-cells, due to a striking increase in the number of CD3+ $\gamma\delta$+ T-cells in a subgroup (7/20) of sarcoid individuals. Importantly, the elevated numbers of sarcoid blood $\gamma\delta$+ T-lymphocytes were mostly

TiγA+ and δTCS1-, consistent with the preferential expansion of T-cells expressing one γ chain variable regions, Vγ9. Despite the increased numbers of blood and lung $\gamma\delta$+ T-cells, Southern analysis of sarcoid and normal T-cell DNA demonstrated no evidence of clonal rearrangements of γ genes, thus excluding the emergence of a single or a few monoclonal populations of $\gamma\delta$+ T-cells. Together with previous observations of subgroups of sarcoid individuals with biases for specific $\alpha\beta$+ T-cells, the observation of a marked skewing in the total $\gamma\delta$+ T-cell populations in a sarcoid subgroup suggests that various specific stimuli may trigger the expansion of different T-cell subpopulations within different groups of individuals with sarcoidosis.

We have also used restriction endonuclease DNA cleavage, Southern blot analysis and β-chain C, D, and J region probes, to evaluated the genotypic configuration of the T-cell antigen receptor β-chain of T-lymphocytes in the lower respiratory tract and blood of individuals with T-cell associated lung diseases (sarcoidosis, chronic beryllium disease, hypersensitivity pneumonitis, chronic pulmonary tuberculosis), individuals with other chronic inflammatory lung diseases (idiopathic pulmonary fibrosis, histiocytosis X) and normals. Southern blot analysis using a constant region (C_β) probe detected β-gene rearrangements in lung and/or blood T-cell DNA in a majority of individuals with active sarcoidosis (14/18), chronic beryllium disease (4/7), hypersensitivity pneumonitis (5/5) and chronic pulmonary tuberculosis (4/5) but rarely in individuals with inactive sarcoidosis (1/7), other inflammatory lung diseases (0/6) or normals (2/21). Further analysis using genomic probes from each J-region ($J_{\beta1}$ and $J_{\beta2}$) and D region ($D_{\beta1}$ and $D_{\beta2}$) revealed that these rearrangements represented uncommon, partial DJ rearrangements of the $D_{\beta1}J_{\beta2}$ type. These rearrangements were likely on the second, non-coding β-chain allele since they were detectable only in T-cell populations with $\alpha\beta$ T-cell antigen receptors (defined by the WT31 antibody). Similar $D_{\beta1}J_{\beta2}$ rearrangements could be observed in T-cell lines derived from lung or blood T-cells of individuals with chronic T-cell associated lung disease, but not from normal individuals. The function of these $D_{\beta1}J_{\beta2}$+ T-cells is unknown, but the fact that they accumulate in individuals with T-cell associated lung diseases provides a novel genotypic marker for T-cells associated with these disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02407-15 PB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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

There are two million individuals in the USA with emphysema. Two percent develop the disease because of inheritance of a deficiency of α1-antitrypsin (α1AT), an antiprotease that protects the lower respiratory tract from destruction mediated by elastase released by neutrophils. Cloning, sequencing, and oligonucleotides together with the polymerase chain reaction, have been used to identify specific mutations in the α1AT gene causing the deficiency states. Of the approximately 75 α1AT alleles that are known, we have now identified the sequence differences for all of the major normal alleles and 15 of the deficiency and null alleles resulting in an increased risk for emphysema. Studies are ongoing to evaluate the control of expression of the α1AT gene mononuclear phagocytes and neutrophils. New approaches to augmentation therapy for α1AT deficiency have been evaluated including plasma exchange, and the aerosolization of recombinant α1AT and plasma α1AT. The α1AT cDNA has been modified to produce α1AT variance with varying numbers of carbohydrate side chains causing differences in physiologic behavior. Strategies for gene therapy of α1AT deficiency have been devised using lymphocytes for the target for the transfer of the normal α1AT gene. Studies in mice have demonstrated the feasibility of this approach, including the ability to target the lymphocytes to specific locations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02533-05 PB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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

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

PI:	R.G. Crystal	Chief	Pulmonary Branch, NHLBI
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	R. Buhl	Guest Worker	Pulmonary Branch, NHLBI
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	I. Nagaoka	Visiting Associate	Pulmonary Branch, NHLBI
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COOPERATING UNITS *(if any)* Victor Ferrans and Tamiko Takemura, Pathology Branch, DIR, NHLBI, NIH, Bethesda, MD; Erik Ottesen, Medical Virology Section, Laboratory of Clinical Investigation, NIAID, NIH, Bethesda, MD; V.K. Vijayan, V. Kumaraswami, R. Prabhakar, Tuberculosis Research Center, Indian Council of Medical Research, Madras, India.

LAB/BRANCH
Pulmonary Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER:
10	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 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 macrophages. The primary group of mediators causing the damage are oxidants. The major growth factors are platelet-derived growth factor, and insulin-like growth factor-I. With the knowledge of the specific processes involved in the release of these mediators by inflammatory cells such as alveolar macrophages, strategies can be developed to modulate the expression of the genes coding for these mediators as therapy for these disorders. Alveolar macrophages also play a critical role in host defense against infectious and inorganic particulates. The role of the alveolar macrophage in host defense can be upregulated with the T-lymphocyte mediator, interferon-γ. Recombinant interferon-γ can be administered by aerosol; this may be a useful strategy for augmenting host defense in infectious disorders such as AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02534-05 PB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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

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

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COOPERATING UNITS *(if any)*
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LAB/BRANCH
Pulmonary Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	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 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.

ANNUAL REPORT OF THE SURGERY BRANCH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
OCTOBER 1, 1988 - SEPTEMBER 30, 1989

Approximately 250,000 persons receive cardiac operations in the United States yearly. The overall mortality rates are in the 5-10% range with reported morbidity rates of 50-80%. Individual procedures carry a wide range of mortality, 1-80%, depending on age, the type and extent of disease, the severity of illness, previous number of cardiac procedures, and the urgency of operation. Nearly all cardiac operations are palliative. Thus, the research efforts of the Surgery Branch are directed not only toward decreasing the mortality and morbidity associated with cardiac operations but toward improved quality of life and longer complication and recurrence-free intervals. To achieve these goals, studies are designed to determine the mechanisms of the pathophysiologic and biochemical abnormalities associated with cardiac disease and its surgical treatment. Additionally, research is ongoing to develop new therapeutic strategies to diminish or prevent long-term complications.
The interests have focused on four areas: prosthetic heart valves, metabolism and function of the ischemic and post ischemic heart and brain, chronic allograft and xenograft rejection, and operative treatment of a genetic disease, hypertrophic cardiomyopathy.

PROSTHETIC HEART VALVES

Approximately 25-30,000 prosthetic heart valves are implanted yearly in the U.S. Durable devices are associated with long-term thromboembolic events and thromboresistant valves are associated with only moderate durability of a decade or less. The goals of the research are to develop accurate methods to assess valve dysfunction, improve the durability of xenograft valves through an understanding of the mechanisms of mineralization and fatigue and to develop and assess new methods and devices for palliating valvular heart disease.

In Vitro Studies

Previous work involved the development of a computer-based pulse duplicator system, the description of which was recently published. This device accurately simulates hydraulic conditions within the heart and arterial system. The dependence of the pressure gradient across a prosthetic heart valve on the location of the downstream pressure measurement has been quantified. The correlations of maximal velocity data to manometric pressure drop data for 18 new mechanical, 33 new bioprosthetic and 12 clinically explanted bioprosthetic aortic valves have been determined over a wide range of pulsatile flow rates. The correlations between the two measurement forms were high ($R_2 > 0.92$). The Doppler data were incorrect in the range of 20-100% by over estimating the pressure drop. The clinical significance is that non-invasive measurement of pressure gradients in patients can lead to incorrect diagnoses of prosthetic valve stenosis and result in unwarranted cardiac catheterizations and/or operations.

Seven new synthetic trileaflet valves were analyzed for possible use in the Utah total artificial heart. None were found satisfactory for this device and one was highly incompetent.

The new thrust is to develop quantitative analytical methods to determine the volume of regurgitation from color continuous wave Doppler data. A new chamber for ultra-sound imaging on both sides of the prosthetic valve has been constructed and tested. These studies will be augmented with computer assisted image processing and steady state hydraulic models. Correlations to Fourier transform phonocardiographic data will be made.

In vivo studies

The past work assessed the effects of various preimplantation treatments of bioprosthetic valves to inhibit calcification after implantation in the mitral position in juvenile sheep for 20 weeks. The treatments, performed after glutaraldehyde fixation included use of surfactants, organic dyes, disphosphonates and polyacrylamide. Only the surfactants significantly retarded (5-10x) the mineralization processes as determined by atomic absorption measurement of calcium. These studies were extended in the reporting interval to determine if the pressure used during glutaraldehyde fixation altered the rate of mineralization and if altering the aldehyde fixation processes would influence the pathologic alterations of porcine aortic bioprostheses. The data demonstrated that high pressure (100 mm Hg) versus low pressure (0-6 mm Hg) resulted in 124 $\pm$ 12.2 vs 66.6 $\pm$ 12.8 mg $Ca^{7}g$ tissue; a result that was not clinically important. The addition of surfactant (polysorbate-80) resulted in a four fold decrease to 28.3 $\pm$ 7.4 mg/g.

Uncrosslinked lysine, hydroxylysine terminal amine residues and residual glutaraldehyde were postulated to promote tissue affinity for calcium and phosphate. The fixation processes were modified by prolonged storage in glutaraldehyde, the addition of formaldehyde, and the removal of glutaraldehyde by Tollen's reagent. None altered the amount of calcification. Studies of new fixation processes and postfixation treatments to mitigate mineralization of bioprostheses continue.

Progress continued on the comparative in vivo hydraulic assessments of generic types of clinical prosthetic valves under similar and clinically relevant hemodynamic conditions. These valve types were tissue valves fabricated from porcine aortic valves and bovine pericardium, mechanical valves with tilting disc occluders and bileaflet hemidisc occluders. A new preclinical device which had the theoretical advantage of high hydraulic efficiency and low thrombogenicity was tested. The collected experience of 662 completed implant studies in sheep with 9 subsets has demonstrated that no device compares favorably with the normal native mitral valve. The comparative superiority of the hemidisc design concept and the excellent hemodynamic efficacy of a new bileaflet tissue valve were shown.

The velocity/flow profile imaging studies of the past five years have been extended and expanded to include normal native valves, prosthetic valves and rings and mitral regurgitation. New data have defined the normal flow related events occurring during diastole. These data permit accurate comparisons to the abnormal flow events found with prosthetic heart valves and ring annuloplasty devices. These findings may be predictive of clinically relevant problems of hemolysis and thrombosis. New studies on fresh and preserved homograft mitral valves have been initiated. Methods of the quantitation of valve regurgitation in vivo will continue using flow convergence shells obtained by aliased Doppler determined velocities.

Mitral valve replacement for mitral regurgitation has a high mortality rate. A two year study has been completed which assessed the effect of preservation of the chordae tendineae (the supporting structures of the mitral valve) on left ventricular function after mitral valve replacement in sheep with chronic mitral regurgitation. 47 sheep had creation of mitral regurgitation, half of which had MVR with resection and half had MVR without resection of the chordal structures. They were matched to control sheep without chronic mitral regurgitation which had MVR with and without chordal preservation. Additional sheep were used for normal data to control for age and weight and technique of acquisition of pressure-volume data. Acute mitral regurgitation increased the work of the right and left sides of the heart significantly without change in size, force of contraction, or elastance. Chronic mitral regurgitation increased cardiac mass and decreased performance. Analyses to determine the influence of chordal preservation are in progress.

The first report of the clinical analog of this experiment was presented in May 1989. The data demonstrated that mitral valve replacement with chordal preservation resulted in preserved left ventricular ejection fraction and improved exercise capacity 6 months after operation compared to those patients that had resection of the mitral valve apparatus during mitral valve replacement.

METABOLISM

Cerebral and Myocardial Energetics

Cardiopulmonary bypass (the heart-lung machine) is required for cardiac operations. To test whether or not modern cardiopulmonary bypass components and techniques were deleterious to metabolism in the heart and brain, 31-P nuclear magnetic resonance spectroscopy was used to assess high energy phosphate concentrations and the intracellular pH of the tissues. Sheep underwent total body perfusion at normal body temperatures and ATP, phosphocreatine and inorganic phosphate concentrations and intracellular pH were measured. Studies of both the brain and the heart demonstrated no alteration of phosphorylation potential of either organ. These data demonstrate that the heart-lung machine system does not alter energy metabolism at normothermia.

The effects of cardiopulmonary bypass at low temperatures on brain and heart metabolism at low temperatures (18 and 26°C) were studied because hypothermia is a standard technique in the clinical setting. The data demonstrated that moderate (26°C) hypothermia did not significantly change the energy state of the brain or the heart whereas deep hypothermia increased the energy status of the brain demonstrating a protective effect.

Further studies demonstrated the effect of manipulating the perfusate pH under moderate and deep hypothermic conditions by alteration of the partial pressure of carbon dioxide. Using NMR, the intracellular pH of the heart and brain were determined. The data demonstrated that independent of blood pH the intracellular pH of the brain and heart paralleled those of the neutral pH of water under cold conditions; i.e., the pH increased. This was the first known demonstration of intracellular pH changes during hypothermia in heterothermic mammals.

The two methods of blood pH management during hypothermia were studied in dogs with respect to hemodynamic variables and oxygen consumption. The data showed that the alpha stat management strategy resulted in more favorable tissue perfusion.

Cerebral Protection

A major problem in cardiac surgery is protection of the brain. Circulatory arrest (cessation of blood flow) at low temperatures is required for many pediatric and adult cardiac operations. The effects of circulatory arrest are poorly understood under these conditions and the safe duration of circulatory arrest is empiric. Several studies are in progress in this area.

A new technique for hypothermic cardiopulmonary bypass and circulatory arrest in sheep undergoing nuclear magnetic resonance spectroscopy was developed and the time-course of high energy phosphate depletion during ischemia and repletion with reperfusion was determined. In addition, the changes in intracellular pH and EEG were measured.

It was hypothesized that perfusion of the brain with a cold asanguinous solution (cerebroplegia) during circulatory arrest would result in preservation of high energy phosphates. Sheep were divided into control (n=8) and cerebroplegia (n=7) groups and were cooled to 15° C. The circulation was stopped for 2 hours during which NMR spectroscopy was continuously performed. The cerebroplegia animals had intermittent perfusion of the carotid arteries. ATP, PCr, Pi, intracellular pH and EEG were measured. When the cerebroplegia animals were compared to the controls the following were found: ATP, PCr and intracellular pH were higher for all points during the arrest period and until 60 minutes of reperfusion. EEG activity returned after 37 minutes in the cerebroplegia group and not until 117 minutes in the controls ($p<.05$). Therefore, both energy metabolism and cerebral function as measured by EEG were improved with cerebroplegia.

Functional and pathological studies were then conducted to compare the acute NMR results with functional outcome in chronic experiments. Sheep underwent sterile operative procedures and circulatory arrest for 2 hours at 15° C either with cerebroplegia (n=8) or as controls (n=6). The animals had EEG and neurological examinations in the early postoperative period and were allowed to recover. Those still alive at one week were sacrificed and the brains examined pathologically. Profound differences in neurologic status and in survival between the two groups (5.3 days in the cerebroplegia animals vs. 1.1 days in the controls, $p<.05$) were found. The significance of these two major studies is that a method has been invented which will extend the time of hypothermic circulatory arrest to two hours with predictable preservation of neurologic function of the brain, the biochemical basis for which has been established. Studies to improve the technique for clinical use are in progress.

Alternate techniques to cerebroplegia for cerebral protection have also been studied with NMR. Data for two of these methods is nearly complete. Intermittent perfusion at 120 ml/kg/min has been compared to very low (5 ml/kg/min) and low (10 ml/kg/min) continuous perfusion during 2 hour intervals. The preliminary data demonstrate low flow completely protects the brain while very low flow has no benefit. Intermittent flow may be slightly protective. These studies will continue.

Myocardial Protection

Newborns, neonates and older children with cyanotic heart disease have been found to tolerate poorly global cardiac ischemia which is required to correct the congenital defects. The mechanisms responsible for this intolerance are poorly understood. Improved techniques would result in improved survivorship and lower morbidity for cardiac surgery in these children. A colony of cyanotic animals was developed which had an oxygen saturation of 55-70% After 4-8 months, the cardiac defects were repaired using two hours of hypothermic global ischemia. Recovery was studied with measurements of the ventricular pressure-volume relation and myocardial high energy phosphate concentrations. The preliminary analysis of the data demonstrated a trend toward poorer performance of the cyanotic hearts compared to a control group of age-matched animals. This model will now serve to evaluate interventions to improve tolerance to ischemia in cyanotic hearts.

Right ventricular dysfunction after intracardiac repair of congenital defects is common and is frequently responsible for perioperative morbidity and mortality. To better understand the pathophysiology of right ventricular failure and its influence on the left ventricle a program to quantitate right ventricular performance in normal and hypertrophied pressure overload states was initiated. Young animals had pulmonary artery banding. Six months later, removal of the band and patching of the pulmonary artery were performed using hypothermic cardiopulmonary bypass and two hours of global cardiac ischemia. Serial measurements of myocardial regional blood flow, oxygen consumption, lactate extraction, and global and regional right and left ventricular function were made. Two groups had severe (N=6) and moderate (N=9) right ventricular hypertension with concomitant increases in right ventricular weights. The hemodynamic and biochemical analyses are in progress. The significance of the program is that baseline data have been obtained which now permit a variety of preoperative and intraoperative interventions to be tested to improve outcome of cardiac surgery in children with obstructive right heart lesions.

A third project applicable to the area of congenital heart disease was undertaken to determine if administration of digitalis compounds, almost universally given to newborns with congestive heart failure, was deleterious to cardiac performance in the simulated cardiac surgical setting of global ischemia and reperfusion using the isolated working heart preparation. The hypothesis tested was that the immature Na/K ATPase system of the newborn was further inhibited by digitalis compounds and that when hearts so treated were subjected to ischemia and reperfusion, abnormal concentrations of sodium and potassium and concomitant cardiac dysfunction would occur. Newborn pigs were treated for five days. The hearts were removed and hemodynamic performance and ATP and myocardial sodium calcium concentrations were determined. The data showed use of digitalis in the newborn decreased ATP levels by more than 10%. There were no differences between groups which received ischemia only or ischemia and reperfusion either treated or untreated with respect to tissue ion concentrations although ATP was decreased in the treated group.

Many agents have been proposed as myoprotective adjuncts when added to a clinical cardioplegia. To assess these, studies using the working isolated rat heart preparation continued. A light stable intravenous dihydropyridine calcium channel blocker, nicardipine was tested for efficacy as an additive

agent to cardioplegic solution. A high degree of effectiveness was shown when used at 37° C. but not under cold conditions (10° C) used in the clinical setting. Hence, pretreatment may be the only method with clinical usefulness. A superoxide radical scavenger was tested for protective effects under normothermic and various hypothermic conditions. Deferoxamine when administered in the cardioplegic solution under all conditions failed to provide significant improvement to hypothermia and hyperkalemic arrest methods. These data suggest that in hyperoxic crystalloid perfusion systems, that either the compounds tested were ineffective or the level of superoxide production was low. The compound was effective when given prior to cardiac excision.

Other studies tested the hypotheses that interlukin-2 (IL-2) and tumor necrosis factor (TNF) were deleterious to cardiac muscle. Various dose response curves were constructed. It was found that the solvents of IL-2 were possibly responsible for the observed clinical findings and that, in the concentrations used for clinical treatment of metastatic cancer, minimal cardiac depression occurred with the pure IL-2 and TNF compounds.

A clinical study of myocardial metabolism as a function of mean arterial pressure and heart rate was completed in the reporting interval. A coronary sinus catheter was used to measure coronary blood flow, myocardial oxygen consumption and lactate metabolism in 20 patients with mean blood pressure was varied between 60 and 90 mm Hg and at heart rates between 60-90 beats per minute using pacing. The data are in the analysis process.

A prior study which measured thyroid hormone concentrations in patients having cardiopulmonary bypass was continued to include free and reverse T-3 concentrations. Eight patients have demonstrated a sustained decrease in free and total T-3 to 24 hours after operation and a rising reverse T-3 value. This is the first demonstration of the changes of the concentrations of reverse T-3, a metabolic end product of T-3, in cardiac patients having cardiopulmonary bypass operations.

CHRONIC REJECTION OF CARDIAC ALLOGRAFTS

Previous work involved studies of acquired specific immune allograft tolerance, IL-2 receptor antibody blockade in cardiac xenografts and demonstration of the hyperacute rejection in an isolated heart system. The IL-2 studies continue in collaboration with NCI immunologists using allografts in subhuman primates. The data demonstrate that neither the antibody (anti-Tac) nor IL-2 prolong rejection significantly. Anti-tac chelated with a beta emitter Yttrium 90, was efficacious and can be used to overcome the initial (1-4 week) rejection response.

Two immunosuppressive agents PGE_2 and succinylacetone were tested for rejection modification efficacy in allograft rat cardiac transplants. PGE_2 doubled the time to rejection suggesting that this naturally occurring compound could be used to decrease the toxicity associated with cyclosporine A, the primary agent in clinical use. Succinylacetone prevented the rejection process indefinitely if daily dosing was used. The trials of this agent continue in subhuman primates.

Further work was performed in the study of the mechanism of the hyperacute rejection using an isolated heart system. The data demonstrate that the presence of complement is not necessary for hyperacute rejection and that the proteins responsible are in the IgG and IgM groups. A system to separate large quantities of these fractions from human sera was developed, tested, and used. Future studies will concentrate on determining the immunoglobin(s) responsible for hyperacute acute rejection.

HYPERTROPHIC CARDIOMYOPATHY

Surgical treatment of patients with this genetic disease continues to be a major area of research for the Surgery Branch. Data published in the past year compared the postoperative hemodynamic results of the standard operation developed by Morrow (the left ventricular septal myotomy and myectomy or LVMM) with mitral valve replacement. The complete experience with the latter operation was also reported. These data demonstrated that LVMM now carries a low mortality (2-3%). Mitral valve replacement resulted in a more predictable relief of obstruction at rest and with provocation although incurring a higher perioperative mortality and long-term morbidity.

A major modification of the LVMM was plication of the anterior leaflet of the mitral valve through the aortic root in an attempt to improve the operation and obviate the need for mitral valve replacement. The rationale was based on the observations by intraoperative echocardiograpy that showed that patients with large anterior mitral leaflets continued to partially obstruct the left ventricular outflow tract after an adequate channel in the upper portion of the septum had been obtained with the myectomy. The postoperative echocardiographic studies conclusively demonstrate that plication prevents systolic anterior motion of the anterior leaflet of the mitral valve and does not create either stenosis or regurgitation. More than 30 patients have had the modified operation and five have returned for complete evaluation. These patients have had marked relief of obstruction to the left ventricular outflow tract. The detailed correlations of phasic hemodynamic events and motion of intracardiac structures continues.

A second clinical program is evaluating the use of automatic internal cardiac defibrillators for patients with HCM and a history of sudden death who are refractory to pharmacologic agents during electrophysiologic testing. Three have been implanted. This study continues in collaboration with the Cardiology Branch.

Two retrospective clinical studies have been completed and two are in progress. These demonstrated that pulmonary hypertension is reliably relieved by the LVMM operation and that preoperative treatment of ventricular arrhythmia with amiodarone was associated with significant increases in morbidity and mortality. Two new studies examine the incidence and natural history of patients who had left bundle branch block as a consequence of operation. A second study has examined the long-term outcome of the LVMM procedure in the young under 18 years of age. Myocardial tissue continues to be supplied to many investigators at NIH and throughout the U.S.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 02714-09 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Evaluations of Prosthetic Cardiac Valves: In Vivo Studies

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

Michael Jones, M.D., Principal Investigator, Surgery Branch, NHLBI

Victor J. Ferrans, M.D., Pathologist and 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 development and application of an in vivo, in situ animal model system using juvenile sheep for evaluating cardiac valves. The studies use several disciplines of investigative techniques: surgery, physiology, pathology, biomechanics, physical/chemical sciences, and device design, implementation, and efficacy. Efforts during the current year have concentrated upon the following areas: 1) continuing studies of the mechanisms of calcification of bioprosthetic valves and the mitigation of this clinically important degenerative process; 2) comparative in vivo hydraulic, hemodynamic assessments of the generic types of prosthetic valves and their respective proprietary designs; 3) utilization of evolving ultrasonic technology to better understand fluid dynamics associated with normally and pathologically occurring intracardiac events particularly as related to the mitral valve; and 4) continuation of collaborative studies of the morphology of pathologic alterations developing with the use of biomaterials for valvular prostheses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02731-07 SU

PERIOD COVERED
October 1, 1988 through September 30, 1989

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 Surgeon, Surgery Branch, NHLBI

Barry J. Maron, M.D., Senior Investigator, Cardiology 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)

XXX ☒ (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, and new for this year, LVMM and plication of the anterior leaflet of the mitral valve. The standard procedure was performed in 17 patients, MVR in 7 and the combined operation in 32. 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02740-06 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Coronary Vascular Resistence and Cardiac Metabolism in the Postoperative Period

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

Chahine Yamine, M.D., Research Fellow, Surgery Branch, NHLBI

Joseph E. Flack, M.D., Senior Staff Fellow, Surgery Branch, NHLBI
Julie A. Swain, M.D., Senior Investigator, 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:
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 hypotheses tested in this study are: 1) Systemic hypertension in the immediate postoperative period is detrimental to myocardial metabolism, 2) The ideal level of systemic arterial pressure with regard to myocardial metabolism can be determined using a thermodilution flow catheter placed in the great cardiac vein, and 3) The mode of pacing alters myocardial metabolism.

Prior to operation the coronary sinus and great cardiac vein are cannulated with a Thermistor catheter. Serial determinations of coronary flow, lactic acid concentrations and systemic hemodynamic variables are made in the postoperative period. Systemic blood pressure is altered to assess myocardial metabolism at various levels of pressure. Pacing the atrium versus the ventricle is used to determine the effects of pacing site on myocardial metabolism.

These studies should determine the effect of blood pressure control and pacing mode on myocardial metabolism in the immediate postoperative interval after coronary artery bypass and other cardiac procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02774-04 SU

PERIOD COVERED

October 1, 1989 through September 30, 1989

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

In vitro Assessment of Noninvasive Methods of Evaluation of Prosthetic Heart Valves

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

Sandy F.C. Stewart, Ph.D., Principle Investigator, Surgery Branch, NHLBI

Edward P. Nast, M.D., Clinical Associate, Surgery Branch, NHLBI
Francisco A. Arabia, M.D., Clinical Associate, Surgery Branch, NHLBI
Thomas L. Talbot, M.M.E., Staff Engineer, Biomedical Engineering and Instrumentation Branch, Division of Research Services
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

BEIB, ORS

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	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.)*

Ultrasound measurement of pressure drops and regurgitation in prosthetic heart valves was assessed in a physiologic pulse duplicator, in 18 mechanical, 33 prosthetic, and 12 explanted bioprosthetic aortic valves. Pressure drop was measured using simultaneous high frequency manometric pressure and CWD velocity measurements. Although the data showed high correlation coefficients (R^2 > 0.92), the calculated pressure drop was always greater than that measured manometrically. The slope was valve type dependent, and varied between 0.46 and 0.85 for mechanical and bioprosthetic valves, but was independent of valve size and flow rate. Comparison of slopes between valve types by analysis of covariance demonstrated significant drops ($p<0.05$). Since no correlations could be drawn between CWD measured and electromagnetic flowmeter measured regurgitation, work is underway to assess two-dimensional color flow Doppler for quantifying regurgitation in bioprostheses with circular holes and in explanted valves. A new ventricular chamber was made which allows ultrasound imaging on the ventricular side of the aortic valve.

Polymeric synthetic stiff, giving mean pressure drops of 12, 18, and 21mm Hg at cardiac outputs of 3.6, 4.8, and 6.0 l/min. Leaflet opening was not simultaneous. Leaflet flutter caused systolic flow disturbance, visible as streaks of color in the M mode color imaging, combined with a 20 hz systolic murmur as shown by phonocardiography. Minor regurgitation (3.8 percent of stroke volume) was found at the joint between leaflets at the struts. A silicone synthetic leaflet valve showed massive regurgitation (77 - 93 percent) due to prolapse of the leaflets giving a diastolic triangular central opening. The pressure drops were very low (3.7 -6.9 mm Hg) but the valve was deemed unusable due to the regurgitation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02777-03 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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 Xenograft

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

Frederick M. Dirbas, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Thomas A. Waldman, M.D., Chief, Immunology Branch, NCI
Otto A. Gansow, Ph.D., Head, Inorganic & Radioimmunochemistry Section,
Radiation Oncology Branch, NCI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Inorganic & Radioimmunochemistry Section, NCI
Immunology Branch, NCI

LAB/BRANCH

Surgery Branch, NHLBI

SECTION

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	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.)*

This study is now in its third year to test the hypothesis that monoclonal antibody binding to IL-2 receptors on activated T-cells will influence graft tolerance especially when chelated to toxins or radioactive substances. Anti-tac, a mouse raised 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 but not in a clinically important way (13 vs 9 days). Pseudomonas exotoxin chelation was not useful. Yttrium-90 chelation to anti-tac markedly prolonged allograft viability to 99 days. The effect was shown to be independent of radiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02779-03 SU

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebral and Myocardial Energetics: Hypothermia and Cardiopulmonary Bypass

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

Thomas McDonald, BS, Research Biologist, Surgery Branch, NHLBI
Robert C. Robbins, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Robert S. Balaban, M.D., Laboratory of Cardiac Energetics, NHLBI
Victoria Hampshire, VMD, Visiting Scientist, 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:
6	5	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.)*

The majority of cardiac surgical procedures are conducted using cardiopulmonary bypass (CPB) and systemic hypothermia. CPB and hypothermia are finding increasing use in the treatment of patients prior to and immediately after cardiac and cerebral ischemia. Neither the effects of CPB nor of systemic hypothermia are known in relation to brain or heart energetics or intracellular pH. Our laboratory is studying these effects using measurements of myocardial function and using 31-P Nuclear Magnetic Resonance Spectroscopy. Four areas of investigation are ongoing in the laboratory: 1) NMR spectroscopy has been used to assess the effects of CPB on 37°C brain and heart energetics by measuring ATP, PCR, and inorganic phosphate levels and the intracellular pH, 2) The effect of moderate and deep hypothermia on brain and heart energetics has been determined by NMR, 3) NMR spectroscopy has been used to determine the relation between the blood pH and the brain and heart pH during hypothermia, and 4) The relation between changes in the acid-base status of the blood on systemic metabolism and hemodynamics has been determined.

Results show that CPB does not change the baseline energy state of the heart and brain, but that deep hypothermia does increase high energy phosphates in the brain. Both the heart and brain maintain their intracellular pH constant relative to the neutral pH of water, despite changing the blood pH during hypothermia. Finally, despite theoretical predictions to the contrary, changing the acid-base status during hypothermia did not produce large changes in systemic oxygen consumption but did affect systemic hemodynamics. The above findings, combined with previous work by the Principle Investigator and others, have important implications in the conduct of cardiac surgery operations that routinely employ CPB and hypothermia. Results suggest that the alpha-stat scheme of pH management should be used to optimize the physiological status of the patient.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02781-02 SU

PERIOD COVERED
October 1, 1988 through September 30, 1989

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

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

Michael Crittenden, M.D., Senior Staff Fellow, Surgery Branch, NHLBI
Thomas McDonald, B.S., Research Biologist, Surgery Branch, NHLBI
Robert S. Balaban, Ph.D., Chief, Laboratory of Cardiac Energetics, NHLBI
Toni Ceckler, Ph.D., Senior Fellow, Surgery Branch, NHLBI
Patrick K. Griffith, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Charles S. Roberts, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Louis Rosa, M.D. Guest Worker, Surgical Neurology, NINCDS

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	4	

CHECK APPROPRIATE BOX(ES)

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

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

A major problem in cardiac surgery is that of brain protection. Circulatory arrest, or the total cessation of blood flow in the body, is necessary for many types of infant and adult cardiac surgical procedures. The effects of and time limit on circulatory arrest are poorly understood. This laboratory has been actively involved in investigating the effect of ischemia on brain metabolism and function to find ways of increasing the tolerance of the brain to ischemia. P-31 nuclear magnetic resonance spectroscopy has been used to assess cerebral energetics. Functional and pathological studies are used to confirm the NMR findings.

Three areas of investigation are currently underway to study various aspects of cerebral preservation. In the first, a model of hypothermic cardio- pulmonary bypass and circulatory arrest in sheep undergoing nuclear magnetic resonance spectroscopy was developed and used to investigate the kinetics of high energy phosphate depletion during ischemia and repletion with reperfusion, and the changes in intracellular pH and the EEG with ischemia and reperfusion. This model was then used to assess the protective effects of intermittent perfusion of the brain with a hypothermic asanguinous oxygenated crystalloid solution (termed cerebroplegia). Cerebroplegia was demonstrated to preserve high energy phosphate levels, prevent the marked decrease in intracellular pH, and to result in a faster return of the EEG after ischemia. A second study found that cerebroplegia improved the functional neurologic status in a chronic animal preparation.

The third area of study is that of the effects of extremely low flow perfusion on cerebral preservation. Clinically, low perfusion rates (5-10 cc/kg/min) are routinely used in some types of cardiac surgery. Studies are underway to determine the safe period of low flow and the level of flow needed. 31-P NMR spectroscopy is being used to determine the consequences of inter- mittent and reduced cerebral perfusion on cerebral energetics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02782-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Amelioration of Hyperacute Rejection

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

Christopher D. Stone, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Robert C. Robbins, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Marc E. Mitchell, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
David Sachs, M.D., Senior Investigator, Immunology Branch, Division of Cancer Biology and Diagnosis, NCI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Transfusion Medicine, CC
Immunology Branch, DCBD, NCI

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

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

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.)*

The use of large domestic animals as heart donors represents a potential solution to the current human donor shortage. Hyperacute graft rejection (HAR) remains a major barrier to xenotransplantation. The purpose of this study was to employ an ex-vivo preparation for the evaluation of HAR in discordant cardiac xenografts. Freshly excised hearts from fifty-one pigs (10-37kg) were perfused at 37 degrees centigrade via the aorta in a retrograde fashion. The hearts were allowed to function in a non-working mode for four hours or until the rejection process resulted in irreversible cardiac dysfunction. The perfusate consisted of Krebs-Henseleit bicarbonate buffer in addition to fresh whole autologous pig blood (n=4) (Group A), dog blood (n=3) (Group B), baboon blood (n=5) (Group C), human PRBCs (n=2) (Group D), human blood and plasma (n=3) (Group E), human whole blood (n=10) (Group F), and human plasma (n=9) (Group G). HAR was uniform for Groups B, E, and G. No evidence of HAR was noted in Groups A, C, or D. Two of ten hearts demonstrated HAR in Group F. An additional twelve hearts have had studies for staining with immunoperoxidase for IgG and IgM. The results are pending. This perfusion circuit provides a means for the analyses of components of human blood to define which component(s) are required for HAR of discordant cardiac xenografts. The results indicate that the HAR of pig hearts perfused with human blood is mediated by a component found in the plasma, probably pre-formed natural antibody. Studies are currently underway to evaluate which class of antibody mediates the rejection process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02784-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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)*

David A. DeBoer, M.D., Principle Investigator, Surgery Branch, NHLBI

Michael D. Crittenden, M.D., Senior Staff Fellow, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch, NHLBI

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, 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 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 at low temperatures. Nicardipine is not a clinically useful agent when added to cold cardioplegic solution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02785-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1988

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

Simultaneous Intraoperative Echo & Hemodynamic Evaluation of Obstructive HCM

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

Christopher D. Stone, M.D., Principle Investigator, Surgery Branch, NHLBI

Barry J. Maron, M.D., Cardiologist, Cardiology Branch, NHLBI
Charles L. McIntosh, M.D., Surgeon and Senior Investigator, Surgery 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:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

XXX ☒ (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 hypotheses to be tested in these intraoperative studies are: a) true obstruction to left ventricular outflow due to mechanical impedance exists in patients with hypertrophic cardiomyopathy (HCM) who have a subaortic pressure gradient and b) operative intervention to relieve this obstruction results in relief of the obstruction and elevated intraventricular systolic pressures, which are detrimental to left ventricular performance. Solid state pressure transducers, electromagnetic flow transducers, and a Hewlett-Packard ultrasonic system are applied under direct exposure intraoperatively in consecutive obstructive HCM patients undergoing first-time operative intervention. Echocardiographic studies preoperatively, intraoperatively (pre-and post-procedure), and postoperatively will include 2-dimensional (2-D), M-mode, and color Doppler imaging, in addition to pulsed Doppler, and continuous wave Doppler (CWD). The intraoperative echocardiography and hemodynamic measurements of left ventricular outflow tract (LVOT) pressure gradient and aortic blood flow are obtained simultaneously. The findings of this study are expected to provide evidence supporting the presence of true mechanical obstruction of the LVOT in patients with HCM. these data should support the increased use of surgical palliation in selected patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02790-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Myocardial Protection of Cyanotic Hearts

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

Joseph E. Flack, M.D., Principal Investigator, Surgery Branch, NHLBI

Chahine Yamine, Research Fellow and Investigator, Surgery Branch, NHLBI
Isabella Liang, Ph.D., Senior Staff Fellow, Surgery Branch, NHLBI
Julie A. Swain, M.D., Senior Surgeon and Investigator, Surgery Branch, NHLBI
Victor J. Ferrans, M.D., Pathologist and Senior Investigator, Pathology Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery 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	2	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.)*

The tolerance to global cardiac ischemia and reperfusion, necessary for repair of congenital defects in the young has been found to be poor in cyanotic patients compared to those without cyanosis. The purpose of these studies was to develop a colony of cyanotic young dogs to determine the effect of chronic hypoxemia on myocardial function and develop methods to improve tolerance to global ischemia.

Foxhound puppies underwent the creation of a right to left shunt between the pulmonary artery and left atrium using absorbable sutures. This created a level of cyanosis which was found to remain constant despite a 3 to 4 fold increase of weight over a 6 month adaptive period.

Currently, we are involved in the second phase of this study. This involves a terminal study of these animals at the end of this 6 month period and measurement of left ventricular function, high energy phosphates and myocardial metabolism via lactate flux and MVO_2. These measurements are done both before and after a period of cross clamp induced ischemia, and correction of the shunt. In this way we hope to gain some insight into the mechanisms responsible for the reported higher morbidity and mortality often reported in children with cyanotic congenital heart disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02794-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Chordal Preservation during Mitral Valve Replacement: Effect on LV Function

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

Hani A. Hennein, M.D. Principal Investigator, Surgery Branch, NHLBI

Michael Jones, M.D., Senior Investigator, Surgery Branch, NHLBI
Christopher D. Stone, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Eric N. Mendeloff, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Julie A. Swain, M.D., Senior Investigator, 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:
4.0	3.0	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 hypothesis tested in this study was that preservation of the chordae tendineae during mitral valve replacement for chronic mitral regurgitation resulted in improved ventricular performance compared to hearts in which the chordal structures were resected. Young sheep had creation of mitral regurgitation and 6-8 months later had a mitral valve replacement with a hemi disc prosthesis. Several months later these animals were assessed by hemodynamic and ultrasonic methods. The data demonstrate that acute mitral regurgitation resulted in increased cardiac work and chronically, cardiac power was diminished. Analyses of comparative data to determine the effect of chordal preservation is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02795-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Thyroid Hormone Concentrations and Cardiac Surgery

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

Richard E. Clark, M.D., Principal Investigator, Surgery Branch, NHLBI

Fred Holland, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Benjamin Synder, B.S., CCP, Clinical Center
Robert Groom, B.S., CCP, Clinical Center
Ronald Elan, M.D., Clinical Pathology, Clinical Center
Bruce Weintraub, M.D., NIDDK, MCNE

COOPERATING UNITS *(if any)*

Clinical Pathology

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	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.)*

The hypothesis tested in this study was that thyroid hormone levels during and after cardiopulmonary bypass (CPBP) would be a function of the mode of flow: steady state versus pulsatile. Three blood samples prior to, during, and after CPBP were used. 25 patients 12 in the pulsatile group were used. The data show that TSH increases during hypothermic hemodilutional CPBP free t_4 remained constant, TBG decreased and recovered to 75% of the pre CPBP by 24 hours as did total T_4. Total T_8 decreased 20% of pre CPBP and remained low for the first 24 hours after operation. Albumin used as a marker of the degree of hemodilution decreased to 55% of pre CPBP levels during CPBP. Pulsatile flow had two effects only a) a transient rise in TSH levels at the mid CPBP interval and b) a lack of increase in albumin concentration after surgery. The latter effect was caused by an increased use of crystalloid solution in the initial post operative in this group. Free and reverse T_3 have been measured in an additional six patients with the previously measured variables. The data show a sustained decrease in free T_3 and a rising reverse T_3 which is highly variable. The conclusions were that total T_3 remains at low levels and may contribute to low cardiac output and pulsativity per se has little effect on the concentrations of thyroid hormones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 02796-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Studies in Myocardial Protection from Super Oxide Radical Injury

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

David A. DeBoer, 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, NHLBI

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.)*

Oxygen-derived free radicals have been implicated as one mechanism of injury associated with global ischemia and reperfusion. Isolated working rat hearts were used to study the effect of chelation of myocardial iron to decrease oxygen free radical formation and enhance post ischemic performance. The drug deferoxamine was used as pretreatment, infused into the aortic root during ischemia or given at the onset of reperfusion. Dose response data were obtained for each mode. The data demonstrate that pretreatment at 10 or 30 mg/kg c.m increased heart survival from 44 to 71-72%. When given during reperfusion, no improvement was found nor was any beneficial effect found in the cardioplegic infusion group except at a dose of 0.46 mmol/l. These data suggest that chelation of myocardial iron to decrease the generation of oxygen radicals by the Haber-Weiss reaction is effective only if given prior to reperfusion and would contribute little to a clinically significant improvement in myocardial protection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02797-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Energy Spectra of Cardiac Sounds

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

Richard E. Clark, M.D., Principle Investigator, Surgery Branch, NHLBI

Sandy F.C. Stewart, Ph.D., Biomedical Engineer, Surgery Branch, NHLBI
Gail E. Greenberg, Statistical Assistant, Surgery Branch, NHLBI
David Caden, Medical Instrumentation Technologist, Surgery Branch, NHLBI
Moshe E. Mehlman, Clinical Engineer, 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:
0.4	0.1	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.)*

It was hypothesized that computer assisted phonocardiography would provide a warning that bioprosthetic valves in patients were becoming dysfunctional. However, frequency analysis of the closing sounds proved not clinically useful. The software was recently improved to provide spectral analysis on consecutive 17 millisecond segments of the acoustic signal. The three-dimensional plot of relative power versus frequency and time shows the frequency response over the entire cardiac cycle, rather than limiting the analysis to just the valve closure. Events throughout the cardiac cycle can be identified such as noise from turbulence due to stenosis or regurgitation. The data from a subpopulation of approximately 40 patients with one prosthetic valve are currently undergoing the new analysis to provide spectra to be correlated to clinical data. Analyses completed to date demonstrate the development of harmonics associated with systolic murmurs that are possibly associated with stenosis. These changes in harmonics are easily identifiable against the peak S1, S2, and S3 landmarks, which remain essentially unchanged in frequency and relative intensity with time. Three dimensional analyses of patient data recorded to date will be performed, from a subpopulation of patients with one bioprosthetic valve who have had two or more serial exams.

Data gathered from previous in vitro tests of valves in a pulse duplicator will also be reanalyzed to determine if the new method can elicit useful data. Spectra will be correlated to hemodynamic variables such as flow velocity and turbulence. Further experiments will examine the ability of phonocardiography to assist in prediction of regurgitation in leaky bioprosthetic valves. Continuous wave Doppler, pulsed Doppler, and color flow mapping will provide additional correlates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02798-02 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

The Effects of Digoxin and Ischemia on the Immature Myocardium

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

Michael D. Crittenden, M.D., Principle Investigator, Surgery Branch, NHLBI

Gerald Kelly, Research Assistant, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Chemistry Department, National Institute of Standards and Technology, Gaithersburg, MD

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	0.4	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.)*

Newborns with congestive heart failure are universally treated with digitalis compounds. The hypothesis tested in these studies is that the drug is deleterious in the setting of ischemia and reperfusion because of Na/K ATPase inhibition leading to abnormally high intracellular concentrations of Sodium and Calcium. Newborn pigs will be treated with digoxin for five days in clinically relevant doses. Treated and non-treated hearts will have intracellular myocardial sodium and calcium concentrations determined using atomic absorption spectrometry. A total of four groups of 8-10 hearts will be placed on the isolated working heart apparatus and two groups of animals will serve as controls (treated and untreated). Two groups (Digoxin treated vs non-treated hearts) will be subjected to 30 minutes of warm ischemia then assessed for adenosine metabolites and tissue cation contents. The remaining two groups (Digoxin treated vs non-treated hearts) will be subjected to 30 minutes of warm ischemia then processed for biochemical analyses as were the first two groups. The control groups will have their hearts harvested and subjected to biochemical testing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 02799-01 SU

PERIOD COVERED
October 1, 1988 through September 30, 1989

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 Liang, M.D., Senior Staff Fellow, Surgery Branch, NHLBI

Joseph E. Flack, M.D., Senior Staff Fellow, Surgery Branch, NHLBI
Chahine Yamine, Research Fellow, Surgery Branch, NHLBI
Julie A. Swain, M.D., Senior Investigator, 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.)*

Despite the growing awareness of the clinical significance of right ventricular dysfunction, attempts to quantitatively assess right ventricular performance to better understand the pathophysiology of right ventricular failure and its influence on the left ventricle have not been successful. 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. This model of right ventricular dysfunction is clinically relevant since pressure overload induced right ventricular hypertrophy is similar to that seen in many congenital cardiac defects. It is the purpose of this study to observe the changes on right and left ventricular performance in the heart with right ventricular hypertrophy before and after global ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02800-01 SU

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Modification of Chronic Rejection Process

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

Christopher D. Stone, M.D., Medical Staff Fellow, Surgery Branch, NHLBI

Robert C. Robbins, M.D., Medical Staff Fellow, Surgery Branch, NHLBI
Bruce R. Rosengard, M.D., Immunology Branch, NCI
David H. Sach, M.D., Immunology Branch, NCI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Immunology Branch, NCI

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	

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 series of studies were undertaken to study the effects of PGE_2 and a newly synthesized compound succinylacetone (SA) on the acute and chronic processes of cardiac transplants. Studies were performed in rats and monkeys. An inbred pig colony was used to determine the effect of MHL matching on allograft cardiac survival. The data demonstrate that PGE_2 nearly doubles the survival time of transplanted hearts. SA provides indefinite survival of hearts in rats and monkeys when given continuously by an implanted osmotic pump system. These data suggest that a naturally occurring prostaglandin may have adjunctive use for preventing chronic rejection whereas succinylacetone may prove to be a new major immunosuppressive agent for transplantation and avoid the significant complications of cyclosporine A.

ANNUAL REPORT OF THE
LABORATORY OF BIOCHEMICAL GENETICS
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1988 through September 30, 1989

Four novel Drosophila melanogaster homeobox genes were found by screening a genomic DNA library with oligodeoxynucleotides that correspond to a conserved amino acid sequence that is part of the putative site of homeobox proteins that recognizes nucleotide sequences in DNA. The portion of each gene around the homeobox was sequenced. The deduced amino acid sequences of NK-2, NK-3, and NK-4 homeoboxes are more closely related to one another (59-66% homology) than they are to other Drosophila homeoboxes (28-54% homology); whereas, the homeobox of NK-1 is most closely related, in order of decreasing homology, to muscle segment homeobox, zerknüllt-1, NK-3, and Distal-less homeoboxes. Three of the genes, NK-1, NK-3, and NK-4 comprise a novel cluster of homeobox genes located in the 93E1-3 region of the right arm of the third chromosome; whereas, the fourth homeobox gene, NK-2, is located in the 1C1-5 region of the X-chromosome. NK-1 and NK-2 poly A^+ RNA are most abundant during embryonic development, then decrease in abundance, but are expressed again in adult flies. In contrast, NK-3 and NK-4 poly A^+ RNA are expressed transiently only during embryonic development.

In addition, portions of 10 novel mouse homeobox genes were cloned and the nucleotide sequence of the homeobox region of each gene was determined.

A selection method for mouse genomic DNA fragments with enhancer or promoter activity was used that is based upon the observation that replication of polyoma DNA in mouse cells is dependent on enhancers associated with the polyoma origin of replication. Murine or rat neuroblastoma, glioma, striated muscle, or fibroblast cell lines were transfected with DNA consisting of a library of mouse genomic DNA fragments ligated to an enhancerless polyoma-pAT153 shuttle vector, $pPyE^o$. Transfected cells were incubated for several days and plasmid DNA then was harvested and the DNA was amplified in E. coli. Recombinants were cloned and characterized after several cycles. The selected populations of recombinants contained some highly abundant clones that each comprised 20-40% of the total plasmid DNA recovered; therefore, the selection method was highly effective. Some of the DNA inserts were subcloned in the 5'-upstream region of an enhancerless chloramphenicol acetyltransferase vector, pA10CAT2, and most were found to increase the expression of the chloramphenicol acetyltransferase gene (2- to 14-fold range). DNA sequence analysis showed that one of the recombinant clones is homologous to the Long Terminal Repeat of intracisternal A-particles, a second clone to the 5'-flanking region of the human vinentin gene, and a third clone has 23 consecutive TG repeats, which is expected to have the conformation of Z DNA. The DNA

insert of one recombinant, selected in mouse neuroblastoma cells, stimulates plasmid DNA replication in neuroblastoma cells, but not in the other cell lines tested.

In previous studies we have obtained cDNA clones that correspond to species of poly A^+ RNA that are more abundant in NG108-15 neuroblastoma-glioma hybrid cells with elevated cAMP levels than in control cells and have shown that prolonged elevation of intracellular cAMP levels shifts the cells to a more differentiated state and increases the abundance of synapses. Sequence analysis showed that clone pNG-10 cDNA corresponds to mitochondrial displacement loop poly A^+ RNA; whereas pNG-32 cDNA corresponds to the mitochondrial ATPase 6 gene. The displacement-loop region of mitochondrial DNA contains nucleotide sequences that regulate the synthesis of mitochondrial mRNA or DNA. The mitochondrial ATPase 6 gene codes for a protein that is part of the proton pump-ATP synthase complex that catalyzes the synthesis of ATP. During the past year we have shown that elevation of cAMP levels in NG108-15 cells by activation of adenylate cyclase, by inhibition of cyclic nucleotide phosphodiesterase, or by simultaneous activation of adenylate cyclase and inhibition of cyclic nucleotide phosphodiesterase, results in approximately a five-fold increase in RNA transcribed from both the mitochondrial light and heavy strands of DNA and a 2-to 3-fold increase in the abundance of mitochondrial DNA relative to values found with control cells. These results show that the number of mitochondria increase in NG108-15 cells treated with cAMP and suggest that the accumulations of mitochondrial RNA and DNA due to cAMP are coupled.

We previously showed that prolonged elevation of intracellular cAMP levels in NG108-15 neuroblastoma-glioma hybrid cells also results in the appearance of functional voltage-sensitive calcium channels that are needed for stimulus-secretion coupling in NG108-15 cells that innervate cultured skeletal muscle cells. Hence, the abundance of synapses and efficiency of transsynaptic communication are regulated presynaptically by cAMP in this model cultured cell system. Cloned cDNAs that correspond to the α_1-subunit of the L-type voltage-sensitive calcium channel were obtained by screening a rat brain λgt11 library with oligodeoxynucleotide probes and the nucleotide sequences of the cDNAs were determined. The deduced amino acid sequence of the calcium channel protein from rat brain was similar to the calcium channel α_1-subunit of rabbit skeletal muscle in transmembrane domains, but less homology was found in cytoplasmic and extracellular domains. These cDNAs can be used as probes for studies on the regulation of the expression of the gene for the α_1-subunit of the voltage-sensitve calcium channel in the nervous system.

Cyclic AMP and phorbol esters that activate protein kinase C synergistically elevate neuropeptide Y levels 20-200-fold within 4-24 hr in PC12 rat pheochromocytoma cells. Treatment of cells

with nerve growth factor (NGF) results in a 40-100-fold increase in neuropeptide Y mRNA within 1-6 days and protein synthesis is required for the increase in mRNA. Treatment of cells with phorbol ester, cAMP and NGF synergistically result in a 300-fold elevation of neuropeptide Y mRNA. In contrast, glucocorticoids potentiate the early (3-10 hr) effects of NGF but profoundly inhibit NGF effects at later times (1-6 days). These findings were confirmed and extended by transcription assays in isolated nuclei. NGF, dexamethasone, cAMP, and phorbol ester were shown to alter the rates of transcription of neuropeptide Y mRNA; their effects on neuropeptide Y mRNA stability were found to be less significant.

Glucocorticoids potentiate preproenkephalin (pEnk) gene expression in several systems including rat brain. We previously showed that glucocorticoids and adenylate cyclase activators synergistically elevate pEnk mRNA levels in C6 rat glioma cells. The pEnk gene transcription rate, assayed in nuclei was not altered by dexamethasone alone, was transiently stimulated by cAMP, and was more persistently stimulated by both dexamethasone and cAMP together (3-6-fold over 1-24 hr). To search for functionally cooperative glucocorticoid and cAMP regulatory elements, chimeric plasmids containing the chloramphenicol acetyltransferase (CAT) reporter gene under the control of rat pEnk gene sequences from nucleotide residues -2800 to +750 were constructed in a promoterless vector. In C6 cells transfected with these constructs, cAMP increased CAT activity 5-17 fold. Unexpectedly, dexamethasone reduced basal and cAMP-stimulated CAT activity by as much as 48 percent. These results suggest that cAMP and glucocorticoids cooperatively elevate endogenous pEnk gene transcription, and that the region of the gene analyzed may contain, in addition to cAMP-responsive elements, a negative glucocorticoid responsive element, suggesting that positive glucocorticoid action may occur via a more distant glucocorticoid responsive element or via an indirect mechanism.

Cotransfection of C6 glioma cells with human T-cell leukemia virus I (HTLV-I) and a rat pEnk-CAT expression vector resulted in a 3-9-fold increase in pEnk-CAT expression that was dependant on the expression of the tax_1 protein of HTLV-I. This suggests that proenkephalin biosynthesis may be activated in certain cells of patients afflicted with diseases caused by HTLV-I infection, such as tropical spastic paraparesis.

Ankyrin, a peripheral membrane protein implicated in linking certain integral membrane proteins to the spectrin-actin membrane cytoskeleton, was shown to be colocalized with voltage-sensitive sodium channels in the troughs of the folds of the postsynaptic membrane. Both ankyrin and voltage-sensitive sodium channel proteins were found to be excluded from the postsynaptic crests opposite the nerve terminal. In contrast, acetylcholine receptors and the receptor-associated 43-kD peripheral membrane protein are concentrated at the crests.

In previous studies we have used embryonic brain extracts to stimulate the formation of acetylcholine receptor aggregates on myotubes in tissue culture. The acetylcholine receptor-enriched domains in newly formed aggregates were shown to contain the 58-kD receptor associated protein and an isoform of β-spectrin, as well as the 43-kD protein and actin. These results suggest that the acetylcholine receptors are immobilized by linkage to a spectrin-actin membrane cytoskeleton at an early stage in the aggregation process. Preliminary electron microscopic studies on these membranes showed that the acetylcholine receptor-enriched domains contain a filamentous meshwork.

Using an immunogold labeling technique the α_1- and α_2-subunits of the voltage-sensitive calcium channel were shown to be localized in the transverse tubule membrane at the junction with the sarcoplasmic reticulum (triad junction). The location of the calcium channels may facilitate the excitation-contraction coupling process by directly juxtaposing the voltage-sensitive calcium channels and the calcium release channels of the sarcoplasmic reticulum.

In E. coli, adenylate cyclase and the DNA binding protein that interacts with cAMP (CRP) are important factors in the regulation of carbon metabolism. Additional presumed components of the regulatory apparatus are proteins involved with the sugar transport system known as the phosphoenolpyruvate:glycose phosphotransferase system (PTS).

Heterofermentative Lactobacilli appear to lack a functional PTS. However, these organisms possess a metabolite-activated vectorial process that displaces intracellularly accumulated galactosides. This regulatory mechanism resembles the expulsion process of homofermentative lactic acid bacteria, which possess a PTS and an ATP-dependent HPr kinase. We investigated the possibility that heterofermentative Lactobacilli may contain HPr and a system for the reversible phosphorylation of HPr. We found that two species of heterofermentative Lactobacilli are deficient in PTS activity but, nevertheless, contain HPr, a kinase, and a phosphatase that catalyze the reversible phosphorylation of HPr.

Protein phosphorylation in Mycoplasma gallisepticum was investigated. A protein kinase was found that phosphorylates an endogenous 55 kD protein substrate as well as a phosphatase that acts on the phosphoprotein.

We previously described forms of CRP that act independently of cAMP (CRP*). 220 CRP has mutations at positions 127 and 170; 222 CRP has the mutations in 220 CRP and, in addition, a substitution of arginine for leucine at position 195. 222 CRP activated a greater number of promoters than did 220 CRP and exhibited increased affinity for DNA. We constructed a mutant CRP with only the substitution at position 195 and found that it was a CRP*. We

concluded that the effects of multiple mutations in CRP can be both cumulative and interactive.

The PTS components that are not sugar specific (enzyme I and HPr) are constitutive and intracellular; purification studies have indicated that the proteins are soluble. Since there is reason to believe that enzyme I may be a multifunctional protein that plays a role in regulating the activity of adenylate cyclase in a membrane-bound complex, we explored the nature of the *in vivo* condition of enzyme I by immunoelectron microscopy. The results of the study indicate that enzyme I is localized to the inner cytoplasmic membrane and that it interacts with a limited number of binding sites associated with the cytoplasmic membrane. Although not yet identified, we predict that the binding site for enzyme I is a component of the sugar transport apparatus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00009-15 LBG

PERIOD COVERED

October 1, 1988 - September 30, 1989

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
Yongsok Kim, Visiting Fellow, LBG, NHLBI
Kohzo Nakayama, Visiting Fellow, LBG, NHLBI
Noriko Nakayama, Guest Worker, 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
G. David Trisler, Special Volunteer, LBG, 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:
13	10	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.)*

Fourteen novel homeobox genes, 4 genes from Drosophila melanagaster and 10 murine genes, were cloned and were sequenced partially. Three of the Drosophila homeobox genes, NK-1, NK-3, and NK-4, comprise a novel cluster of homeobox genes located in the 93E1-3 region of the right arm of the third chromosome; whereas the fourth homeobox gene, NK-2, is located in the 1C1-5 region of the X-chromosome. NK-1 and NK-2 poly A+ RNA are most abundant during embryonic development, then decrease in abundance, but are expressed again in adult flies. NK-3 and NK-4 poly A+ RNA also are expressed during embryonic development, but not thereafter.

A selection method for mouse genomic DNA fragments with enhancer or promoter activity was used that is based upon the observation that replication of polyoma DNA in mouse cells is dependent on enhancers associated with the polyoma origin of replication. Murine or rat cell lines were transfected with DNA consisting of a library of mouse genomic DNA fragments ligated to an enhancerless polyoma-pAT153 shuttle vector. Plasmids that replicate in mammalian cells were cloned and characterized. Most of the selected clones that were tested were found to have promoter or enhancer activity when subcloned in the upstream region of an enhancerless chloramphenicol acetyltransferase vector.

Prolonged elevation of cAMP in NG108-15 neuroblastoma-glioma cells was shown to increase the abundance of mitochondrial mRNA and DNA relative to values found with control cells.

cDNA clones were obtained that correspond to the α-1 subunit of the L-type voltage-sensitive calcium channel of rat brain and the nucleotide sequence of most of the cDNA was determined. The deduced amino acid sequence of the α-1 subunit of the calcium channel from rat brain was shown to be similar to the α-1 subunit of rat skeletal muscle in transmembrane domains, but less homology was found in the putative cytoplasmic and extracellular domains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00018-12 LBG

PERIOD COVERED

October 1, 1988 - September 30, 1989 (actually July 1, 1988 to July 1, 1989)

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
Dong-Ping Tan, Ph.D., Visiting Fellow

COOPERATING UNITS *(if any)*

Hsiu-Ying Yang, Ph.D., LBG, NIMH, St. Elizabeth's Hospital, Washington, D.C.
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:
2.5	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.)*

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 large increases in NPY mRNA levels and NPY gene transcription rates in PC12 rat pheochromocytoma cells are elicited by the synergistic action of cyclic AMP, protein kinase C activators, and nerve growth factor (NGF). The action of NGF is profoundly inhibited by glucocorticoids, illustrating an important antagonism between NGF and glucocorticoids in neural development.

A second group of projects concerns regulation of transcription of the gene coding for proenkephalin, the precursor of the enkephalin opioid peptides. Glucocorticoids and cyclic AMP synergistically increase the transcription of the proenkephalin gene and the abundance of proenkephalin mRNA in C6 rat glioma cells. Sequences near and within the gene were analyzed for a glucocorticoid regulatory element, but none was found within 2800 bases upstream from the promoter. The trans-activator protein (tax_1) of the human T-cell leukemia virus I (HTLV-I) was found to be capable of transactivating the proenkephalin gene promoter in cotransfection transient-expression assays. This suggests that proenkephalin biosynthesis may be activated in certain cells of patients afflicted with diseases caused by HTLV-I infection, such as tropical spastic paraparesis.

A third project is an attempt to isolate and sequence cDNA clones coding for the precursors of bovine brain morphine-modulating peptides, which are amidated octa- and octadecapeptides recently characterized by Dr. H.-Y. Yang. These peptides have been shown to antagonize the central analgesic effect of opiates, and they may be involved in opiate tolerance and dependence.

These studies will hopefully light on the control of biosynthesis of peptides and 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 00151-19 LBG

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

COOPERATING UNITS *(if any)*

University of Conn., Storrs, CT (A. Romano); University of Cal., San Diego, CA, (J. Reizer); Hebrew University, Jerusalem, Israel (S. Rottem); FDA, Bethesda, MD, (M. Barile); Texas Tech Univ., Lubbock, TX (J. Harman); Robert Wood Johnson Med. Sch., Piscataway, NJ (B. Ghosh).

LAB/BRANCH

Laboratory of Biochemical Genetics

SECTION

Macromolecules Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	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. Evidence for the Presence of HPr, a Component of the Phosphoenolpyruvate: glycose Phosphotransferase System (PTS) in Heterofermentative Lactobacilli Lacking Glucose Transport Activity. An analysis of the biochemical basis for the lack of PTS activity in heterofermentative lactobacilli showed that enzyme I was absent, but that both HPr and a kinase that phosphorylates HPr were present. It was proposed that, in lactobacilli, HPr plays a role in the regulation of pathways other than the phosphotransferase system.

B. Protein Phosphorylation in Mycoplasma. Incubation of extracts of Mycoplasma, the smallest self-replicating prokaryote, with radioactive ATP resulted in the phosphorylation of a 55 kDa protein at a serine residue. An endogenous protein phosphatase reversed the action of the protein kinase.

C. Substitution of Arginine for Leucine at Position 195 Produces a cAMP-independent Form of the E. coli Cyclic AMP Receptor Protein (CRP). Our previous studies showed that CRP mutants having amino acid substitutions at positions 127 & 170 had a cAMP independent phenotype (CRP*). An additional substitution at position 195 increased the expression of the CRP* phenotype and showed a high affinity for a cAMP responsive promoter. A mutant CRP containing only the substitution at position 195 was constructed and found to exhibit the CRP* phenotype but not a high affinity for the lacP promoter.

D. Localization of Enzyme I of the PTS of E. coli to the Inner Cytoplasmic Membrane. Studies in this laboratory have led to the proposal that PTS proteins interact with adenylate cyclase to form a functional regulatory complex at the membrane. Evidence in support of this proposal has been accumulated by electronmicroscopic studies showing the localization of enzyme I at the site of the cytoplasmic membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00153-02 LBG

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Differentiation of Excitable Membranes (New Title)

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
M. Terasaki	" "
D. Pumplin	U.Md. Medical School, Dept. of Anatomy
S. Froehner	Dartmouth Med. School, Dept. of Biochem.

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).

Immunogold labeling at the electron microscopic level shows that the postsynaptic membrane of the rat neuromuscular junction contains two distinct membrane domains, one at the crests of the postsynaptic folds (close to the nerve terminal) with a high concentration of acetylcholine receptors and a cytoplasmic 43-kD protein, the other in the troughs of the folds, with a lower concentration of voltage dependant sodium channels, and another cytoplasmic protein, ankyrin. Thus, the 43-kD protein and ankyrin may be involved in segregating the acetylcholine receptors and sodium channels into complementary membrane domains, an arrangement which could serve to facilitate initiation of the muscle action potential.

Immunofluorescence labeling of isolated membranes shows that the cytoplasmic face of the acetylcholine receptor-enriched domains in newly formed acetylcholine receptor aggregates is enriched in an isoform of β-spectrin and a 58-kD protein as well as the 43-kD protein and actin demonstrated previously. Thus, immobilization of acetylcholine receptors by binding to a spectrin-actin membrane cytoskeleton probably occurs early in aggregate formation.

Immunogold labeling shows that the voltage-sensitive calcium channels (dihydropyridine receptors) of skeletal muscle are concentrated in the transverse tubule membrane at the triad junction, where they may interact directly with the calcium release channels (ryanodine receptors) of the sarcoplasmic reticulum.

SUMMARY REPORT OF THE LABORATORY CHIEF

Laboratory of Biochemistry
National Heart, Lung, and Blood Institute
October 1, 1988 to September 30, 1989

Section on Enzymes

Oxygen radical-mediated oxidation of proteins, lipids and nucleic acids is implicated in a number of pathological processes, including: rheumatoid arthritis, aging, carcinogenesis, broncho-pulmonary dysplasia, adult respiratory distress syndrome, retinopathy of prematurity, atherosclerosis, reperfusion-mediated ischemic damage, and hypertension.

In previous studies, highly sensitive methods for the detection and quantitation of oxygen-radical mediated protein damage were developed and evidence was presented that such damage marks enzymes for proteolytic degradation and is implicated in the accumulation of altered forms of enzymes during aging and in normal neutrophil/macrophage function. Protein oxidation is also associated with tissue damage provoked by exposure of animals to 100% oxygen (i.e., oxygen toxicity). Reported here are results of continuing studies in these areas, with particular attention to the role of MCO systems in some pathological disorders, namely, ischemic–reperfusion tissue damage, demyelination associated with rapid correction of severe hyponatremia, and oxygen toxicity.

Mechanism Studies. The proposal that MCO provoked protein modification involves a "cage-like" reaction at metal binding sites on protein was confirmed by the demonstration that metal-catalyzed inactivation of ***E. coli*** glutamine synthetase is associated with the oxidation of histidine and arginine residues which are situated at metal binding sites on the enzyme. The histidine residue is converted to an asparagine residue and the arginine residue is converted to a glutamic semialdehyde residue.

Manganese Ion Provoked Oxygen Radical Generation. In the presence of physiological concentrations of bicarbonate/CO_2 buffer, Mn(II) was found to catalyze the rapid decomposition of hydrogen peroxide and also the oxidation of amino acids by hydrogen peroxide. Both processes are absolutely dependent upon the presence of bicarbonate ion, which probably explains why they were not recognized earlier. In other studies (see P. B. Chock's report), it was demonstrated that in bicarbonate buffer and in the presence of hydrogen peroxide, Mn(II) catalyzes the generation of hydroxyl and superoxide anion radicals; amino acids quench superoxide anion radical trapping and lead to the formation also of amino acid derived radicals, probably a nitroxyl radicals.

Ischemia-Reperfusion. It becomes increasingly apparent that the tissue damage during ischemia-reperfusion is due to oxygen-free radicals. Direct verification of this concept was obtained in collaborative studies with Drs. Robert Floyd (Oklahoma Medical Research Foundation) and John Carney (University of Kentucky). In these studies two biomarkers of oxygen-radical damage; namely, the conversion of amino acid side chains of protein to carbonyl derivatives and a loss in glutamine synthetase activity were observed during ischemia-reperfusion of gerbil brains. Some protein oxidation and loss of glutamine synthetase activity occurred during ischemia; however, most of the damage occurred during 15 to 60 minutes reperfusion.

Demyelination. Rapid correction of sodium levels following hyponatremia leads to localized myelinolysis in the brain. Support for the view that demyelination is due to oxygen-radical-mediated oxidation of myelin was obtained in studies carried out with Hugh Mickel, Laboratory of Experimental Neuropathology, and with Johanna Moller and Richard Quarles of the Laboratory of Molecular and Cellular Neurobiology, NINDS, NIH. The loss of myelin in rat brain which occurs during rapid correction of hyponatremia is accompanied by an appreciable increase in the level of oxidized protein. Since serum iron levels increase during hyponatremia and then decrease during the correction phase, it is proposed that correction may lead to mobilization of iron to the brain and hence to an increase in MCO activity. The susceptibility of myelin protein to oxygen-radical oxidation was confirmed by results of ***in***

vitro experiments showing that myelin isolated from normal rat brain is rapidly oxidized by several different iron-containing MCO systems. After oxidation, the myelin protein was rapidly degraded by endogenous calcium activated protease.

Prevention of Oxygen-Radical Damage by Bacterial Endotoxin. Treatment of rats with bacterial endotoxin leads to tissue specific increases in the levels of catalase and superoxide dismutase and to increased resistance to oxygen toxicity. The ability of endotoxin to protect rats against oxygen toxicity was confirmed by showing that a single injection of endotoxin (1/40 the lethal dose) increased the life span of rats by more than 5-fold. After 15 days in 100% oxygen, the treated animals appeared perfectly normal with no visible signs of lung injury, and the levels of oxidized proteins and of glutamine synthetase activity in hepatocytes and brain tissue were comparable to those observed in untreated animals exposed to air.

As previously reported (last year's annual report), in the absence of endotoxin treatment, rats are highly sensitive to oxygen toxicity. They do not survive for more than 60 hours in 100% oxygen and during such exposure the level of oxidized proteins increases sharply and the level of glutamine synthetase declines.

AIDS Research. Taking advantage of knowledge gained on the mechanism of enzyme inactivation and of nucleic acid damage by metal-ion oxidation systems, efforts to develop effective therapeutic agents against the AIDS virus are in progress (see last year's annual report). To this end, small amounts of the viral protease which is responsible for the processing of viral gene products has been obtained in a nearly homogeneous form.

Section on Metabolic Regulation

The research projects of the investigators in the Section on Metabolic Regulation are concerned mainly with the physical, chemical, immunological, and biological approaches to resolve the mechanism of enzyme action and its regulation. In the past year, research activities have been concentrated on (1) investigating the mechanisms of enzyme action and regulation which includes Ca(II)-calmodulin-dependent protein kinase II, and Mg(II)-dependent Ca(II)-inhibited protein phosphatase; (2) studying the role of multienzyme complexes in the transfer of metabolites in the glycolytic pathway; (3) purification and characterization of ubiquitin activating enzyme and ubiquitin carrier proteins; (4) EPR study of free radicals in oxidative modification of amino acids and in the mechanism by which protector protein protects enzyme from sulfur radicals; (5) immunological study of ubiquitin and ubiquitin-activated enzyme antibodies; and (6) developing analytical and technical methods for biomedical research. Together these projects will provide a better understanding of how enzymes in living cell function are regulated.

I. Mechanism of Enzyme Action and Regulation

1. The Role of Multienzyme Complexes in Metabolite Transfer in the Glycolytic Pathway. Reinvestigation of the evidence in support of a proposed mechanism for direct transfer of NADH between its complexes with α-glycerol-3-phosphate dehydrogenase and with lactate dehydrogenase revealed that the data can be explained with a free-diffusion mechanism without invoking a direct transfer mechanism.

2. Mechanism of Ca(II)-Calmodulin-Dependent Protein Kinase II. The kinetic mechanism of this enzyme has been studied in detail using the catalytic domain of the kinase and a synthetic peptide as substrate. The mechanism was found to be a steady-state ordered substrate binding in both forward and reverse directions with the nucleotides first to bind.

3. Purification and Characterization of Ubiquitin Activating Enzyme and Ubiquitin Carrier Proteins. In order to study the ubiquitination/deubiquitination cascade and to explore the physiological role of ubiquitin, we have purified the ubiquitin activating enzyme, E1, from human red blood cells and from rabbit reticulocytes, and four isoproteins of ubiquitin carrier protein, E2, from rabbit reticulocytes. Preliminary attempts to sequence the 20 kDa and the 32 kDa E2 isoproteins indicated that the N-terminuses of both proteins were blocked.

4. Purification and Characterization of a Ca(II)-Inhibited Protein Phosphatase. A novel Mg(II)-dependent Ca(II)-inhibited protein phosphatase has been purified to homogeneity from bovine brain. It is a 78,000 Da protein and is composed of three nonidentical subunits. A protein activator which stimulates the phosphatase activity by about 4-fold has also been identified.

II. EPR Study of Free Radicals in Biology

1. Oxidative Modification of Amino Acids. To study the fundamental mechanism of protein oxidative modification by oxygen-free radicals, EPR and spin trapping methods were used to monitor the formation and utilization of free radicals and to identify the radical species. In the Fe(II)/EDTA/hydrogen peroxide system, hydroxyl radicals were trapped and Leu enhanced the rate of this radical generation. In the Mn(II) system, both superoxide and hydroxyl radicals were trapped. Leu inhibits the formation of superoxide radical, while it was converted to a Leu-derived free radical. Using various isotope enriched Leu, this new radical was identified as $HOOCC(R)CHNHO^{\cdot}$, a hydro nitroxide.

2. Mechanism of Protector Protein. A 27 kDa protector protein has previously been purified from yeast. It protects enzymes from oxidative inactivation by the Fe(III)/O_2/thiol system. EPR data show that hydroxyl radicals and sulfur radicals were generated in the Fe(II)/O_2/DTT system. Addition of an enzyme, glutamine synthetase, enhanced the formation of these free radicals. The function of the protector protein is to catalyze the removal of sulfur radicals formed.

III. Immunological Study of Ubiquitin and Ubiquitin-Activated Enzyme

The highly purified human E1 ubiquitin and ubiquitin-BSA conjugate were used to raise antibodies in rabbit. The anti-E1 antiserum exhibits high affinity and specificity for E1 and is capable of immunoprecipitating E1 from solution without inhibiting E1 activity. Although the anti-ubiquitin antibodies were specific for ubiquitin, its affinity was relatively poor.

IV. Analytical and Technical Methods

1. A Modified Job Plot for Differentiating Various Protein-Ligand or Protein-Protein Binding Modes. Job plot is a method for determining stoichiometry of two interacting components. However, at low total reactant concentrations, the value obtained may not be the true value. This method has been modified by using the apex or the intersecting point of two lines with limiting slope of Job plot as a function of total reactant concentrations. The modified method permits differentiation of various binding modes.

2. Development of an AC Electroporation Instrument. Based on our study on the effect of oscillating electric field on cell membrane, we are developing an instrument which can use low amplitude, bipolar sinusoidal electric fields with variable pulse times as a means of introducing molecules of interest into cells.

Section on Intermediary Metabolism and Bioenergetics

One of the major research areas addressed has to do with the biosynthesis and biological activities of selenoenzymes and seleno-tRNAs. Selenocysteine incorporation into both prokaryotic and eukaryotic selenoenzymes is directed by the UGA termination codon. Unique tRNAs (anticodon UCA) that recognize UGA are involved. In collaborative experiments with August Böck's group of the University of München and Dolph Hatfield of the National Cancer Institute, the unique tRNAs were shown to be esterified with serine and, after activation of the serine hydroxyl group by phosphorylation, the latter was substituted by selenium to form selenocystyl-$tRNA_{UCA}$. Thus, the interconversion occurs at the level of esterified amino acids and free selenocysteine is not directly aminoacylated to the tRNA. Selenium incorporation into certain tRNA molecules per se (i.e., lysine- and glutamate-accepting tRNAs) involves the specific incorporation of selenium into the "wobble position" of the anticodons of the tRNAs. This is an ATP-dependent process that results in the removal of sulfur from a 5-methylaminomethyl-2-thiouridine residue and substitution with selenium. ***Escherichia coli*** and ***Salmonella typhimurium*** mutants defective in

various steps of the UGA translation process and also in the selenation of tRNAs have been characterized. A single mutation in the selD gene that prevents both seryl-$tRNA_{UCA}$ conversion to selenocystyl-$tRNA_{UCA}$ and 2-thiouridine conversion to 2-selenouridine in tRNAs was studied. A 37 kilodalton protein of unknown function is the product of the selD gene in both organisms. To explain the requirement of this single gene product for the two different selenation processes it is presumed that it is required for the generation of an active selenol donor utilized for both. Early steps in selenium uptake and incorporation into free selenocysteine and selenomethionine were shown to be unaffected by the mutation. Instead the selD mutants synthesize selenocysteine and selenomethionine and incorporate these amino acids non-specifically into many proteins of the cells. Up to 30% of the selenite added to the culture media (0.5 μM) was recovered as these selenoamino acids in the bacterial proteins. This extensive incorporation of selenocysteine observed in the Salmonella cell proteins is the first non-specific occurrence of this selenoamino acid to be carefully documented. Presumably a normal regulatory process that prevents substitution of selenocysteine for cysteine in proteins is defective in the selD mutants. A possible relevance to the known toxic effects of selenium is under consideration.

One of the selenium-dependent formate dehydrogenases of ***E. coli*** is a component of the formate hydrogenase lyase complex and is linked to a hydrogenase in the complex. Although the gene encoding the selenocysteine-containing subunit of this enzyme (an 80,000 M.W. subunit) has been cloned and shown to contain the TGA (UGA) codon, the enzyme had never been purified. This membrane-bound, highly oxygen-sensitive enzyme has now been isolated in almost homogeneous form. In addition to the 80,000 M.W. subunit, there is a smaller subunit of about 40,000 making up the native enzyme of about 100,000. Replacement of the TGA codon with TGC by site-directed mutagenesis results in the replacement of selenocysteine with cysteine in the formate dehydrogenase. The sulfur-containing enzyme is only 20 to 25% as active catalytically as the selenium-containing enzyme. This provides an example of the advantage of selenium over sulfur in a catalyst that operates at a low redox potential. In view of the marked oxygen sensitivity of the ***E. coli*** formate dehydrogenase and numerous problems encountered because of the unsatisfactory chemical properties of this protein, studies to locate the gene that encodes clostridial selenoprotein A were continued. The latter small, very stable selenoprotein is much more abundant and is ideal for protein chemistry and gene expression studies. Additional protein sequence information needed to generate multiple oligonucleotide probes to hybridize with the gene were obtained. A 17 amino acid sequence from the amino terminus of selenoprotein A purified from ***Clostridium purinolyticum*** was determined. Selenoprotein A highly enriched in ^{77}Se was isolated in pure form from bacteria grown in the presence of [^{77}Se]selenite. The pure biologically active labeled protein exhibited a strong ^{77}Se NMR signal, the first observed for a native [^{77}Se]protein.

Based on the recent report of Abeles that the intermediate in the conversion of glycine to acetate is acetyl phosphate, it was shown that the highly purified protein C component of the glycine reductase complex is the acceptor of the acetyl group generated from glycine. This was demonstrated by showing that protein C catalyzes an arsenate- dependent cleavage of acetyl phosphate. Since a thiol-reducing agent is required for the reaction and alkylation of the protein destroys it ability to catalyze the reaction, it is concluded that a thiol ester on the protein reacts with phosphate to generate acetyl phosphate in the overall reaction. In the reverse direction the acetylthiol ester is generated from acetyl phosphate and this can react with arsenate to form an unstable arsenate ester. Unexpectedly the protein C supplies a heat labile component as well as the group that is alkylated and both are required for the arsenate-dependent cleavage of acetyl phosphate. This is consistent with the known inactivation of protein C as an essential component of the glycine reductase complex by either heat treatment or alkylation.

A research project on the mechanism of acetate conversion to methane that encompasses studies on the biological roles of trace elements such as nickel and cobalt (in vitamin B_{12}) in the anaerobic redox process has been continued. One of the intermediate steps in the conversion of the methyl group of acetate to methane is catalyzed by a specific methyltransferase enzyme. This enzyme transfers the methyl group of methyl-B_{12} to a thiol group on thioethane sulfonate in an ***in vitro*** reaction that mimics the normal reaction involving an enzyme-bound methylcobalamin (methyl-B_{12}) intermediate. When the methyl group originates from methyl alcohol, a separate methyltransferase serves to catalyze the transfer to the thioethane sulfonate acceptor. Specific antibodies to the two methyltransferases were elicited in sheep and used to show that, although low constitutive levels of the enzymes normally are present, growth on acetate induced high

levels of one of the transferases and growth on methanol induced the other one. Both transferases were isolated in homogeneous form and their properties characterized.

As a collaborative effort with other investigators of the Laboratory of Biochemistry who are characterizing carbonyl group products generated by metal ion-catalyzed oxidation of proteins, model carbonyl compound adducts were prepared. These are needed as authentic reference compounds for identification purposes. One of the products identified was a derivative of glutamic semialdehyde which can be generated in proteins by oxidation of arginine or proline residues.

Section on Protein Chemistry

The Section on Protein Chemistry is studying the physical and chemical properties of macromolecules of biological interest and the roles of ligand binding and of protein-protein and inter- and intra-subunit interactions in enzyme catalysis and regulation. The energetics of ligand binding to proteins involve contributions from both ligand-protein and protein-protein interactions. Ligand-promoted changes in protein-protein interactions underlie the phenomenon of cooperativity in ligand binding to proteins and, in addition, give rise to many examples of stabilization and destabilization of protein structures by ligands and metal ions.

Glutamine synthetase (622000 M_r), a strictly regulated enzyme in ***E. coli***, contains 12 identical subunits arranged in 2 superimposed hexagonal rings ~140 Å in diameter with centers of adjacent subunits. An unusual feature of the enzyme structure is that the 12 active sites are formed at heterologous interfaces between subunits within a hexagonal ring of the two face-to-face eclipsed rings (Almassy et al., Nature **323**, 304-309, 1986). The two divalent cations (Mn^{2+} or Mg^{2+}) at an active site as well as the site of adenylylation are in the C-terminal domain, whereas the nucleotide binding site is in the N-terminal region (near Lys 47) of an adjacent subunit. This explains how the binding of substrates stabilizes the inter-subunit contacts of the enzyme.

Temperature-induced UV spectral changes of the Mn^{2+}-form of ***E. coli*** glutamine synthetase in 100 mM KCl are reversible at pH 7.0 (50°C) and involve exposure of 0.7 of the 2 Trp residues/subunit and 2 of the 17 Tyr residues/subunit without dissociation or aggregation of the dodecamer. Monitoring changes in Trp or Tyr exposure independently gives data that conform to a two-state model for partial unfolding with T_m values (where $\Delta G_{unfolding} = 0$ differing by 2-3°C at each level of Mn^{2+} studied and with average ΔR_{vH} values of 80 and 94 kcal/mol, respectively. The data can be fit equally well by a random model in which two regions of the oligomeric structure unfold separately as independent transitions or by a sequential model in which the trp transition occurs first upon heating. Substrate additions to Mn•GS increased T_m values by preferential binding to the folded form, whereas high concentrations of Mn^{2+} stabilize the partially unfolded form by Mn^{2+} binding weakly to additional sites exposed by heat (T_m decreased). The thermally induced transitions of dodecameric Mn•enzyme appear to involve a loosening of active site structures that are stabilized through the free energy of substrate binding.

The metallochromic indicator 4-(2-pyridylazo)resorcinol (PAR) has been used at pH 7.0 to monitor the mercurial-promoted Zn^{2+} release from ***E. coli*** aspartate transcarbamoylase (ATCase: c_6r_6) and from regulatory dimers (r_2) after separating these subunits from catalytic trimers (c_3). As previously reported, the properties of PAR-Zn^{2+} interactions make PAR a generally useful reagent for studying Zn^{2+} release from proteins. Zn^{2+} is tetrahedrally bonded to the four thiol groups of each r chain and the mercurial-promoted Zn^{2+} release from r_2 is about 77-fold greater than the corresponding rate of Zn^{2+} release from intact ATcase in which Zn^{2+} is bound near regulatory:catalytic chain (r:c) contacts. This and other observations indicate that Zn^{2+} bonding domains in isolated r_2 subunit are much more accessible to mercurial attack than are Zn^{2+} clusters in the intact ATCase molecule. The rate of mercurial promoted Zn^{2+} release from r_2 dimer is ~200-fold faster than is the "spontaneous" rate of Zn^{2+} release measured in the presence of 25-fold excess quin-2. This indicates a direct attack of mercurial reagents on thiols bonded to Zn^{2+} which facilitates Zn^{2+} release. The affinity constant of r_2 for Zn^{2+} at pH 7.0 and 20°C was determined to be 10^{12} $(M\ r\ chain)^{-1}$ ($\Delta G' = -16$ kcal/mol) by using steady equilibria with the EGTA

analogues indo-1 and quin-2; these have been found to be both high-affinity and sensitive spectral indicators for Zn^{2+}. These indicators remove Zn^{2+} from $r_2 \cdot Zn^{2+}$ but not from intact ATCase, which is consistent with the fact that EDTA removes Zn^{2+} from r_2 but not from ATCase. Indo-1 also was used to measure the effects of ATP and CTP ($\pm$ Mg^{2+}) on the apparent association constant of r_2 dimer for Zn^{2+}.

The methods developed during our studies on Zn^{2+} interactions with ATCase and isolated r_2 subunits are being applied to studies of other biologically important Zn^{2+} binding proteins. An example is the transcriptional factor TFIIIA from immature frog ***Xenopus laevis***, which is responsible along with at least two other factors (TFIIIB and TFIIIC) for directing the synthesis of oocyte 5*S* RNA genes by RNA polymerase III. Since TFIIIA is a member of a growing list of important DNA-binding proteins with characteristic structures involving a large number of "Zn^{2+} fingers" (9-11), it is of interest to study the structure-function relationships of this small protein (M_r 38,500). Factor IIIA has been purified and isolations of Factors IIIB and IIIC are in progress; antibodies to each factor are being prepared. In addition, fluorescence probes will be introduced into TFIIIA as well as into DNA fragments of the 5*S* RNA gene in order to use fluorescence resonance energy transfer and emission anisotropy techniques to monitor protein-protein and DNA-protein interactions.

Section on Signal Transduction

Extracellular signalling molecules such as neurotransmitters, growth factors, and pharmacological agonists elicit a variety of cellular responses (secretion, excitation, motility, metabolism, sensory mechanism, differentiation, and DNA synthesis). The signal is transmitted to the inside of the cell through a chain of protein molecules and ultimately activates an intracellular enzyme to generate intracellular signalling molecules. Alteration in this signal relay system often causes cancer, diabetes, and infectious diseases.

Phospholipase C is one of the several enzymes known for generating intracellular signalling molecules in response to a variety of external signals. Phospholipase C cleaves an inositol-containing lipid molecule and generates two signal molecules, inositol trisphosphate and diacylglycerol. We have purified three distinct phospholipase C enzymes (PLC-ß, γ, and δ) from bovine brain. The three enzymes are quite dissimilar in molecular size and in amino acid sequences, and expressed differently between tissues and in individual cells. This dissimilarity suggests that there are different means of regulation for the phospholipase C isozymes. Thus, different modes of modulation may account in part for the diversity of responses observed in different tissues and individual cell types to an external signalling molecule.

Direct support for this hypothesis came from the study on growth regulatory systems. Platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) initiate proliferative response in cells by binding to specific cell surface receptors. The initial biochemical response after binding of the growth factors to the receptor is the stimulation of the intrinsic tyrosine kinase activity of the receptors. Since PDGF and EGF are capable of modulating the inositol phospholipid signalling system in a variety of cell types, a likely substrate for the growth factor-receptor tyrosine kinase is phospholipase C. Indeed, PLC-γ (but not PLC-ß and PLC-δ) was found to be rapidly phosphorylated on tyrosine following the growth factor treatment of cells and coprecipitate with the receptors upon immunoprecipitation. This suggests that tyrosine phosphorylation of PLC-γ constitutes the mechanism by which external growth signal is converted to intracellular signal. The fact that PLC is an important enzyme in the growth signal pathway was further supported by the microinjection of PLC to cells. Introduction of exogenous PLC to NIH 3T3 cells induced DNA synthesis and caused morphologic transformation of growth arrested fibroblast cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 00202-18 LB

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.)
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

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Luz Hermida, Special Volunteer, LB, NHLBI
Markus Hoefer, Special Volunteer, LB, NHLBI
Xiaomao Wu, Ph.D., Visiting Fellow, LB, NHLBI
Ephrem Tekle, Ph.D., NRC Fellow, LB, NHLBI

COOPERATING UNITS (if any)

D. Yang, Georgetown University, Washington, D.C.; H. Gutfreund, Fogarty Scholar-in-Residence (Bristol University, Bristol, England)

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Section on Metabolic Regulation

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	4.25	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.)

Protein ubiquitination is believed to play a role in a number of important cellular processes in eukaryotes. To study the ubiquitination/deubiquitination cascade and to explore the physiological roles of ubiquitin, we have purified the ubiquitin activating enzyme, E1, from outdated human red blood cells and from rabbit reticulocytes, and four isoproteins of ubiquitin carrier proteins, E2, from rabbit reticulocytes. The highly purified human E1 was used to prepare antibodies for immunological studies. The antiserum exhibits high affinity and specificity for E1. Preliminary attempts to sequence two of the E2 isoproteins, the 20,000 Da and the 32,000 Da protein, indicated that the N-terminus of both proteins was blocked.

In view of the fundamental importance of a recently proposed metabolite channeling mechanism for the glycolytic pathway, we have reinvestigated the evidence for direct transfer of NADH between its complexes with α-glycerol-3-phosphate dehydrogenase and with lactate dehydrogenase. The data are consistent with a free-diffusion mechanism.

Oxygen-free radicals have been implicated in oxygen toxicity, aging, and protein turnover. To study the fundamental mechanism of the oxidative modification of proteins by oxygen-free radicals, we used EPR and spin trapping methods to monitor the formation and utilization of free radicals and to identify the radical species. The results showed that in the Fe(II)/EDTA/-hydrogen peroxide system hydroxyl radicals were trapped and Leu enhanced the rate of hydroxyl radicals generation. When Fe(II)/EDTA was replaced by Mn(II), superoxide radicals and hydroxyl radicals were trapped. Addition of leucine eliminated the signal for trapped superoxide radicals and the formation of a leucine-derived free radical was formed. Using various isotope enriched leucines, this radical was identified as HOOCC(R)CHNHO•, a hydro nitroxide.

We are developing an instrument which uses low amplitude, bipolar sinusoidal electric fields with variable pulse times as a means of introducing molecules of interest into the interior of cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00203-16 LB

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.)
Cellular Regulation of Enzyme Levels

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

PI: Cynthia N. Oliver, Ph.D., Special Volunteer, Enzyme Section, LB, NHLBI

Others: Pamela E. Starke-Reed, Ph.D. Staff Fellow, Enzyme Section, LB, NHLBI

COOPERATING UNITS (if any)
Richard Clark and David DeBoer, Surgery Branch, NHLBI; Robert Floyd, Oklahoma Medical Research Foundation; John Carney, University of Kentucky; Emily Shacter, Laboratory of Genetics, NCI

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

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☐ (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 undertaken studies to examine the possible role of protein oxidation and enzyme inactivation in ischemia/reperfusion injury in both heart and brain. Preliminary studies have been carried out with Dr. Robert Floyd, Oklahoma Medical Research Foundation and Dr. John Carney, University of Kentucky using a gerbil brain model of ischemia/reperfusion injury. Initial results indicate that loss of glutamine synthetase activity is correlated with increased carbonyl content during ischemia followed by reperfusion. Although a small amount of protein oxidation is associated with the ischemic event, most of the oxidation occurs during 15 to 60 minutes of reperfusion. It is important to point out that these represent intracellular changes and suggest that both ischemia and reperfusion triggers a cellular response which leads to protein oxidation and loss of enzyme activity. Other studies have been initiated with Dr. Richard Clark and Dr. David DeBoer of the surgery branch of the NHLBI, NIH using a working rat heart model. The initial studies have involved method development.

In collaboration with Dr. Moon Bin Yim and Dr. Emily Shacter, we have undertaken additional studies to identify the radicals produced by activated neutrophils under a variety of conditions using electron spin resonance (ESR) and the spin trap, 5,5-dimethyl-1-pyrroline-N-oxide. Although the production of superoxide radical by activated neutrophils has been demonstrated by this method, there are conflicting results with respect to the production of hydroxyl radical by this technique. Again, most of the initial studies have been devoted to method development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 00204-22 LB

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.)*
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 (5/88/89–), LB, NHLBI
Francis P. Cyran, FAES Fellow (8/1–9/31/88); Chemist (12/4/88–), LB, NHLBI
Steven C. VanNoord, NHLBI Summer Program (5/23/89–~8/20/89), 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; J.R. Knutson, Lab. Technical Development, NHLBI

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.2	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.)*

(1) Nucleotide analogs have been introduced as structural probes into active sites and adenylylated sites of dodecameric glutamine synthetase from ***E. coli***. These enzyme derivatives are being used for X-ray structural analysis in D. Eisenberg's laboratory at UCLA and for determining relative intra- and inter-subunit distances between the active sites and Trp 57 and Trp 158.

(2) Reversible temperature-induced transitions of glutamine synthetase involve the exposure of 0.7 of the 2 Trp residues/subunit and 2 of the 17 Tyr residues/subunit without dissociation or aggregation of the dodecameric enzyme. Partial unfolding appears to loosen active site structures that are stabilized by the free energy of substrate binding. Two regions of the oligomeric structure unfold separately--either randomly or sequentially with Trp exposure occurring before Tyr exposure upon heating.

(3) Isolated regulatory subunits of ***E. coli*** aspartate transcarbamoylase bind zinc with high affinity: log K = 12 moles/liter at pH 7.0 and 293K as determined from rapid steady state equilibria with metallochelator indicators indo-1 and quin-2.

(4) Fluorescence and spectral studies of zinc-dependent structures of transcriptional factor IIIA (TFIIIA) purified from immature oocytes of ***Xenopus laevis*** are in progress. TFIIIA interactions with 5*S* RNA, DNA encoding for 5*S* RNA, RNA polymerase III, and purified TFIIIB and TFIIIC will be studied also.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00205-34 LB

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.)*

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:	Dr. Richard S. Glass	NRC Senior Fellow (Visiting Scientist)	LB, NHLBI
	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

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	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 space provided.)*

Unique serine-accepting tRNAs of eukaryotic and prokaryotic origin that have anticodons complimentary to the UGA termination codon have been implicated in the cotranslational insertion of selenocysteine into proteins. That these tRNAs are esterified with serine and, after activation of the hydroxyl group by phosphorylation, are then converted to selenocysteyl-tRNAs was demonstrated in collaborative in vivo studies using cultured rat mammary tumor cells and Escherichia coli. Thus, the conversion of serine to selenocysteine occurs at the level of esterified amino acids and free selenocysteine is not directly aminoacylated to the UGA-recognizing tRNAs.

Selenoprotein A of the glycine reductase complex was labeled in vivo with the stable isotope Se-77. The pure protein exhibited a strong Se-77 NMR signal, the first observed for a biologically active native selenoprotein.

The protein C component of the glycine reductase complex was shown to catalyze the arsenate-dependent decomposition of acetyl phosphate indicating it is the protein that accepts the acetyl group generated from glycine in the overall reductase reaction. The requirement of an added thiol for the arsenolysis reaction and inhibition of the enzyme by alkylation suggest that an acetyl thiol ester derivative of the protein is the intermediate generated from glycine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00206-30 LB

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.)*

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.)*

To characterize the carbonyl function generated by metal ion–catalyzed oxidation of proteins, 4–aminofluorescein was reacted under reducing conditions with oxidized glutamine synthetase. A fluorescein derivative was isolated and its mass spectrum was consistent with a compound derived from glutamyl γ–semialdehyde. In order to confirm this assignment, a synthesis of this compound was undertaken so as to provide an authentic sample for comparison. Although the derivative of glutamyl γ–semialdehyde with p–aminobenzoic acid was successfully synthesized and characterized, the synthesis of the corresponding derivative with 4–aminofluorescein has not yet been accomplished. Therefore, the confirmation of the structure assigned to the derivative isolated form oxidatively modified glutamine synthetase awaits further experimentation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 00211-16 LB

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.)*

Mechanisms of Metal-Ion Catalyzed Oxidation of Amino Acids

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, LB, NHLBI

Others: Barbara S. Berlett, Biologist, Enzymes, Section, LB, NHLBI

COOPERATING UNITS *(if any)*

M. B. Yim and P. B. Chock, Section on Metabolic Regulation, Laboratory of Biochemistry, NHLBI

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Laboratory of Biochemistry

SECTION

Section on Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.0	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.)*

It is generally assumed that the antioxidant properties of manganese ion (MnII) complexes are due to their ability to catalyze the dismutation of superoxide anion radicals. The present research demonstrates that in addition to its superoxide dismutase activity Mn(II) is also capable of catalyzing the decomposition of hydrogen peroxide in a catalase-like manner, and also to catalyze the hydrogen peroxide-dependent oxidation of amino acids to mixtures of α-ketoacids, ammonia, carbon, and aldehydes containing one less carbon atom. The catalase-like activity and the oxidation of amino acids are both dependent upon the presence of bicarbonate ion; the rates of both processes increase exponentially as a function of the bicarbonate ion concentration. By means of electron paramagnetic resonance spectroscopy, it was established (see M.B. Yim's annual report), that, in the presence of bicarbonate ion and hydrogen peroxide, Mn(II) catalyzes the generation of both hydroxyl and superoxide anion radicals. Upon addition of amino acid, the superoxide anion radical is quenched as an amino acid radical (nitroxyl radical) is produced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 00212-18 LB

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.)*

The Metabolic Signal and Genetic Hierarchy of Nitrogen Control in *E. coli*

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

PI: Mary Anne Berberich, Ph.D., Research Chemist, LB, NHLBI

COOPERATING UNITS *(if any)*

John Smith, Senior Investigator, Seattle Biomedical Research Institute, Seattle, Washington

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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 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Although most of the major genes of the nitrogen control system have been identified, cloned, and sequenced, neither the identity of the metabolic signal nor the mechanism of signal transduction has been elucidated. This work is primarily concerned with (a) identifying the metabolic initiator of the regulatory process and (b) defining the function of the PII protein in the regulation of synthesis of glutamine synthetase (GS).

Previous studies indicated that an increased serine synthesis is associated with the ability of some D-amino acids to elicit an increased synthesis of GS in the presence of excess ammonium nitrogen. It was also observed that strains carrying mutations in glyA, the gene for serine hydroxymethyltransferase, are deficient in response to the D-amino acids. Subsequent studies with inhibitors and mutants suggest an involvement of the threonine utilization (TUT) cycle, including induction of the glycine cleavage system. However, the distribution of the amino nitrogen from D-glutamate, D-lysine, or D-threonine into serine is not as yet understood.

Studies with strains devoid of PII protein show that the absence of PII protein does not lead to an elevation in the level of GS. Therefore, it is not likely that PII has a repressor function. However, it appears that PII is required for the increased synthesis of GS during growth on "derepressing" medium or upon addition of the effector D-amino acids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00224-12 LB

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.)*

Calcium-Regulated Phosphorylation-Dephosphorylation

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

PI: Charles Y. Huang Research Chemist LB, NHLBI

Others: Francesca Santini Visiting Fellow LB, NHLBI

COOPERATING UNITS *(if any)*

Marita King, Chemistry Department, Ohio State University, Columbus, Ohio

LAB/BRANCH

Laboratory of Biochemistry

SECTION

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. A novel magnesium-dependent, calcium-inhibited protein phosphatase (CIP) from bovine brain has been purified to homogeneity. This phosphatase may serve an important physiological function because of its involvement in two major cellular regulatory processes: Phosphorylation-dephosphorylation and calcium release. Furthermore, since there are a group of calcium and calmodulin activated protein kinases, the calcium-inhibited phosphatase should work well with these kinases in a compensatory manner. CIP is a protein of 78,000 molecular weight and is composed of three nonidentical subunits. A protein activator that stimulates the phosphatase activity by approximately four-fold has also been identified.

2. The kinetic mechanism of type II calmodulin-dependent protein kinase has been studied in detail. The studies were performed with the catalytic domain of the kinase and a synthetic substrate to avoid various complications. The mechanism was found to be ordered substrate addition with the nucleotides binding first in both forward and reverse direction. The work should serve as a basis for understanding the catalytic and regulatory properties of calmodulin-regulated kinases. Theoretical analysis of the observed apparent rapid-equilibrium ordered binding in the reverse direction also established conditions for seeing such a kinetic pattern and the pitfall involved in its interpretation.

3. Job plot is a method for determining the binding stoichiometry of two interacting components. A method utilizing the special points in a Job curve as a function of total reactant concentrations has been developed for differentiating various protein-ligand or protein-protein binding modes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00225-12 LB

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.)*

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	Senior Investigator	LB, NHLBI
Others:	John Boutelje	Visiting Fellow	LB, NHLBI
	Isabel Climent	Visiting Fellow	LB, NHLBI
	Anders Karlström	Visiting Fellow	LB, NHLBI
	Miranda Marsh	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, Massachusetts

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	4.2	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. 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 will allow assessment of 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 00261-04 LB

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.)*

CO Dehydrogenase and Acetoclastic Methanogenesis in *Methanosarcina barkeri*

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

PI: David A. Grahame Staff Fellow 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.)*

Utilizing cells of *Methanosarcina barkeri*, studies have been carried out on the system of enzymes generating methane from acetate. Experiments have focused on a specific methyl group transfer step. Improved methods have been developed for purification of the methyltransferase enzyme involved. The chemical mechanism of methyl group transfer has been probed using spectrophotometric techniques to identify the oxidation state of the product cobalamin (a vitamin B_{12} compound). Characteristics of the enzyme active site have been determined in studies which uncovered two new methyl acceptor substrates and a novel inhibitor compound. Two isoenzyme forms of methyltransferase were purified and antibodies were produced against both forms. Studies using the antibodies demonstrated different levels of the isozymes in cells using different carbon and energy substrates for metabolism. Affinity-purified, immobilized antibody preparations were prepared and characterized for use in experiments designed to demonstrate directly the exact roles of the two methyltransferase isozymes in methanogenic metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 00263-04 LB

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.)*

Signal Transduction Mechanism Involving Phosphoinositide

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

PI: Sue Goo Rhee, Chief, Section on Signal Transduction, LB, NHLBI
Kwan Young Choi, Ph.D., NRC Fellow, LB, NHLBI
Uh Hyun Kim, 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., Visiting Fellow, LB, NHLBI

COOPERATING UNITS *(if any)*

Tony Hunter, Salk Institute; Graham Carpenter, Vanderbilt University; Joseph Schlessinger, Rohr Research Laboratory; Hsiang-Fu Kung, NCI

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Signal Transduction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.8	5.2	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 space provided.)*

Phospholipase C (PLC) is a key enzyme in converting extracellular signals to intracellular messengers as it hydrolyzes phosphatidylinositol bisphosphate in response to a variety of calcium-mobilizing agonists and generates 2 second messenger molecules, inositol trisphosphate and diacylglycerol. We have purified 3 immunologically distinct phospholipase C enzymes, PLC-α, PLC-ß, and PLC-γ from bovine brain. The 3 enzymes are quite dissimilar in molecular size and in amino acid sequences, and expressed differently between and within tissues, suggesting that there are different means of regulation for the phospholipase C isozymes. We found that PLC-γ (but not PLC-ß or PLC-δ) is regulated by two protein kinases, growth factor receptor kinase and cAMP-dependent kinase. By immunoprecipitation from 32P-labeled cells, PLC-γ was found to be rapidly phosphorylated on tyrosine and serine residues following PDGF and EGF treatment of NIH 3T3 mouse fibroblasts and A431 human epidermoid. In the presence of the growth factors, their receptors coimmunoprecipitate with PLC-γ. This suggests that PLC-γ may be directly phosphorylated by the growth factor receptors. Indeed, purified PLC-γ (but not PLC-ß or PLC-δ) was phosphorylated by either purified PDGF or EGF receptors in vitro at sites of tyrosine phosphorylation identical to those found in vivo. We concluded that tyrosine phosphorylation of PLC-γ by the PDGF and EGF receptors leads to its activation and a consequential increase in phosphatidylinositol turnover. It had been shown that enhanced intracellular levels of cAMP inhibits the formation of inositol trisphosphate. When C6Bu1 cells were incubated with cholera toxin, forskolin, or Br-cAMP to enhance the intracellular concentration of cAMP, phosphoserine content in PLC-γ was dramatically elevated, while the levels in PLC-ß and PLC-δ were not changed. The specificity of cAMP-dependent kinase for PLC-γ was also confirmed in vitro. These results suggest that direct phosphorylation of PLC-γ by cAMP-dependent kinase is responsible for the cross-talk between the phosphoinositide- and cAMP-signalling cascades.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00265-03 LB

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.)*

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

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 mechanism by which selenocysteine is incorporated into protein remains a mystery. 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 recently purified this formate dehydrogenase to near homogeneity. This will allow 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. 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 00266-03 LB

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.)*

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 of the glycine reductase complex from Clostridium sticklandii has been purified and partially sequenced around the selenocysteine residue. An oligonucleotide probe to this region has been synthesized and shown by Southern blot analysis to hybridize to a single 2300 base pair (bp) fragment of clostridial DNA digested to completion with Hind III. The fragment was cloned and digested with restriction enzyme TaqI to yield four smaller fragments. A 400 bp fragment was found to hybridize to the probe. It was subcloned into bacteriophage M13. Sequencing of this fragment revealed that it does not contain the sequence for selenoprotein A, but does contain numerous adenine- and thymidine-rich regions that are capable of binding multiple copies of the probe. This led to the strategy of obtaining more peptide sequence to generate oligonucleotide probes for a multi-probe selection of the gene. Since the selenoprotein A of C. sticklandii is blocked at its N-terminus, the analogous protein from C. purinolyticum was investigated. The protein was purified but found not to be blocked at its N-terminus. The first 17 amino acids were determined to be: --MetIleLeuGlnGlyLysLys-ValIleAlaIleGlyAspAspAspGlyIle--.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 00267-03 LB

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.)*
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, Section on Metabolic Regulation, LB, NHLBI

Others: P. Boon Chock, Chief, Section on Metabolic Regulation, LB, NHLBI
Luz Hermeda, Special Volunteer, LB, NHLBI
Markus Hoefer, Special Volunteer, LB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Section on Metabolic Regulation

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.)*

We have isolated "Ubiquitin Activating Enzyme" ("E1") in submilligram quantities from human red blood cells. Highly purified E1 was used to raise antibodies in rabbits for use in immunochemical studies of the protein. The antiserum exhibits high titer and specificity for E1, and is capable of immunoprecipitating E1 from solution.

Purification schemes for purification of "Ubiquitin Carrier Enzyme" ("E2") are being explored. Preliminary attempts to sequence a purified E2 indicate a blocked N-terminus.

Immunochemical analysis of endogenous ubiquitin and ubiquitin-conjugates ubiquitin has not been possible due to insufficient sensitivity of detection and/or insufficient levels of antigen present in lysates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00268-03 LB

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.)*
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 ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Homopolymers of L-alanine, L-arginine, L-histidine, L-lysine, and L-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. Polymers thus derivatized exhibit characteristic spectra which may be measured. Oxidation proceeds best when the ferrous iron to citrate ratio is 1:2. Ferric iron will also support the reaction. Addition of Manganous ion enhances the oxidation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 00269-02 LB

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.)*

Conformational Analysis of Altered Proteins Generated by Metal-Catalyzed Oxidation

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 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

The effects of oxygen radical damage to proteins is largely unknown. The presence of iron inside cells can be detrimental to proteins if these iron stores become labilized (freed) in the presence of reducing equivalents and oxygen. The resultant damage to proteins mediated by Fenton chemistry can result in localized oxygen radical induced destruction in and around protein metal binding sites. A number of metal binding proteins were subjected to iron catalyzed oxidation, and concomitant losses of activity and structural changes were monitored. Glutamine synthetase, phosphoglycerate kinase, carbonic anhydrase, myoglobin and cytochrome b562 were the proteins used to explore the consequences of oxidative events. These proteins represent particular classes of proteins which can potentially serve as sites for oxygen radical production (i.e., metalloproteins, oxygen binding proteins and electron transfer proteins). Various physiochemical aspects of protein structure were examined before and after oxidation. Oxygen radical damage to these proteins ranges from substantial (multiple reactions observed along with loss of activity e.g. glutamine synthetase, phosphoglycerate kinase, Zn free carbonic anhydrase) to undetectable (e.g., myoglobin, cytochrome b562, Zn bound carbonic anhydrase).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00271-01 LB

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.)*: Protein Oxidation during Aging, Oxidative Stress, and Hyponatremia

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

Pamela E. Starke-Reed, Ph.D. Staff Fellow, Enzyme Section, LB, NHLBI

Others: Cynthia N. Oliver, Ph.D., Special Volunteer, Enzyme Section, LB, NHLBI

COOPERATING UNITS *(if any)*

Johanna Mollen and Richard Quarles, Laboratory of Molecular and Cellular Neurobiology, NINDS; Hugh Mickel, Laboratory of Experimental Neuropathology, NINDS

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 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

We have continued studies on the oxidation of proteins and specific enzymes in hepatocytes from rats treated with 100% oxygen (oxygen toxicity model) and rats of various ages 3-26 months (aging model). The results show that loss of specific activity of glutamine synthetase (GS) and glucose-6-phosphate dehydrogenase (G-6-PDH) in both models correlates with increased carbonyl content of soluble proteins. Proteases which selectively degrade the oxidized proteins are induced or activated in young rats (3 months) treated with 100 % oxygen. However, old rats (26 months) possess only 20 % of the selective protease activity of young rats (3 months) and these proteases cannot be induced or activated in response to 100% oxygen treatment. Treatment of young rats with endotoxin (1/40 lethal dose) renders rats tolerant to 100 % oxygen. Moreover, the levels of oxidized proteins in hepatocytes is not increased and enzyme specific activity is not lost.

Other studies have been carried out with Dr. Hugh Mickel, Laboratory of Experimental Neuropathology, and Dr. Johanna Moller and Dr. Richard Quarles, both of the laboratory of Molecular and Cellular Neurobiology, NINDS, NIH. Previous studies by Norenberg and his colleagues indicated that rapid correction of serum sodium levels following vasopressin-induced hyponatremia leads to highly localized myelinolysis in brains of experimental animals. Rapid correction of serum sodium is also associated with increased protein oxidation of soluble proteins (whole brain) as well as solubilized myelin basic protein (MBP). *In vitro* experiments also indicate that the major myelin proteins (from normal animals) are highly susceptible to oxidation by various metal catalyzed oxidation (MCO) systems and that the oxidized proteins are readily degraded by Ca(II)-activated proteases whereas myelin proteins from control incubations are highly resistant to proteolysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 00272-01 LB

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.)*

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, Section on Metabolic Regulation, LB, NHLBI

Others: P. Boon Chock, Chief, Section on Metabolic Regulation, LB, NHLBI
Earl R. Stadtman, Chief, Laboratory of Biochemistry, NHLBI
Sue Goo Rhee, Chief, Section on Signal Transduction, LB, NHLBI
Cynthia N. Oliver, Ph.D., Special Volunteer, LB, NHLBI

COOPERATING UNITS *(if any)*

Emily Shacter, Laboratory of Genetics, NCI

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Metabolic Regulation

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.)*

In order to examine the structural identities of reactive free radicals and mechanism of the oxidative modification of proteins, we investigated using EPR and spin trapping methods on (a) oxidation of L-leucine as a model system, (b) protector protein that protects enzymes from sulfur radicals, and (c) activated neutrophils. The compound, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was used as the spin trap throughout these investigations.

Hydroxyl radical, and both hydroxyl and a new leucine-derived radical were observed, respectively, in the reaction mixtures of Fe(II)/EDTA/hydrogen peroxide/DMPO and Fe(II)/EDTA/hydrogen peroxide/L-leucine/DMPO in bicarbonate buffer. With Mn(II) in place of Fe(II)/EDTA, superoxide as well as hydroxyl radicals were trapped in the absence of L-leucine. Addition of this amino acid resulted in production of the leucine-derived radical that replaced superoxide radical anion. By employing various isotope enriched leucine, we have successfully identified this radical as HOOCC(R)CHNHO•, a hydro nitroxide.

In the investigation on the protector protein, we have found that addition of this protein to the Fe(III)/oxygen/DTT/DMPO or Fe(III)/oxygen/DTT/glutamine synthetase/DMPO completely quenched EPR signals from hydroxyl and sulfur radicals, indicating antioxidant activity. Preliminary experiments on activated neutrophils were concerned on method development.

Annual Report of the Laboratory of Cardiac Energetics

National Heart, Lung and Blood Institute
October 1, 1988 through September 30, 1989

The major goal of the Laboratory of Cardiac Energetics is to develop a better understanding of the cellular processes involved in the conversion of energy to useful forms of work in the heart and other tissues. With this insight we hope to develop new strategies for studying this "energy economy" of the cell as well as for the prevention and treatment of heart disease. Our technological approach to these problems is the use of non-invasive nuclear magnetic resonance (NMR) and optical imaging and spectroscopy techniques. These methods permit the non-invasive monitoring of several critical metabolites of cellular energy metabolism in intact biological tissues or man. The application of these techniques to humans also allows us to evaluate these tools as non-invasive diagnostic modalities.

The cellular metabolic process we have been concentrating on over the last year is the complex interaction between energy conversion processes of the heart (i.e. oxidative phosphorylation) and muscle contraction (i.e. pumping of blood). Myocardial muscle contraction is believed to occur by utilizing the energy in adenosine triphosphate (ATP) produced predominantly by oxidative phosphorylation in the heart. To use this energy for muscle contraction, ATP is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate (Pi). However, as we have shown in our previous work, the increase in ATP hydrolysis can occur in the healthy myocardium without a significant increase in the products of its ATP hydrolysis. Thus, the cytosolic signal between the contractile elements and oxidative phosphorylation is not a simple end-product feedback of the hydrolysis products of ATP to oxidative phosphorylation as previously believed. Hence some other parameters must be responsible for the orchestration of these two critical processes. Over the last year we have concentrated on oxygen delivery as a potentially rate limiting aspect of this process, and the possible use of the high energy phosphate metabolites as indicators of <u>functional</u> coronary blood flow for clinical studies using NMR. In addition we have also worked on the further technical development of these non-invasive tools. The highlights of these two projects are outlined below.

In a joint project with the Cardiology Research Center of the USSR, Dr. Kuprianov and Dr. Heineman have determined the net tissue oxygenation of perfused hearts during work "stress" tests. In these studies, the oxygenation of the heart was directly monitored using optical spectroscopy techniques which detected the redox state of the mitochondrial cytochromes and oxygenation of myoglobin. These studies revealed that as the work demands, or ATP hydrolysis rate, of the heart was increased the net oxygenation of the heart also augmented. This was indicated by a net oxidation of mitochondrial cytochromes and an increase in the heart myoglobin oxygenation. These results are consistent with an increase in oxygen delivery being partially responsible for the work induced increased

rate of oxidative phosphorylation required to "feed" the increased contractile activity. This suggests that the actual delivery of oxygen to the heart tissue may be one of the major rate limiting steps for metabolism even in the normal heart in vitro, whether this is also the case for the heart in vivo is yet unknown, however, the technical developments required to make these measurements in vivo are currently underway.

With regard to using the high energy phosphates as an index of functional cardiac perfusion, Dr. Heineman has monitored the high energy phosphates (i.e. creatine phosphate (CrP), ATP, and Pi) using 31P NMR techniques on a canine model in vivo. In these studies the high energy phosphate metabolites were monitored with a 16 sec time resolution simultaneously with coronary blood flow during step increases in work induced by ventricular pacing or various drugs. The results of this in vivo stress test demonstrated that the high energy phosphates do not change until coronary blood flow is not adequate to support oxidative phosphorylation. At this point, where the workload exceeds the metabolic capacity of the heart, a rapid and dramatic decrease in CrP is observed reflecting the inadequacy of coronary blood flow. These studies indicate the 31P NMR monitoring of high energy phosphates maybe a very effective non-invasive manner of detecting the metabolic consequences of inadequate coronary perfusion in humans. In collaboration with Dr. P. Bottomly at the Corporate Research Center of General Electric, we have been successful in detecting these cardiac phosphate metabolites in humans at our In Vivo NMR Center. Clinical evaluation of these NMR procedures are now underway.

To make these non-invasive measurements of tissue biochemistry and function, our laboratory also has an ongoing program of technical development in this area. In the last year this development program has resulted in several new techniques including: the determination of cardiac tissue oxygenation via myoglobin and the mitochondrial cytochromes, a non-invasive method of determining aldose reductase activity in vivo and a method of determining the magnetization exchange rate between water protons and macromolecules in NMR imaging (MRI) studies. This latter technique has become a clinical standard in evaluating the morphology of the body actually replacing many x-ray based techniques. However, little was known about the basis of the tissue contrast generated by these techniques and this lack of knowledge has limited the interpretation of these images. This recent development has begun to provide a molecular basis for the generation of this contrast. The highlights of our work in the latter two areas are presented below.

Aldose reductase is an important enzyme in the normal metabolism of glucose and formation of sorbitol. This enzyme is of special interest in the diabetic when its activity may result in some of the numerous complications of the disease. Using a 19F labeled 3F-deoxyglucose (3-FDG) label, Dr. Berkowitz has been able to monitor the production of sorbitol by aldose reductase in different tissues using 19F NMR. We have determined that this particular probe is not metabolized by the major glycolytic, glycogen synthesis or hexomonophospate shunt pathways, but is readily metabolized by aldose reductase to 19F labeled sorbitol. Thus, the amount of 19F labeled sorbitol in tissue provides a specific measurement of aldose reductase activity in vivo after administration of

the non-toxic 3-FDG probe. This technique should be extremely useful in the determination of aldose reductase activity distribution in vivo under normal and disease states. It will also be applicable to the evaluation of aldose reductase inhibitors in intact tissues, a currently developing technique in the treatment of diabetes.

The relaxation properties of water 1H nuclei are the basis for most of the contrast obtained by NMR imaging (MRI) techniques. Conventional 1H NMR images of biological tissues usually reflect a combination of spin-lattice (T1) and spin-spin (T2) water 1H relaxation. In this study, Dr. Wolff attempted to gain further insight into the molecular mechanisms responsible for the variation in tissue water 1H relaxation and thereby help establish the molecular basis of the contrast observed in NMR images. Toward this goal, Dr. Wolff determined the exchange between 1H magnetization in "free" water (1Hf), the pool usually monitored in an NMR imaging experiment, and the 1H pools with restricted motion (1Hr) (i.e. proteins, membranes, etc.) in vivo using NMR saturation transfer methods previously developed in this laboratory. Significant magnetization exchange between these two pools was directly demonstrated in these studies. The pseudo-first-order rate constants for the movement of magnetization from 1Hf to 1Hr was ~1 s-1 in kidney and ~3 s-1 in skeletal muscle in vivo. Proton MR images, collected at 4.7 Tesla, of the kidney, leg and brain, demonstrated that this exchange was tissue specific and generated a novel form of NMR image contrast, magnetization transfer contrast (MTC). This approach provided high contrast images similar to heavily T2 weighted images, as well as provided excellent fat-water, blood flow, and brain grey-white matter contrast. The extent of exchange between 1Hf and 1Hr are well as the topological correlation of the exchange with relaxation weighted images suggests that this pathway (i.e. magnetization exchange between 1Hf and 1Hr) is a major determinant of the observed relaxation properties of water 1H in vivo. This implies that much of the contrast observed in MRI images is due to variations in magnetization exchange between the macromolecules and "free" water in different biological tissues. Our recent studies have demonstrated that this exchange process is dependent on the types of macromolecules and their relative mobility indicating that the NMR image provides not only chemical information on the macromolecular matrix but also it's mobility. Further investigation on the types of macromolecular structures and utilization of MTC to detect different disease states are currently underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04601-02 CE

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.)*
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
	J. Eng	Research Scholar	HHMI, NHLBI
	T. Fralix	Guest Worker	IRTA
	V. Kuprianov	Guest Worker	USSR, Cardiology Research Center
	F. Heineman	Medical Staff Fellow	LCE, NHLBI
	P. Bottomly	Senior Scientist	G.E. Corporate Research Center

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:
3.25	3	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 understand of the metabolism of energy in tissues in vivo. Toward this goal this laboratory concentrates on the use of non-invasive or non-destructive techniques to evaluate the biochemical function of tissues. These techniques include optical and nuclear magnetic resonance (NMR) spectroscopy to monitor various aspects of tissue energetics including high energy phosphate and other metabolite contents and turnover, oxygenation and blood flow. In the past year we have studied the relationship between coronary blood flow and high energy phosphates during increases in cardiac work. These studies have revealed that the high energy phosphate compounds are extremely sensitive indicators of cardiac ischemia. This method is unique since it does not simply rely on the absolute amount of blood supplying the heart but evaluates the intermediary metabolism to establish whether any given blood flow is adequate for normal cardiac function. Also in the last year we have successfully obtained 31P NMR data from the human heart using a totally non-invasive approach. Using this approach we are not able to evaluate the use of NMR in the detection of early cardiac ischemia in various clinical conditions. The role of oxygen in the control of cardiac metabolic rate was also evaluated in perfused heart models in vitro using optical spectroscopy techniques. These studies indicate that the delivery of oxygen may contribute to the control of cardiac metabolic rate even under "normal" conditions. These results suggest that the regulation of blood flow and subsequent oxygen delivery may be more important than previously believed even in the normally functioning heart.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04602-02 CE

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.)*
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:	B. A. Berkowitz	Staff Fellow	LCE, NHLBI
	J. Eng	Research Scholar	HHMI, NHLBI
	S. D. Wolff	Research Scholar	HHMI, NHLBI
	T. A. Fralix	Guest Worker	IRTA
	V. Kuprianov	Guest Worker	USSR, Cardiology Research Center
	F. Heineman	Medical Staff Fellow	LCE, NHLBI
	S. Simon	Professor	Duke University

COOPERATING UNITS *(if any)*
Howard Hughes Medical Institute, Duke University,
USSR Cardiology Research Center

LAB/BRANCH
Laboratory of Cardiac Energetics

SECTION
Non-invasive Technology

INSTITUTE AND LOCATION
Nation Heart, Lung and Blood Institute, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	4	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 non-invasive 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 developed and demonstrated the following NMR techniques: (1) A new form of NMR image contrast was developed, magnetization transfer contrast (MTC), which is based on the property of water interaction with macromolecules in intact tissues. This technique has been demonstrated to improve the contrast present in NMR images of brain, kidney and tumors. This research has led to a better fundamental understanding of the NMR image as it applies to the study of biological tissues, including clinical evaluations. (2) A method of monitoring aldose reductase activity in vivo was developed using 3-Fluorodeoxyglucose. This method provides a non-invasive assay of aldose reductase activity in tissues. This should prove useful in the evaluation of diabetes in patients. (3) An NMR method was developed that permits the detection of metabolites in proton-exchange with water, this permits the monitoring of metabolites as low as 1 micromolar in vivo. (4) NMR images of the perfused heart with resolution on the order of 100 microns were obtained indicating the resolution limits of in vivo cardiac imaging studies. Using optical spectroscopy the following advancements were made: (1) The oxygenation of the perfused heart was directly evaluated by monitoring the optical absorbance of cytochrome and myoglobin in the intact heart using gated rapid scanning spectrophoto-metric techniques. (2) Topological maps of intracellular Ca and mitochondrial NADH were also measured using optical imaging techniques.

Annual Report of the Laboratory of Cell Biology
National, Heart, Lung, and Blood Institute
October 1, 1988 to September 30, 1989

The Laboratory of Cell Biology presently has 4 major research interests: (1) the structure, enzymatic properties, regulation and biological role of non-muscle myosins in cell motility, (2) the mechanism of polymerization of actin, its regulation by actin-binding proteins, and the organization of actin filaments in the cytoskeleton; (3) the biochemical properties of the 70-kDa heat shock proteins and their role in normal cellular processes and heat shock; (4) bioenergetics: the process by which the energy released by the oxidation of metabolites is converted into a form that can be utilized by the cell.

Non-muscle Myosins: Myosins are a family of enzymes that, together with actin filaments, provide the mechanochemical basis for many motile activities of non-muscle cells as well as for muscle contraction. Actin forms long polymeric filaments that activate the ATPase activity of the myosin. The energy released by the hydrolysis of ATP causes a conformational change in the actomyosin complex that results in movement at the cellular level. Work in the Laboratory of Cell Biology has established the existence of at least two classes of non-muscle myosins. Both myosins I and myosins II contain heavy and light chains but they differ in the size of the heavy chain and the structure of the molecule.

The heavy chains of all myosins contains an N-terminal region of 85-95 kDa that has a globular conformation and which contains the catalytic (ATPase) site, an actin-binding site and binding sites for the associated light chains. We have shown that there is strong sequence similarity among this domain of all myosins. The heavy chain of the myosin II class has a long C-terminal region (90-120 kDa) through which two heavy chains interact to form an α-helical coiled-coil. The helical tails of multiple myosin molecules self-associate to form the myosin filaments that are the functional forms of the myosin II class: the globular heads that project from the myosin II filaments bind to and cross-link actin filaments. The heavy chains of the myosin I class are much smaller (about 50 kDa), do not form helical structures and do not interact with one another so that myosin I molecules remain single-heavy chain monomers in their functional state. However, the short, C-terminal region of the myosin I heavy chains contains a second actin-binding site so that monomeric myosin I molecules can cross link 2 actin filaments (analogous to the way in which the multiple globular heads of myosin II filaments can cross-link actin filaments). The actin bound to the globular heads of myosins I and myosin II activate the myosins' ATPase activities and the hydrolysis of ATP by the actomyosin complexes moves one actin filament relative to another.

Acanthamoeba myosin II, discovered in this laboratory, remains the best studied non-muscle myosin II. Of special interest, is the fact that the actin-activated ATPase activity of the enzyme is inactivated by phosphorylation of three serine residues in a short non-helical tailpiece that extends from the α-helical coiled-coil rod. Subsequently, similar phosphorylation sites have been discovered in the tails of the heavy chains of many non-muscle myosin II isoforms. A major effort of this laboratory has been to understand how the ATPase activity of the globular head is regulated by the phosphorylation state of amino acid residues far away at the tip of the tail. Recent work has shown that the properties of Acanthamoeba myosin II are essentially unchanged upon

removal of a C-terminal region containing 2 of the 3 regulatory serines. The reason for the apparently redundant occurrence of 3 phosphorylation sites remains obscure. Efforts to remove the third site continue in order to determine if the non-helical tailpiece is essential for activity.

Earlier evidence had indicated that the level of actin-activated ATPase activity of each myosin II molecule depends on the state of phosphorylation of the filament as a whole rather than the phosphorylation state of the molecule itself. This inference was proved this year by the following experiments. Under carefully controlled conditions, a 35,000-dalton C-terminal peptide was proteolytically produced from the tail of the myosin II heavy chain. This peptide contained 40% of the coiled-coil region and a portion of the non-helical tailpiece containing 1 regulatory serine. The dephosphorylated and phosphorylated forms of this peptide were purified to homogeneity and shown to be capable of polymerization and of co-polymerization with native myosin II molecules. The phosphorylated peptide inactivated the actin-activated ATPase activity of dephosphorylated myosin II when the two were co-polymerized while dephosphorylated peptide had no effect. Conversely, the dephosphorylated peptide activated the ATPase activity of phosphorylated myosin II when they were co-polymerized. It remains to understand the details of the conformational changes that phosphorylation and dephosphorylation cause in the myosin II heavy chain that alter the ATPase activity.

In contrast to myosin II, the actin-activated ATPase activity of Acanthamoeba myosin I is activated by phosphorylation of one residue in the heavy chain: a threonine in myosin IA and a serine in myosins IB and IC. A small phosphorylated peptide has been isolated from each myosin I heavy chain, sequenced and the location of the phosphorylated amino acid identified by comparison of the amino acid sequence to the complete sequence previously deduced from the genomic DNA sequence. The phosphorylated residue is in the same position of each isoform - a region that has the lowest degree of sequence similarity to the heavy chains of myosin II isoforms. This suggests the possibility that phosphorylation activates myosin I isoforms by converting this region of the heavy chain into the conformation already present in myosin II heavy chains. Additional credence to this proposal comes from the fact that the phosphorylation site lies between the actin-binding site and the ATPase site.

In addition to at least 3 myosin I isoforms and 1 myosin II isoform, Acanthamoeba has been found to contain at least one member of what seems to be another class of myosin molecules. A DNA has been cloned and sequenced and the predicted 170-kDa protein has a globular head typical of all myosins and a C-terminal region that is unlike both the helical coiled-coil of myosins II and the glycine/alanine/proline-rich region of myosins I.

In part because of the complexity of the myosin isoform composition of Acanthamoeba, efforts to determine the differential functions of myosins I and II have shifted to Dictyostelium, which had previously been shown also to contain both myosin I and myosin II. Work in another laboratory had shown that Dictyostelium contains only 1 myosin II gene. We have now found that Dictyostelium probably contains only 1 myosin I gene. It has been cloned and sequenced and found to be very similar in sequence to the Acanthamoeba myosin I isozymes. The isolated protein is also almost identical in properties to the Acanthamoeba

myosin I enzymes. Immunofluorescence microscopy of migrating cells has shown that myosin I is highly concentrated in the leading edge of pseudopodia and lammelopods while myosin II is at the rear of the cell; in dividing cells myosin II is exclusively in the contractile ring, while myosin I is again concentrated in the pseudopodal extensions; in phagocytosing cells, myosin I is greatly enriched in the region of the phagocytic cup. Thus, actomyosin I may provide a projectile force at the leading edge while actomyosin II provides a contractile pressure at the rear. The specific involvement of myosin I in migratory events is consistent with the observation that steady state mRNA levels for myosin I increase 7-fold between 5-10 hours after starvation-induced aggregation. These studies are being pursued by multiple approaches involving genetic engineering of the amoebae.

Actin Polymerization: The 42,000-dalton actin monomer polymerizes under the ionic conditions present in cells to long filaments that are a major component of the membrane-cytoskeletal complex of eukaryotic cells where, together with myosin, they provide the mechanochemical basis for many movements including muscle contraction. Previous work in this and other laboratories has shown how the hydrolysis of actin-bound ATP to actin-bound ADP that accompanies polymerization serves to regulate the process, stabilize the filaments, and confer specific and different characteristics to the two ends of the filaments. There is now considerable information about the nature of the process by which filaments elongate by addition of monomers to the two growing ends, but rather little is known about the process by which monomers are initially converted into the small oligomers that begin the elongation process.

We have now found conditions of low Mg ions and/or low Ca ion concentrations in which monomers below their apparent critical concentration seem to be converted into small oligomers, perhaps containing about 10-15 subunits. Hydrolysis of ATP is associated with their formation. These "oligomers" are either intermediates in the polymerization process or can be converted to intermediates because they facilitate formation of long filaments when the Mg ion concentration is increased.

Actobindin is a protein previously isolated from Acanthamoeba that seems to inhibit actin filament formation primarily by interacting with a small, intermediate in the polymerization process. The complete amino acid sequence of this 89-residue homodimer has been obtained. Interestingly, it contains an almost identical internal repeat of about 34 amino acids. Preliminary data suggest that actobindin can form both 1:1 and 1:2 actobindin:actin complexes.

Heat-shock Proteins: There is a class of 70-kDa proteins in a wide variety of species from yeast to man, some of which are constitutively present while others occur only when the organism is subjected to heat-shock or other stress. The specific functions of the 70-kDa proteins are not known, but in yeast the constitutive isoform is essential for viability. All bind tightly to ATP and one of the 70-kDa heat-shock proteins has been found to have ATPase activity and to be capable of removing clathrin from coated vesicles. Coated vesicles are formed by the internalization of the coated pit regions of plasma membranes which are the sites of ligand-binding in the process of receptor-mediated endocytosis. Clathrin is the protein that coats the cytoplasmic surface of the coated pit/vesicle and its dissociation from the membrane is one of the

earliest steps in the processing of the internalized vesicles. The 70-kDa heat shock proteins have also been found to associate with the nucleolus of heat-shocked cells, and to be involved in the transport of newly synthesized proteins across the membrane of the endoplasmic reticulum. Thus, the concept has developed that the 70-kDa heat-shock proteins may function in an ATP-dependent manner to keep proteins in a dissociated, or disaggregated state. Research in this Laboratory has concentrated on three aspects: (a) characterization of the bovine brain uncoating ATPase (a 70-kDa heat-shock protein), (b) similar studies of the 70-kDa proteins from yeast, (c) molecular genetics studies of the bovine brain uncoating ATPase and the 70-kDa proteins from Dictyostelium. These studies are all in their initial stages.

When the bovine brain uncoating ATPase is incubated first with ATP and then with intact bovine brain coated vesicles, an initial burst of uncoating is followed by a much slower steady-state process. The initial burst is stoichiometric rather than catalytic - one clathrin molecule removed for each enzyme molecule - and is strongly inhibited by the presence of ADP and Pi. ADP and Pi slowly dissociate from the 70-kDa ATPase. The working model is that 70-kDa enzyme with bound ATP interacts with tho coated vesicle forming a complex with clathrin and dissociating it from the vesicle (the rapid burst). The ATP is then hydrolyzed, ADP and Pi are slowly released from the ATPase-clathrin-ADP-Pi complex (the rate-determining steady-state step), ATP binds to the ATPase dissociating the clathrin and allowing the cycle to repeat. The 70-kDa proteins from yeast are only 10-20% as active as the bovine enzyme and the several yeast 70-kDa proteins differ in activity.

Bioenergetics: One of the important recent findings from this Laboratory was that the single redox center of enzyme cytochrome aa_3 displays two different redox potentials. The redox potential appears to be regulated by the state of oxidation of another redox center which may correspond to the "extra" Cu recently described in several other laboratories. When this second center is oxidized the cytochrome has a high E_m of about 770 mV and when the second center is reduced the cytochrome has low E_m of about 185 mV. Because the reduced form of the cytochrome has a lower redox potential than the oxidized form of the cytochrome, cytochrome aa_3 shows the paradoxical behavior of going from the reduced to the oxidized form as the voltage is lowered from 450 mV to 200 mV.

Because these observations were so unexpected, it was deemed absolutely essential to make every possible effort to ensure that they were not the result of some subtle experimental artifact. For example, to control the redox potentials in the experiments, ferri- and ferrocyanide complexes were used which raises the possibility that CN complexes of the cytochrome aa_3 were responsible for its anomalous behavior. Thus far, however, all evidence indicates that this cannot be the cause of the phenomenon and no evidence for any other alternative explanation has been found.

Dr. M. Zasloff has found that frog skin contains a class of small peptides (23 amino acids), called magainins, with potent toxicity against a broad spectrum of bacteria and protozoa. From their amino acid sequences, it was suggested that these peptides might form an amphipathic helix that could span biological membranes and thus interfere with essential membrane processes, specifically disrupt the bioenergetic reactions. Studies have been performed on the effect

of the magainins on respiration and energy transduction using rat liver mitochondria, E. coli, and reconstructed systems of cytochrome oxidase in lipid vesicles. In general, the magainins were found to permeabilize the membranes to a variety of ions thus dissipating membrane potentials, causing a loss of respiratory control and the ability to synthesize ATP. The simplest quantitative explanation of their effects involves the formation of a membrane channel through the cooperative interaction of 3-5 magainin molecules.

The normally non-pathogenic Acanthamoeba, an ubiquitous organism, has recently been identified as the causative agent in keratitis associated with the use of contact lenses. The possibility that the magainins might be a therapeutic agent has been explored. Magainin is toxic to Acanthamoeba amoeba at a concentration of about 40 μg/ml and oxygen consumption is abolished within 30 seconds of exposure. These results are consistent with the studies on the effects of magainins on membrane potentials, etc., but the basis of the toxicity has yet to be determined.

The presently accepted mechanism for coupling of ATP synthesis to electron transport depends on the primary formation of a membrane potential which then drives the synthesis of ATP. A simple system for studying the behavior of membrane potentials has recently been established in this Laboratory. Phospholipid membrane vesicles (liposomes) were formed in the presence of 120 mM K ions (trapping K ions inside) and the external solution replaced by 120 mM Na ions. The vesicles were essentially impermeable to ions until the addition of valinomycin created a slow diffusion of K ions from inside to outside. This caused a diffusion potential of about 200 mV. Similarly, a pH gradient could be established by forming the liposomes in solutions of one pH and then replacing the external solution with one of a different pH. In this way, it will be possible to study the properties of membrane potentials and pH gradients separately and together, and then to study their effects on liposomes that contain cytochrome oxidase and/or ATP-synthesizing enzymes in the lipid membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00401-23 LCB

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.)*
Thermodynamic studies of electron and proton affinities of cytochromes

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)*
None

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	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 were conducted to determine whether CN- can be released from metallo cyanide redox mediators that are used during potentiometric titrations, in sufficient quantities to form complexes with cytochrome c oxidase. Specifically, the question is whether spectral changes seen during high potential titrations of the enzyme are due uniquely to the binding of electrons or whether they may be due to the binding of CN- instead. It was found that the spectral changes seen during the titration are due mainly (if not entirely) to electron binding. This is based on the observations that ~67% of the same change in the spectrum occurs under conditions where CN- release is prevented and that the extent of spectral shift which occurs during a titration in the presence of CN- is not correlated to the amount of CN- present. The possibility that released CN- could be converted to cyanogen (C2N2) and that C2N2 complex formation could occur with the enzyme was also tested and found to not occur. Feasibility studies were conducted to see if an ultra high speed recording spectrometer could be built. This instrument is designed to accumulate optical spectra repeatedly every 10 μs. It is intended to study the kinetics of cytochrome oxidase with this instrument and our singular value decomposition procedures. On the basis of the preliminary studies we have decided that the instrument can be built and intend to proceed with its development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00418-09 LCB

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.)*

Energetic and stoichiometric relationships involving respiration, $\Delta\mu H^+$ and ATP

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

PI:	Richard W. Hendler	Section Head	LCB, NHLBI
Others:	Davor Juretic	Visiting Associate	LCB, NHLBI
	Frits Kamp	Visiting Fellow	LMB, NIDDK
	Hans V. Westerhoff	Special Volunteer (and Huygens Fellow, Dutch Cancer Inst., Amsterdam, Holland)	LMB, NIDDK

COOPERATING UNITS *(if any)*

Richard I. Shrager, Mathematician, LAS, DCRT and Mike Zasloff, University of Pennsylvania

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.)*

A class of peptides which have a broad spectrum bacteriocidal activity has been isolated from the skin of the frog Xenopus lavis by Mike Zasloff and collaborators. Our laboratory has investigated the question of whether this bacteriocidal activity is explainable by the ability of these peptides to disrupt a basic membrane-associated activity, namely respiratory energy transduction. For this purpose, we have examined several kinds of active peptide and some related but non bacteriocidal analogues. Energy transduction in mitochondria, cytochrome oxidase containing liposomes and E. coli was studied as well as the inhibition of growth of E. coli. All of the active peptides were able to permeabilize all of the membranes to a variety of ions, leading to the dissipation of $\Delta\Psi$, the loss of respiratory control, and the loss of ability to form ATP. We found that some protection against these peptides is provided by proteases in the bacteria, that there is a cooperative phenomenon which indicates that 3 to 5 peptide units interact to form a membrane channel, and that a strong synergistic action between two different peptides markedly enhances the energy disrupting and bacteriostatic activities of each one. In separate studies, we have found how to create and maintain sizeable $\Delta\Psi$'s in pure (non-enzyme-containing) liposomes. We can also form and maintain sizeable ΔpH's in the bare liposomes. This tool will be immensely useful in trying to dissect the roles of $\Delta\Psi$ and ΔpH on proton fluxes through membranes and specific H+-pumping enzymes and in separating the potential of each of the two forms of $\Delta\mu H^+$ in the formation of ATP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00419-09 LCB

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-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

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	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.)*

The small soil amoeba, Acanthamoeba castellanii, is being used as a model system to examine the flow of membrane through successive cellular compartments during endocytosis. We have made and characterized two monoclonal antibodies (MC1 and MC3) that are specific for Acanthamoeba membranes. The present study used electron microscopic immunocytochemistry to measure the relative abundance of the two classes of epitopes on the plasma membrane and the internal vacuolar membranes to determine if some proteins might be selectively concentrated in one membrane compartment. The labeling was done on thin-sections of plastic embedded cells so that internal membranes and plasma membranes had equivalent access to the colloidal gold label. The results indicate that proteins localized by MC1 are about 60% more concentrated in the intracellular membranes than in the plasma membrane, whereas those localized by MC3 have approximately the same distribution in both membrane compartments. The results suggest that the amoeba differentially sorts some, but not all, membrane proteins during the rapid exchange between surface and internal membrane. In other studies, toxicity of the magainins, a class of small peptides particularly toxic to protozoa and bacteria, was examined. Doses of magainin as low as 40 μg/ml for about one million cells were toxic to Acanthamoeba trophozoites, but the encysted form of the organism appeared resistant to levels up to four times higher. Killing of trophozoites, as judged by cessation of oxygen consumption, was very rapid and occurred within 30 seconds of application of appropriate doses of the peptide. The cellular mechanism of the peptide toxicity is being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00501-16 LCB

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.)*
Actin Polymerization

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

PI:	Edward D. Korn	Chief	LCB, NHLBI
Others:	Michael Bubb	Medical Staff Fellow	LCB, NHLBI
	Arun K. Attri	Visiting Associate	LCB, NHLBI

COOPERATING UNITS *(if any)*
Laboratory of Technical Development, NHLBI
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:
2.15	2.15	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.)*

Earlier reports by other laboratories on the formation of actin oligomers at low Mg ion concentrations with the actin concentration below the critical concentration have been confirmed. These oligomers appear to be converted to F-actin when the Mg ion concentration is increased. However, analytical ultracentrifugation and fluorescence anisotropy measurements indicate that the oligomers are larger than the dimers or trimers that were previously suggested by others.

Actobindin, a protein from Acanthamoeba castellanii, that forms a 1:1 complex with actin monomer and also inhibits actin polymerization by interacting with an early intermediate has been found by analytical ultracentrifugation to be a monomer of about 9500 daltons, and not, as previously reported, a homodimer of a 12,500-dalton peptide. The 89-residue amino acid sequence of the polypeptide has been determined. It shows an almost perfect internal repeat of 34 amino acids, at least one residue of which can be crosslinked to actin by the zero-length cross-linker EDC. The sequence of actobindin seems to have no similarity to the sequences reported for any other actin-binding proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00503-17 LCB

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, assembly and function of microtubules

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

PI: Martin Flavin	Section Head	LCB, NHLBI
Others: Ravi Kambadur	Visiting Fellow	LCB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Organelle Biochemistry

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.75	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.)*

Interaction between microtubules and membranes is essential to many cell functions yet, in contrast to cytoskeletal actin, nothing is known about how it occurs. We chose a Trypanosomatid protozoan because its skeletal microtubules are found in close opposition to the plasma membrane, and electron microscopy had revealed periodic crosslinks between tubules and membrane (as well as between adjacent microtubules). We began by studying 3 proteins that appeared prominent in isolated cytoskeleton. Antibodies to one of them stained glycosomes in cell sections, and the protein proved to be a glycolytic enzyme which adhered to microtubules when released by homogenization. The other 2, detergent-soluble proteins, are absent from glycosomes. One of them cross-links microtubules in vitro. Both bind to soluble tubulin or microtubules with -log KD = 7 (one prefers tubulin, -log KD =8). Binding curves show positive cooperativity with maximum of 0.5 to 1.0 mol bound per mol tubulin. However, we have not yet established whether either protein is part of the cellular crosslinks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00506-14 LCB

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.)*

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

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:
6.15	6.15	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 actin-activated ATPase activity of the globular heads of filamentous Acanthamoeba myosin II is known to be inactivated by phosphorylation of 3 serines in the non-helical tailpiece at the end of the coiled-coil helical rod. Myosin II remained fully active after removing 2 of these serines with an arginine-specific protease, and the cleaved myosin was regulated normally by phosphorylation of the one remaining serine. A dimer of the 35,000-dalton C-terminal peptide was produced by tryptic cleavage the tails of myosin II. When co-polymerized with native myosin, the phosphorylated peptide inactivated dephosphorylated myosin II and the dephosphorylated peptide activated phosphorylated myosin II. This proves that the activity of myosin II heads is independent of the state of phosphorylation of the tail of the same molecule and depends only on the overall state of phosphorylation of the filament of which it is a part.

The exact location of the regulatory threonine in myosin IA and the regulatory serines in myosin IB and IC have been determined by sequence analysis of the phosphorylated tryptic peptides obtained from each isoform. In the course of this work, it was discovered that the heavy chains of myosins IB and myosin IC correspond to the genes previously identified as coding for the heavy chains of myosin IL and myosin IB, respectively.

Immunofluorescence studies with Dictyostelium amoebae show differential localization of myosin I in the leading edge of pseudopods and lamellopods of migrating cells and in the phagocytic cup of cells ingesting bacteria whereas myosin II is concentrated at the rear of the same migrating cells and in the contractile ring of dividing cells. Thus, actomyosin I may provide an extensive force at the front of locomoting cells while actomyosin II provides a contractile force at the rear.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00514-06 LCB

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.)*
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
	David Halsall	Visiting Fellow	LCB, NHLBI
	Jill Horowitz	IRTA 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:
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 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) using peptides generated by in vitro transcription/translation, (3) testing models of myosin II filament formation and enzymatic regulation using site-directed mutagenesis and expression in E. coli, and (4) full characterization of a third type of nonmuscle myosin distinct from myosin I and myosin II. 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 00516-03 LCB

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.)*: 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 — LCB, NHLBI
Lois E. Greene — Research Chemist — LCB, NHLBI

Others: Winifred W. Barouch, IRTA Fellow; Yumiko Emoto, Visiting Fellow; John A. Evans, Staff Fellow; Bao-chong Gao, Visiting Fellow; and Myrna Mandel, Staff Fellow (LCB, NHLBI

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:
5.75	5.75	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. The initial burst of uncoating is essentially stoichiometric with each enzyme apparently binding to and dissociating one leg of the clathrin triskelion. ADP and Pi together, strongly inhibit both the initial burst and steady-state uncoating. However, kinetic studies suggest that ADP and Pi dissociate from the enzyme relatively rapidly unless clathrin is also bound to the enzyme. These data suggest a model where the enzyme with bound ATP interacts with the coated vesicles and then rapidly removes a stoichiometric amount of clathrin as ATP at the active site is hydrolyzed. This is followed by slow release of ADP and Pi from the resulting enzyme·clathrin·ADP·Pi complex which limits the rate at which further uncoating can occur. ATP then dissociates the bound clathrin allowing further cycles of uncoating to occur. 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. In addition to these studies, we have investigated the ability of several 70-kDa proteins isolated from various yeast clones to uncoat bovine brain clathrin coated vesicles. Our results show that, in general, 5-10-fold more yeast enzyme is required to carry out the same amount of uncoating as the brain enzyme. This shows that the uncoating activity is a specific property of the brain uncoating ATPase and is not a general property of all 70 kDa proteins.

ANNUAL REPORT OF
THE LABORATORY OF CELLULAR METABOLISM
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

October 1, 1988 through September 30, 1989

Research in the Laboratory of Cellular Metabolism is largely focused on the guanine nucleotide-binding (G) proteins that function in the adenylyl cyclase and other systems to transmit signals from the exterior of the cell to internal effectors. The objective of this effort is to elucidate mechanisms for control of synthesis, assembly and operation of these ubiquitous regulatory proteins. Major subjects of current studies are G_o, a G protein whose physiological role is at present unclear, and several recently recognized, so-called ADP-ribosylation factors that appear to be members of a different family of guanine nucleotide-binding proteins. In addition, work is continuing on characterization of specific cyclic nucleotide phosphodiesterase that play an important regulatory role in cells.

1. Regulatory Properties of Cyclic Nucleotide Phosphodieterases

By regulating degradation of cAMP and/or cGMP, phosphodiesterases terminate the cyclic nucleotide signals generated by a number of hormones, neurotransmitters, autocoids, physiologically important peptides, and therapeutic agents and thus can regulate cellular responses to these effectors. Phosphodiesterases are a family of enzymes with distinct structural and regulatory properties and specific cellular/subcellular localizations. Over the past several years, we have described and purified so-called cGMP-stimulated phosphodiesterases from soluble and particulate factions of liver and brain and elucidated some of their allosteric properties. Current studies with photoaffinity labeling and selective proteolysis are characterizing structural relationships among enzymes of this type in regulatory and catalytic domains. This particular group of phosphodiesterases may be of importance in regulating cyclic nucleotide metabolism in a number of tissues including heart, adrenal, and brain and perhaps play a role in the mechanisms through which cell cAMP content is regulated by agonists such as atrial peptides that increase cGMP.

It was shown in this laboratory a number of years ago that fat cell membranes contain a high affinity cAMP phosphodiesterase that can be activated by insulin or isoproterenol. Activation by isoproterenol was believed to be secondary to isoproterenol activation of adenylyl cyclase and resulting cAMP accumulation. Insulin activation was implicated in the mechanism of the anti-lipolytic action of this hormone. In collaboration with scientists at the University of Lund, this phosphodiesterase was purified from rat and bovine adipose tissue; the bovine enzyme ($\sim$62 and 77 kDa) was used to produce specific polyclonal

antibodies that react with the 64 kDa rat enzyme. In current studies, the antibodies are used for immunoprecipitation of the phosphodiesterase from rat fat cells. It has been found that both insulin and isoproterenol cause phosphorylation of a 135 kDa protein that appears to be the native enzyme (which was presumably proteolyzed during purification). There is a close correlation between phosphorylation and phosphodiesterase activation (temporally and in relationship to hormone concentration). With either hormone, only serine phosphorylation is detected. Findings have been confirmed by isolating the phosphodiesterase using affinity chromatography with a specific inhibitor as the ligand, a procedure utilized in the original purification.

The affinity ligand and several other specific inhibitors of this phosphodiesterase were characterized earlier in this laboratory. Some of these inhibitors also specifically inhibit high affinity cAMP phosphodiesterases in platelets and in heart that are very similar to the adipose tissue hormone-sensitive enzyme. It appears that this inhibition is important in the mechanism of action of certain drugs that alter platelet aggregation and myocardial contractility. Thus, elucidating the molecular basis of activation and inhibition of this adipose tissue phosphodiesterase may provide information relevant as well to the regulation and role of the analogous platelet and cardiac enzymes in normal and pathophysiological states, certain types of diabetes, obesity, cardiac failure.

2. Structure and Function of $G_{o\alpha}$

One family of guanine nucleotide-binding (G) proteins functions to transduce signals initiated by a variety of hormones, neurotransmitters, autocoids, and drugs in virtually all mammalian cells. These G proteins are heterotrimers of α, β and γ subunits that couple transmembrane receptors to intracellular effectors. The α subunits, which bind and hydrolyze GTP, define functional specificity and serve as substrates for certain bacterial toxins that catalyze their ADP-ribosylation and thereby disrupt normal cell function. Members of this family include G_s and G_i, the stimulatory and inhibitory G proteins of the adenylyl cyclase system, G_t (transducin) which mediates visual excitation, and G_o, which is most abundant in neural tissues and appears to play a role in regulation of ion channels. Our studies on $G_{o\alpha}$, initiated with isolation of a $G_{o\alpha}$ cDNA clone from bovine retina, have recently been focussed on 1)*in vitro* mutagenesis to establish structure-function relationships and 2)understanding the molecular basis for the presence of multiple species of $G_{o\alpha}$ mRNA as well as the cell-specific expression of the protein.

It has been suggested that the carboxyl terminus of the G protein α subunit is critical for receptor interaction. As shown earlier in this laboratory, pertussis toxin catalyzes the ADP-ribosylation of a cysteine very near the carboxyl terminus (Cys-

351 of $G_{o\alpha}$ which has 354 amino acids). This results in uncoupling of G protein and receptor. Three different procedures for mutagenesis of $G_{o\alpha}$ have been used. The proteins expressed in E. coli were isolated by the methods we previously used in study of recombinant wild type $G_{o\alpha}$. Replacement of Cys-351 with glycine or of Gly 352 with aspartate prevented ADP-ribosylation by pertussis toxin as did deletion of two carboxyl terminal amino acids (Leu-353 and Tyr-354). After replacement of Gly 350 by aspartate or arginine, however, the protein was ADP-ribosylated and this was enhanced by $\beta\gamma$ subunits as is the case for native $G_{o\alpha}$. Thus far it has not been possible to carry out other functional studies with these mutant proteins because the $G_{o\alpha}$ produced in E. coli is obtained in an aggregated state that has resisted purification.

In the hope that expression in a eukaryotic cell capable of post-translational modification might yield a non-aggregated protein, we turned to the baculovirus (BCV) system, which uses insect (Spodoptera frugiperda, Sf-9) cells infected with virus containing the gene of interest (and a viral promoter) to express large amounts of the foreign protein. The insect cells are capable of glycosylation, leader sequence cleavage, phosphorylation, and secretion of proteins. No immunoreactive $G_{o\alpha}$ was detected in uninfected Sf-9 cells or in those infected with BCV. Cells infected with BCV-$G_{o\alpha}$ contained an immunoreactive protein of the same apparent size as bovine brain $G_{o\alpha}$ which was ADP-ribosylated by pertussis toxin. This process was not, however, influenced by $\beta\gamma$ subunits. The BCV-$G_{o\alpha}$-infected cells also contained a larger immuno-reactive protein that was not a toxin substrate. Purification and further characterization of the $G_{o\alpha}$-like protein will determine whether this baculovirus system is suitable for continuing mutagenesis and structure-function studies of $G_{o\alpha}$.

For other studies of structure-function relationship in G protein α subunits, we have used transducin (G_t), which is structurally very similar to G_o and is relatively easily purified from retinal rod outer segments. Several years ago we prepared monoclonal antibodies against $G_{t\alpha}$ and have recently more precisely defined the epitope recognized by one of these as lying with the first 18 amino acids of the protein. This antibody inhibits rhodopsin-stimulated GTP hydrolysis by $G_{t\alpha}$ as well as pertussis-catalyzed ADP-ribosylation, both of which depend on effective interaction of $G_{t\alpha}$ with $\beta\gamma$ subunits. Further studies with synthetic peptides and site-directed mutagenesis, as well as monoclonal antibodies should identify the $G_{t\alpha}$ sequences critical for this interaction.

We had earlier found that neural tissues contained multiple $G_{o\alpha}$ mRNAs. We have now established that size heterogeneity of $G_{o\alpha}$ mRNA is a function primarily of differences in the 3'-untranslated regions (UTRs). Using PCR (polymerase chain reaction) and RNase H mapping, it was confirmed that a single mRNA fragment (1.9 kb) accounts for the $G_{o\alpha}$ coding regions (1062 bass) and 5'-UTR ($\sim$800 bases) of all three major mRNA species.

Three different 3'-UTRs were isolated, UTR-B (250 bases), UTR-A (1,250 bases), and UTR-C (2,000 bases) which contained UTR-A and UTR-B with additional unique sequences. The different UTRs account for the size differences in the three major $G_{o\alpha}$ mRNAs of ∿2, 3, and 4 kDa. Based on differences in the $G_{o\alpha}$ fragments amplified by PCR, selective probes for the 3'-UTRs were constructed and used to confirm assignment of UTR sequences to individual mRNAs. Hybridization of specific oligonucleotide probes with restriction fragments of DNA from mouse-human somatic cell hybrids mapped both the $G_{o\alpha}$ coding region and different 3'-UTRs to the same small region of human chromosome 16. Thus, the multiple mRNAs appear to arise by alternative splicing of transcripts from a single $G_{o\alpha}$ gene.

The $G_{o\alpha}$ 3'-UTRs are highly conserved in bovine, murine, and human, as determined by direct sequencing of PCR-amplified DNA, and the relative abundance of individual $G_{o\alpha}$ mRNAs differs in different tissues. Current investigations focus on the role of the different 3'-UTRs in tissue-specific expression of $G_{o\alpha}$ and the stability of $G_{o\alpha}$ mRNA.

3. Structure and Function of Small GTP-binding Proteins: ADP-ribosylation Factors (ARF) and substrates for Clostridium Botulinum C 3 ADP-ribosyltransferase.

Cholera toxin catalyzes the ADP-ribosylation $G_{s\alpha}$, the stimulatory guanine nucleotide-binding protein of the adenylyl cyclase system. The ADP-ribosylation of $G_{s\alpha}$ is enhanced by ∿20 kDa guanine nucleotide-binding proteins termed ARF by Kahn and Gilman who originally described a membrane ARF. Subsequently, we purified one membrane and two soluble ARF proteins from bovine brain and showed that they interact directly with the toxin A_1 catalytic protein in a GTP-dependent fashion to alter its allosteric properties and increase activity. Although ARF proteins are widely distributed in animal cells, their physiological function is unknown. Our current studies approach this question in several ways.

Last year we isolated from a bovine retinal library, an ARF cDNA termed ARF2; we refer to a cDNA isolated by others from a bovine adrenal library as ARF1. Coding region nucleotide sequences of the two clones are 80% identical and deduced amino acid sequences 96%. We recently used oligonucleotides specific for ARF1 or ARF2 (coding region and 3'-UTR) and the ARF2 cDNA to investigate the size, number, and relatedness of ARF-like mRNAs in bovine tissues and brain of several species. These studies revealed that multiple ARF-like mRNAs, two of which (∿1.7 and ∿2.1 kb, respectively) correspond to the ARF1 and ARF2 cDNAs, are relatively ubiquitous. Nucleotide sequences of the coding regions of the ARF-like mRNAs are more highly conserved than are those of the 3'-UTRs.

In the past year, we have cloned two ARF cDNAs from a human cerebellum library. These are 84% identical in the putative coding regions, but there is little or no similarity in 3'- and

5'-untranslated regions. One clone encodes a protein with an amino acid sequence identical to bovine ARF1 (coding region nucleotide sequence 91% identical). It is referred to as human ARF1. The other encodes a protein different from ARF1 and bovine ARF2. This newly identified form we call human ARF3. Among the three different ARFs (human and bovine) there is 94-95% identity of amino acid and 79-84% identity of nucleotide (coding region) sequences. Most of the differences among deduced amino acid sequences are at the amino and carboxyl termini. All of the ARFs contain putative consensus sequences for GTP binding and hydrolysis. Because of these regions, they are significantly related to both the heterotrimeric G protein α subunits and to the ras p21 and ras-like proteins, although they appear not to share effector domains.

The human ARF1 cDNA, like bovine ARF1, hybridized with a ∿ 1.7 kb mRNA from human brain. The human ARF3 cDNA hybridized predominantly with a ∿3.7 kb mRNA whereas the bovine ARF2 corresponds to a ∿2.1 mRNA. At present, the cDNAs (or mRNAs) cannot be unambiguously identified with any of the three ARF proteins that we have purified from bovine brain. In vitro translation of mRNAs synthesized from human ARF1 and 3 and bovine ARF2 cDNAs produced proteins with mobilities on SDS-PAGE identical to that of a purified soluble ARF from bovine brain, which are being further characterized.

In preparation for mutagenesis studies, we used the baculovirus (BCV) system with Sf-9 insect cells to express the bovine ARF2 cDNA. Although neither cDNA or mRNA from uninfected cells (or from cells infected with wild type BCV) hybridized with the ARF2 cDNA, hybridization with Sf-9 genomic DNA was observed and all Sf-9 cells (infected and uninfected) contained an ARF-immunoreactive 20 kDa protein. After infection with different ARF-BCV contructs, all cells contained both DNA and mRNA that hybridized with ARF2 cDNA. In almost all cases, however, amounts of ARF protein were similar to those in non-infected cells or in cells infected with BCV. Infection with one recombinant ARF-BCV resulted in >200% increase in ARF protein, indicating that this may be useful for production of mutant ARFs. In addition, elucidation of the mechanism of regulation of ARF expression in these cells, which appears to be at the level of translation may provide clues to the function of ARF.

To obtain information about the interaction of ARF with cholera toxin that might aid in identifying cellular proteins related to its physiological function, isolation of a toxin-ARF complex was undertaken. It was found that in the presence of sodium dodecyl sulfate and a non-hydrolyzable GTP analogue stable aggregated complexes of ARF and the toxin catalytic protein (CTA1) could be isolated. The toxin in these complexes displayed a substrate specificity different from that of the uncomplexed toxin and ARF. Only a small fraction of ARF (or toxin) is capable of forming these stable complexes and it appears that this is not the result of alterations in ARF during purification.

The significance of this seemingly functional heterogeneity of ARF remains to be determined.

The ARF proteins resemble the ras oncogene products (p21) in size and structure at least in regions of sequence believed to be involved in GTP binding. It has been reported that injection of ras p21 into Xenopus oocytes induces maturation whereas injection of monoclonal antibody against ras p21 prevents insulin- but not progesterone-induced maturation. To determine whether ARF might have functional effects like those of ras p21, we examined the effects of microinjected ARF on progesterone- and insulin-stimulated maturation of Xenopus oocytes. In contrast to the findings with ras, maturation was inhibited by injection of a purified bovine brain ARF 3 to 8 h before exposure of oocytes to progesterone or insulin. ARF inhibition was dependent on progesterone concentration but not on insulin concentration. Inhibition was enhanced by concomitant injection of GTP and to a greater extent guanosine 5'-0-(3-thiotriphosphate)($GTP\gamma S$) which, itself in the absence of ARF, inhibited somewhat. The demonstration of this effect of ARF on both progesterone- and insulin-stimulated oocyte maturation may provide a clue to its physiologic role(s).

Clostridium botulinium C3 ADP-ribosyltransferase specifically ADP-ribosylates 25-22 kDa proteins in animal tissues. Injection of C3 ADP-ribosyltransferase into NIH3T3, and PC-12 cells causes morphological changes, whereas injection into Xenopus oocytes causes maturational changes similar to those induced by injection of activated ras. Recent reports indicate that the human rho A and C gene products, members of the ras superfamily of small guanine nucleotide-binding proteins, and rho-like proteins purified from bovine brain or adrenal are substrates for C3 ADP-ribosyltransferase. We isolated high and low molecular weight forms of rho-immunoreactive proteins (RIP) from bovine brain supernatant. ADP-ribosylation of the larger form (RIP-L) by purified C3 transferase was enhanced by GTP or nonhydrolyzable analogues; adenine nucleotides were ineffective. C3-catalyzed ADP-ribosylation of the smaller rho-immunoreactive protein (RIP-S) in the presence of $MgCl_2$ was insensitive to guanine nucleotides. RIP-S behaved as a 22 kDa protein on SDS-PAGE and gel filtration. RIP-L behaved as a 77 kDa protein on gel filtration and on SDS-PAGE as a 23 kDa protein. These findings are consistent with the possibility that RIP-L was isolated as part of a complex in which it exhibits sensitivity to guanine nucleotides. Characterization of this complex may provide dues to the physiological function(s) of the rho family of GTP-binding proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00622-12 CM

PERIOD COVERED
October 1, 1988 through September 30, 1989

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:	Lucia Monaco, Ph.D.	Special Volunteer	CM, NHLBI
	Sally J. Stanley	Chemist	CM, NHLBI

COOPERATING UNITS *(if any)*
H.-C. Chen, Endocrinology and Reproduction Branch, NICHD, NIH

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:
2.3	1.3	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 hormone-sensitive adenylyl cyclase system is the target of bacterial toxins that alter its activity by catalyzing the ADP-ribosylation of critical guanine nucleotide-binding (or G) proteins that couple stimulatory and inhibitory receptors to the cyclase catalytic unit. The stimulatory G protein, termed Gs, is ADP-ribosylated by cholera toxin and E. coli heat-labile enterotoxins, etiological agents in cholera and "travelers' diarrhea", respectively. The toxins transfer ADP-ribose to arginine and a critical arginine residue in the G protein. ADP-ribosyltransferases present in animal cells catalyze reactions similar to cholera toxin and the E. coli enterotoxins; these enzymes ADP-ribosylate free arginine and arginine residues in proteins, although they do not have, when isolated, the protein substrate specificity of cholera toxin. In animal tissues, ADP-ribosylation of arginine residues appears to be a reversible modification of proteins. Enzymes exist, termed ADP-ribosylarginine hydrolases, that cleave the ADP-ribose-arginine linkage, regenerating the free arginine residue. In this laboratory, hydrolases were purified from avian erythrocytes and rat brain and their regulatory, kinetic, and physical properties determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00627-11 CM

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
GTP-Binding Proteins and Adenylate Cyclase

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

PI:	Su-Chen Tsai, Ph.D.	Research Chemist	CM, NHLBI
Others:	Ronald Adamik	Biologist	CM, NHLBI
	Patrick P. Chang	Chemist	CM, NHLBI
	Mikako Tsuchiya, M.D., Ph.D.	Visiting Fellow	CM, NHLBI
	Mary Walker, Ph.D.	PRAT Fellow	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. Mol. Mechanisms	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
NHBLI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	2.9	1.4

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☐ (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 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 (or GTP analogue), by ∿20 kDa proteins, termed ADP-ribosylation factors or ARFs. In this study, an active complex of ARF with the cholera toxin A subunit (CTA) was isolated by gel permeation chromatography in the presence of SDS and GTPγS, but not GDPβS. Only a fraction of the ARF was capable of complex formation. The substrate specificities of complexed and non-complexed CTA differed; complexed CTA exhibited markedly enhanced auto-ADP-ribosylation. In the presence of GTPγS and DMPC/cholate no ARF-CTA complex was detected. A GTPγS-dependent ARF aggregate was observed, however, which differed from monomeric ARF in its effects on substrate specificity of the toxin. These studies support the hypothesis that in the presence of guanine nucleotide and either SDS or DMPC/cholate, ARF and toxin exist as multiple species which exhibit different substrate specificities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00634-09 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

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: 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

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Biochemical Physiology

INSTITUTE AND LOCATION

NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.6	0.7

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☐ (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 purified particulate cGMP-stimulated phosphodiesterase (PDE) exhibits a slightly greater subunit Mr than do soluble forms from calf liver or bovine brain, based on Coomassie staining of PAGE-SDS gels or Western immunoblots. Characteristics of cGMP-stimulated PDEs from bovine brain and calf liver were further investigated by photoaffinity labelling and peptide mapping (Cleveland et al., J. Biol. Chem. 252, 1102, 1977). The purified particulate PDE from bovine brain was directly photolabelled with 32 nM [32 P]cGMP or 700 nM [32P]cAMP in the presence of 200 μM IBMX, suggesting that [32P]cAMP was interacting with high affinity, presumably regulatory, sites. Photolabelling with [32P]cGMP was inhibited by much lower concentrations of cGMP (more than ∿100 fold) than of 8-Br-cGMP or cAMP. Exposure of bovine brain particulate and calf liver soluble PDEs to V8 protease generated 5-6 major peptides of similar sizes from both PDEs. Two unique peptides of Mr ∿27,000 from bovine brain particulate PDE and Mr ∿22,000 from calf liver soluble PDE were produced by treatment with V8 protease, suggesting at least some differences in amino acid sequences of the PDEs. Photolabelling of the brain particulate and liver soluble PDEs, followed by digestion with V8 protease, indicated that [32P]cGMP was associated with the same two peptides of ∿24,000 kDa. The [32P] cGMP was not associated with those peptides that differed in digests from brain particulate and liver soluble PDEs. Taken together, these findings suggest the existence of cGMP-stimulated PDE isoenzymes with similar (perhaps conserved) and different domains in brain particulate and liver soluble cGMP-stimulated PDEs. We further infer that cGMP binding sites are apt to be located in conserved regions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00636-08 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Hormonal Regulation of the 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.	Staff Fellow	CM, NHLBI
Others:	Vincent Manganiello, M.D., Ph.D.	Head, Sec. on Biochem. Physiology	CM, NHLBI
	Valeria Vasta, Ph.D.	Special Volunteer	

COOPERATING UNITS *(if any)*

Eva Degerman 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:
1.7	1.4	0.3

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☐ (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 insulin- and isoproterenol-activated, cGMP-inhibited, low Km cAMP phosphodiesterase (PDE) has been isolated and purified from rat and bovine adipose tissues and utilized for the production of specific polyclonal rabbit antibodies (anti-cAMP PDE). Incubation of intact rat fat cells with maximally effective concentrations of insulin (0.1 nM) or isoproterenol (100 nM) increased particulate (100,000 g) cAMP PDE activity by ∿50 and 100%, respectively. Under these conditions, in 32P-labeled rat adipocytes both hormones induced [32P]-phosphoserine phosphorylation of a predominant 135 kDa and a minor 44 kDa particulate protein immunoprecipitated by anti-cAMP PDE. Little or no phosphorylation was detected in the absence of hormones. The two phospho-proteins were identified as or closely related to cAMP PDE (with the 44 kDa likely a proteolytic fragment) by the following findings: 1) Immunoprecipitation of the 135 kDa phosphoprotein paralleled loss of enzyme activity in the supernatant, and preincubation of the anti-cAMP PDE with pure rat or bovine PDE selectively blocked the immunoprecipitation of the phospho-proteins. 2) These proteins copurified with cAMP PDE activity through DEAE-Sephacel chromatography and were obtained by highly selective affinity chromatography on CIT-agarose, the agarose-immobilized isothiocyante derivative of cilostamide, a specific and potent inhibitor of the particulate enzyme. These results indicate that catecholamines and insulin induce phosphorylation of cAMP PDE in fat cells through activation of cAMP-dependent protein kinase and, presumably, an insulin-sensitive serine protein kinase, respectively, and suggest that these phosphorylations are related to activation of the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00638-07 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

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)*

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	Inez Serventi, Ph.D.	Staff Fellow	CM, NHLBI
	Maria Nightingale	Chemist	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)*

H.-C. Chen, Endocrinology and Reproduction Research Branch, NICHD

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:
3.8	2.3	1.5

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☐ (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.)*

Choleragen catalyzes the ADP-ribosylation of Gsα, the stimulatory guanine nucleotide-binding protein (G protein) responsible for coupling hormone and neurotransmitter receptors to the catalytic subunit of adenylyl cyclase. This ADP-ribosylation results in increased cAMP production by inhibiting the intrinsic GTPase of Gsα, thereby maintaining the activated form of the G protein subunit which in turn activates the cyclase. Recently, the ADP-ribosyltransferase activity of the A1 protein of cholera toxin has been shown to be enhanced by ∿20 kDa guanine nucleotide-binding proteins called ADP-ribosylation factors (ARFs). These proteins allosterically activate the toxin catalytic protein. Two bovine cDNA clones, ARF-1 isolated from adrenal and ARF-2 from retina, exhibit 80% and 96% identity in their nucleotide coding regions and deduced amino acid sequences, respectively. Tissue and species distribution of ARF-like mRNAs was investigated by Northern analysis using cDNAs and oligonucleotide probes designed to distinquish between ARF-1 and ARF-2 cDNA coding and 3'-untranslated regions. Based on hybridization with specific oligonucleotide probes, all bovine tissues contain mRNAs of ∿1.7 and ∿2.1 kb that are related to ARF-1 and ARF-2, respectively. Hybridization of brain poly(A)+ RNA from different animal species with the ARF-2 cDNA under low stringency identified several bands varying in size between 0.9 to 3.7 kb. A ∿1.7 kb mRNA from all species hybridized with an ARF-1 coding region-specific oligonucleotide but not with a probe specific for the 3'-untranslated region. In contrast, ARF-2 probes analogous to the ARF-1 coding and 3'-untanslated region oligonucleotides hybridized with an ∿2.1 kb mRNA present only in bovine; other ARF-2-specific oligonucleotides hybridized with an ∿2.1 kb mRNA in rat, mouse, and human brain poly(A)+ RNA. Thus, there appear to be at least two distinct, yet highly homologous ARF genes expressed in a variety of tissues and species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00643-03 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters 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 professional 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
	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:
2.0	1.2	0.8

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☐ (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.)*

Goα is a guanine nucleotide-binding protein that is relatively abundant in neural tissues. It's function has so far not been demonstrated, but it is likely to be similar to that of other proteins of the same type, namely the transduction of certain hormonal signals to cells, or possibly, control of ion flux through cell membranes. The process is carried out through complex interactions with other components of the system, the β and γ protein subunits, guanine nucleotides, hormone receptors, the effector enzymes and their co-factors. In previous work, we achieved expression of bovine Goα in E. coli, partially purified the protein and studied its function in an in vitro system. It has been proposed for other guanine nucleotide-binding proteins that hormone receptors react with the Gα subunit at its carboxy end. As previously shown in this laboratory, carboxy-proximal cysteine in transducin-α is ADP-ribosylated by pertussis toxin. We have continued work on recombinant Goα producing specific mutations in the carboxy terminal region of the molecule to determine their effects on function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00645-02 CM

PERIOD COVERED: October 1, 1988 through September 30, 1989

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:	James Murtagh, M.D.	Md. Staff Fellow	CM, NHLBI
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	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:
1.9	1.9	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.)

Guanine nucleotide-binding regulatory (G) proteins regulate cell growth and function through the transduction of receptor-initiated signals across cell membranes. Go is a member of this family whose physiologic function has not been defined. Go exists in the brain in large quantities (as much as 1% of membrane protein); it can interact with rhodopsin and muscarinic receptors. Like other known GNPs, Go is a heterotrimer of α, β, and α subunits. Goα mRNAs of 4.0, 3.0, and 2.0 kb have been found in bovine brain and retina (Price et al., Biochemistry 38: 3803-3907, 1989). Size heterogeneity in Goα mRNA is a function primarily of differences in the 3'-untranslated regions (UTRs), as we have determined by four different methods. First, exhaustive screening of two different cDNA libraries from bovine retina identified Goα clones with identical coding regions, but divergent 3'-UTRs. Second, hybridization of mouse-human somatic cell lines with exon-specific oligonucleotides mapped both the Goα coding region and the divergent 3'-UTRs to the same small region of chromosome 16. Third, amplification of Goα mRNA by the polymerase chain reaction (PCR), produced a single coding region and three different 3'-UTRs. Finally, mapping of Goα mRNA with RNase H and site-specific oligonucleotides confirmed the PCR results and indicated that alternative splicing of a single Goα transcript gives rise to multiple RNA species. Current investigations focus on the role of these different 3'-UTRs in tissue-specific expression and stability of Goα mRNA transcripts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00646-02 CM

PERIOD COVERED
October 1, 1988 through September 30, 1989

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:	David Bobak, M.D.	Md. Staff Fellow	CM, NHLBI
Others:	James Murtagh, M.D.	Md. Staff Fellow	CM, NHLBI
	S. Russ Price, Ph.D.	Staff Fellow	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. 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:
2.1	1.6	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.)*

ADP-ribosylation factors (ARFs) are small guanine nucleotide-binding proteins that enhance the enzymatic activities of cholera toxin. Two ARF cDNAs, ARF1 and ARF3, were cloned from a human cerebellum library. Based on deduced amino acid sequences and patterns of hybridization of cDNA and oligonucleotide probes with mammalian brain poly (A)+ RNA, human ARF1 is the homologue of bovine ARF1. Human ARF3, which differs from bovine ARF1 and bovine ARF2, appears to represent a newly identified, third type of ARF. Hybridization patterns of human ARF cDNA and clone-specific oligonucleotides with poly (A)+ RNA are consistent with the presence of at least two, and perhaps four, separate ARF mRNAs in human brain. In vitro translation of ARF1, ARF2, and ARF3 produced proteins that behaved on SDS-polyacrylamide gel electrophoresis, similar to purified bovine brain ARF. Deduced amino acid sequences of human ARF1 and ARF3 contain regions, similar to those in other GTP-binding proteins, that are believed to be involved in GTP binding and hydrolysis. Our observations support the conclusion that the ARFs are members of a multigene family of small guanine nucleotide-binding proteins. Definition of the regulation of ARF mRNAs and of function(s) of recombinant ARF proteins will aid in the elucidation of the physiologic role(s) of ARFs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00647-01 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Synthesis of ADP-ribosylation Factor Protein in a Baculovirus Expression System

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

PI:	Barbara Kunz, Ph.D.	Visiting Fellow	CM, NHLBI
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	0.5

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☐ (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.)*

ADP-ribosylation factor (ARF), a 21 kDa GTP-binding protein of unknown function, present in most eukaryotic tissues is identified by its ability to enhance the ADP-ribosyltransferase activity of cholera toxin. An attempt was made to express a bovine retinal gene for ARF in the baculovirus (BCV) cloning system for subsequent mutagenesis and protein production. In the BCV system, insect (Spodopter frugiperda=Sf-9) ovary cells are infected with a recombinant baculovirus containing the gene to be expressed. Due to a viral promoter, large amounts of the desired protein are made. When Sf-9 cells were infected with BCV containing the ARF gene, only a small amount of ARF, above that which is made endogenously in the cells, was detected by Western blot.

From these preliminary observations, experiments were proposed to investigate the regulation of ARF expression in Sf-9 cells at the level of DNA, RNA, protein synthesis and protein degradation. Given the homology between ARFs from different species it was speculated that understanding the regulation of the bovine retinal ARF in Sf-9 cells might have a broader implication for the regulation of ARF expression in native tissues. To add credibility to this speculation, bovine retinal ARF and Sf-9 ARF were compared on the basis of antigenicity, enhancement of cholera toxin activity, and isoelectric point.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00648-01 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Effects of a Small GTP-binding Protein Injected into Xenopus Oocytes

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

PI:	Tristram Bahnson, M.D.	Md. Staff Fellow	CM, NHLBI
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	Ronald Adamik	Biologist	CM, NHLBI
	Joel Moss, M.D., Ph.D.	Head, Sec. on Mol. Mechanisms	CM, NHLBI
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SECTION

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NHLBI, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

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☐ (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 proteins are involved in many pathways including signal transduction mediated by hormones, drugs and neurotransmitters (heterotrimeric "G" proteins) and in the regulation of cell growth and proliferation (~21 kDa ras and ras-like proteins). ADP-ribosylation factors (ARFs) are ~20 kDa guanine nucleotide-binding proteins that were isolated based on their ability to enhance the enzymatic activity of cholera toxin. The physiological function(s) of ARF proteins is unknown. Injection of mRNA and protein into Xenopus oocytes has been used to investigate metabolic regulation and protein structure-function relationships. It has been shown that injection of ras p21 into Xenopus oocytes induces maturation whereas injection of monoclonal antibody against ras p21 prevents insulin- but not progesterone-induced maturation. To determine whether ARF might have functional effects like those of ras p21, we examined the effects of microinjected ARF on progesterone- and insulin-stimulated maturation of Xenopus oocytes. In contrast to the findings with ras, maturation was inhibited by injection of ARF 3 to 8 h before exposure of oocytes to progesterone or insulin. ARF inhibition was dependent on progesterone concentration but not on insulin concentration. Inhibition was enhanced by concomitant injection of GTP and to a greater extent guanosine 5'-O-(3-thiotriphosphate)(GTPγS) which, in the absence of ARF, inhibited somewhat at early time points. The demonstration of this effect of ARF on both progesterone- and insulin-stimulated oocyte maturation may provide a clue to its physiologic role(s).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00649-01 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Analysis of Functional Domains in GTP 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
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	Martha Vaughan, M.D.	Chief	CM, NHLBI

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H.-C. Chen, Endocrinology and Reproduction Research Branch, NICHD

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

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☐ (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 proteins (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. Transducin (or Gt), a G protein, couples the photoreceptor rhodopsin to the cyclic GMP phosphodiesterase in retinal rods. Like all G proteins, transducin is a heterotrimer composed of α, β, and γ subunits.

Recent evidence suggests that the amino-terminus of transducin α (Gtα) may be involved in binding the βγ subunit. To identify domains responsible for functional interactions between the subunits, monoclonal antibodies against Gtα were prepared. One monoclonal, MSN1, inhibited photolyzed rhodopsin-stimulated GTP hydrolysis catalyzed by Gtα in the presence of Gβγ. It also inhibited βγ-stimulated pertussis toxin-catalyzed ADP-ribosylation of Gtα. Proteolytic fragments of Gtα generated with V8 protease and trypsin were not immunoreactive with MSN1. Synthetic peptides derived from the amino terminal sequence of Gtα appear to inhibit reactivity of MSN1 with Gtα. MSN1 failed to cross-react with Gsα, Giα, or Goα. We conclude that the epitope recognized by MSN1 appears to be in the amino terminus of Gtα, and that there is immunological heterogeneity among the Gα subunits in this important domain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00650-01 CM

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.)*
Go Expression in the Baculovirus Cloning System

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

PI:	Kimberly A. Muczynski, M.D., Ph.D.	Md. Staff Fellow	CM, NHLBI
Others:	Barbara Kunz, Ph.D.	Visiting Fellow	CM, NHLBI
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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:
0.8	0.8	0.0

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☐ (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.)*

Goα, a GTP-binding protein of unknown function, present in relative high concentration in brain, has been cloned in this laboratory and expressed in E. coli. Go produced in E. coli, however, aggregated making purification and assessment of its GTPase activity impossible. It was proposed that aggregation might be due to properties of E. coli and that the gene expressed in a eukaryotic system capable of post-translation modifications, might produce non-aggregated Go protein. The baculovirus (BCV) cloning system uses insect (Spodoptera frugiperda = Sf-9) cells infected with virus containing the gene of interest to express large amounts of foreign proteins off of a viral promoter. The insect cells are capable of glycosylation, leader sequence cleavage, phosphorylation and extracellular secretion. Hence, the purpose of the following work was to express Go in the BCV system to see whether a non-aggregated, functional protein could be obtained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00651-01 CM

PERIOD COVERED

October 1, 1988 through September 30, 1989

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
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Leonard A. Smith, Ph.D., Head, Laboratory of Molecular Biology, Pathology, USAMRIID

LAB/BRANCH

Laboratory of Cellular Metabolism

SECTION

Molecular Mechanisms

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

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☐ (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.)*

Clostridium botulinium C3 ADP-ribosyltransferase specifically ADP-ribosylates 25-22 kDa proteins in a wide range of tissues. Injection of C3 ADP-ribosyltransferase into NIH3T3, and PC-12 cells causes morphological changes, whereas injection into Xenopus oocytes causes maturational changes similar to those induced by injection of an activated ras protein. The human rho A and C gene products, members of the ras superfamily of small guanine nucleotide-binding proteins, and rho-like proteins purified from bovine brain or adrenal are substrates for C3 ADP-ribosyltransferase. High and low molecular weight forms of rho-immunoreactive proteins (RIP) were isolated from bovine brain supernatant. ADP-ribosylation of the larger form (RIP-L) by purified C3 transferase was enhanced by GTP or nonhydrolyzable analogues > GDP; adenine nucleotides were ineffective. C3-catalyzed ADP-ribosylation of the smaller rho-immunoreactive protein (RIP-S.) in the presence of MgCl2 was insensitive to guanine nucleotides. RIP-S behaved as a 22 kDa protein on SDS-PAGE and gel filtration. RIP-L behaved as a 77kDa protein on gel filtration and on SDS-PAGE as a 23kDa protein. These findings are consistent with the possibility that RIP-L was isolated as part of a complex in which it exhibits sensitivity to guanine nucleotides.

Annual Report of the
Laboratory of Chemical Pharmacology
October 1, 1988 to September 30, 1989

A major objective of the Laboratory of Chemical Pharmacology has been to elucidate possible mechanisms by which drugs, other foreign compounds and their metabolites may evoke various kinds of toxicities. In recent years most of the resources of the Laboratory have been devoted to studying the mechanisms of immune reactions. One section has directed attention on mechanisms by which chemically reactive metabolites are synthesized in cells and react with cellular components to form putative antigens that may serve either as immunogens or as putative targets in immune mediated mechanisms of cellular toxicity. Another section has focused attention on the mechanisms by which antigens cause the release from mast cells of histamine and other mediators of immediate hypersensitivity reactions. During the past year, the section has focused on studies of various G-proteins, and adenine receptors that mediate and modulate the responses evoked by antigens. Another section has devoted its efforts toward mechanisms by which toxicants decrease intracellular ATP concentrations and subsequently cause cell death. The fourth section has initiated a program in which predictions obtained from theoretical calculations are compared with the metabolism of model substrates by cloned isozymes of cytochrome P-450. The objective of the program is to elucidate factors that govern the substrate of specificity and product formation by the various isozymes.

Mechanisms of Toxicity

Halogenated anesthetic gases. There is considerable evidence that the fulminant form of hepatitis caused by halothane in humans is mediated by an immune reaction. In support of this view, sera of patients manifesting halothane hepatitis have been shown to contain several antibodies that react to different extents with several trifluoroacetylated proteins (100 kDa, 76 kDa, 59 kDa, 57 kDa and 54 kDa) in liver of rats treated with halothane. The antibodies are not present in sera of patients who have received halothane but do not manifest hepatitis, nor are they present in sera of patients who have developed other hepatotoxicities. During the past few years we have concentrated efforts toward the purification, identification, and cloning of the various rat proteins. Last year we reported that the 59 kDa protein was a carboxy-esterase. During the past year, clones of the cDNA that encodes the esterase has been completely sequenced. A similar cDNA has been found in a human liver library and its sequence is approximately 70% homologous to that of the rat cDNA. The 57 kDa protein has been identified as protein disulfide isomerase. The 100 kDa and 76 kDa proteins, have been purified, but have not yet been identified. Two other trifluoroacetylated proteins have been recently detected in liver microsomes from rats treated with halothane: one is a 58 kDa protein; the other is a 62 kDa protein. The amino acid sequence of these proteins do not correspond to any known protein and therefore their functions are unknown.

The purified trifluoroacetylated proteins have been used in the development of an ELISA assay for detecting antibodies in sera of patients who manifest halothane-induced hepatitis. Preliminary results indicate that the assay is more sensitive and specific than methods that utilize as test antigens either trifluoroacetylated rabbit serum albumin or liver microsomes from rats treated with halothane. Once developed, the method is may prove useful in identifying patients who are sensitive not only to halothane, but also to other halogenated anesthetics, such as enflurane, the covalently bound metabolites of which also react with human antibodies.

Mechanism of heme destruction. Many substances, including allylic compounds that cause porphyria, and carbon tetrachloride, which causes hepatic necrosis, inactivate isozymes of cytochrome P-450 by causing the destruction of heme, probably through a free radical mechanism. In these reactions, not only is the heme converted to alkylated heme products that can be dissociated from the protein of the isozymes, but also it becomes covalently bound to the protein of the isozymes. Because the mechanisms by which these reactions occur are complex and difficult to elucidate, we have focused attention on a model in which reduced myoglobin converts trichlorobromomethane to trichloromethyl radical. Our studies suggest that the radical reacts with a specific vinyl group of the heme to form a dichloromethyl-heme radical that becomes covalently linked to the histidine 93 of myoglobin. The reduced form of the altered myoglobin is more easily oxidized by oxygen and hydrolyzed by trypsin. Thus the alteration of the hemoprotein caused by the trichloromethyl radical results in a "mechanism based activation" of myoglobin. These results with myoglobin are thus consistent with our previous finding that the number of moles of reactive metabolite bound to isozymes of cytochrome P-450 can exceed the number of moles of cytochrome P-450 present. In addition, our findings are also consistent with our previous results indicating that the covalent binding of heme to the protein of cytochrome P-450 results in rapid degradation of the proteins <u>in vivo</u>.

Mechanism of toxicity caused by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropuridine). MPTP causes a Parkinson-like syndrome in humans and primates. Studies by many laboratories, however, revealed that the toxic effects of MPTP are mediated by 1-methyl-4-phenyl pyridinium ion (MPP^+), which is formed from MPTP by monoamine oxidase B. In the past we have used hepatocytes as a model for studying the lethal effects of MPTP. We discovered that MPTP and MPP^+ causes a marked decrease in cellular ATP before the cells leak cellular enzymes, such as lactic acid dehydrogenase. During the past year, we have obtained similar results with cultures of a neuroblastoma-glioma hybrid cell, NG 108-15. We have demonstrated that in these cells, MPTP is converted to MPP^+ and that both MPTP and MPP^+ deplete cellular ATP before they cause leakage of lactic acid dehydrogenase. Moreover, the toxicity evoked by MPTP is blocked by a combination of inhibitors of monoamine oxidase A and monoamine oxidase B, indicating that the toxicity must be caused by MPP^+ rather than MPTP. We also found, however, that high concentrations (25 mM) of glucose prevented the toxicity in these cells by preventing the depletion of intracellular

ATP induced by MPTP and MPP+.

Maitotoxin-induced cellular toxicities. Maitotoxin is a polyhydroxy, polyether, disulfate that has a molecular weight of about 3424. In mice it is highly toxic with an LD_{50} of about 170 ng/kg. Last year we reported that in both hepatocytes and cardiac myocytes, maitotoxin enhances the influx of extracellular Ca^{2+}, which leads to a decrease in intracellular ATP and ultimately to the release of lactic acid dehydrogenase. We also reported that the toxicity could be decreased by lowering the extracellular Ca^{2+} or by the addition of high concentrations of verapamil, a voltage dependent calcium channel blocker. During the past year we have repeated these studies with several cell lines, including Reuber hepatoma, rat glioma C6 and a neuroblastoma-glioma hybrid, NG 108-15, and have obtained similar results.

Doxorubicin-induced myocardiotoxicity. Other investigators have suggested that doxorubicin causes myocardiotoxicity by a reduction-oxidation cycle that produces superoxide, hydrogen .peroxide and hydroxyl radicals. Studies with cultures of neonatal cardiomyocytes, however, have revealed that cell death is not prevented by deferoxamine, cysteamine or dithiothreitol, all of which are known to prevent the toxicity caused by the oxygen cascade evoked by menadione. Moreover, in contrast to the toxic effects of maitotoxin, the toxicity evoked by doxorubicin does not appear to be mediated by an influx of Ca^{2+}. Instead, the toxic effects of doxorubicin are enhanced by decreasing extracellular Ca^{2+} and by the addition of the Ca^{2+} entry blocking agents, nifedipine and fluarizine. The increase in the toxicity caused by these treatments appears to be associated with an increase in the amount of doxorubicin entering the cells, which suggests the presence of a Ca^{2+}-modulated transporter of doxorubicin in cardiac myocytes.

Mechanism of Mast Cell Activation

The mast cell, and its blood-borne counterpart, the basophil, play a primary role in mounting the immediate allergic response to IgE-directed antigens, whether originating from the type of drug interactions described here or from environmental sources. The activation of these cells leads to a rapid Ca^{2+}-dependent release of histamine and other mediators of immediate hypersensitivity reactions. The subsequent production of interleukins and growth factors by mast cells give them the additional capacity of recruiting and activating other inflammatory cells such as neutrophils, macrophages and eosinophils for the delayed response to antigens (Nature 339:150,1989). The study of the intracellular signalling mechanisms in mast cells is important not only for developing new therapies against these types of allergic reactions, but also because these cells are in themselves versatile experimental models for the study of Ca^{2+}-dependent secretion. During the past few years our studies with a cognate, the RBL-2H3 cell, have provided information that is relevant to the basic mechanisms of Ca^{2+}-dependent responses in many types of cells. Of particular promise has been our ability to permeabilize RBL-2H3 cells with full retention of functional responses to antigens. By manipulating the intracellular environment, we have gained considerable insight into

the mechanisms by which the different intracellular signals are generated and orchestrated.

Previous studies showed that the addition of antigen to RBL-2H3 cells activates both phosphoinositide-dependent and phosphoinositide-independent processes. The net effect is a rapid increase, followed by a slow decay in $[Ca^{2+}]_i$, a slow Na^+-dependent increase in pH and the activation of protein kinase C. Studies with permeabilized cells indicated that these 3 events provide the primary combination of signals required for secretion: At resting pH_i (7.05) elevated levels of Ca^{2+} (0.33 μM) is required for maximal secretory responses to antigen. At elevated pH_i, 7.4, however, 0.1 μM $[Ca^{2+}]_i$ is sufficient to sustain near maximal responses to antigen. Thus an increase in $[Ca^{2+}]_i$ to about 0.33 μM is required to initiate secretion, but once the pH_i is elevated, secretion is sustained at near basal levels of $[Ca^{2+}]_i$. Our studies, however, indicate that a third potentiating signal is generated, because antigen stimulated hydrolysis of phospholipids and secretion were inhibited equally by GDPβS and neomycin. This phosphoinositide-dependent signal could be the activation of protein kinase C, as both protein kinase C and the secretory response to antigen were lost after permeabilized cells were washed, but both were retained when the cells were briefly exposed to phorbol myristate (PMA) before permeabilization. Consistent with this idea, the secretory response correlates with the phosphorylation of light and heavy chains of myosin at sites that are phosphorylated specifically by protein kinase C.

Studies performed during the past year clearly indicate that Ca^{2+} is mobilized by the release of intracellular Ca^{2+}, induced by inositol 1,4,5-trisphosphate, and by influx of Ca^{2+} through a nonselective cation, G-protein-regulated, channel that operates only when the plasma-membrane is held in a polarized state. An influx of Ca^{2+} is necessary for both the amplification of antigen-induced stimulatory signals and the secretion from intact cells. Studies of G-proteins and the effects of adenosine analogs have also revealed that G-proteins have an important role in promoting coordinated signals within RBL-2H3 cells.

During the past year we have found that RBL-2H3 cells possess mRNA for $G\alpha_o$, $G\alpha_s$, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_z$ and possibly $G\alpha_t$. The mRNAs for $G\alpha_{i2}$ and G_z are especially abundant. Antigen stimulation causes the activation of a phosphoinositide-specific phospholipase C by a mechanism that is resistent to the effects of pertussis and cholera toxins (i.e. via a G_z-like reaction). The probable consequence of this reaction is the release of intracellular Ca^{2+} by inositol 1,4,5-trisphosphate to produce a modest increase in $[Ca^{2+}]_i$ and the initial activation of only one isozyme of protein kinase C (type II) by diacylglycerol. Diacylglycerol, however, is derived from the inositol phospholipids at only the early stages of the response to antigen. At later stages, phosphatidyl-choline is the major source of diacylglycerol. The actions of the phorbol ester, PMA, are exerted via the activation of the second isozyme (type III) of protein kinase C that is present in RBL-2H3 cells. The influx of Ca^{2+}, which markedly reinforces the increase in $[Ca^{2+}]_i$, is enhanced by a cholera toxin-sensitive, pertussis toxin-insensitive mechanism (i.e. via a G_s-like

reaction). The role of this influx in amplifying the stimulatory signals was apparent from studies with adenosine analogs, such as NECA. In contrast to antigen, NECA provoked a substantial, but transient, production of inositol phosphates and a transient increase in $[Ca^{2+}]_i$ by a mechanism sensitive to both pertussis and cholera toxins (i.e. via a G_i-like reaction), but it failed to produce a sustained influx of Ca^{2+} or secretion. NECA markedly enhanced all responses to antigen.

Unexpectedly, we found that phospholipase C could be activated by more than one G-protein through different receptors (i.e. the IgE-receptor and an adenosine-receptor), and that the receptors for adenosine on RBL-2H3 cells appear to be coupled to phospholipase C instead of adenylate cyclase. The adenosine-receptors have characteristics different from the classic A_1 and A_2-adenosine-receptors, as determined from studies with antagonists of these receptors. However, the xanthine antagonists blocked antigen-induced responses at micromolar concentrations by a mechanism that is distinct and separate from their action on adenosine receptors. This action may have therapeutic importance, because it may lead to the development of drugs that diminish the release of histamine and other mediators of immediate hypersensitivity reactions initiated by all IgE-directed antigens.

Biochemistry and Kinetics of Drug Metabolism

Whether a given isozyme of cytochrome P-450 catalyzes the metabolism of a compound to a given pattern of metabolites depends on several interrelated factors. The substrate specificity of an isozyme depends on the size, shape and binding groups of the active site. The pattern of metabolites depends on the orientations of the enzyme-substrate complex relative to the heme in the active site, the rigidity of the complex, and the energies required either to abstract a hydrogen atom from alkyl groups of substrate or to form tetrahedral carbon intermediates from unsaturated groups of the substrate. During the past year, we have performed studies designed to elucidate many of these factors.

Studies with cDNA clones that express isozymes of P-450. $P\text{-}450_b$ oxidizes testosterone at the 17-,16α- and 16β-positions. But a variant of $P\text{-}450_b$ developed by F. Gonzales (LMC,NCI) fails to form 16β-hydroxytestosterone even though it differs from the parent $P\text{-}450_b$ by only two amino acids. Only a few changes in the amino acid sequence at critical positions, therefore, can alter the orientation of the substrate at the active site of the P-450 enzymes and thus alter the pattern of metabolites formed.

Derivation of equations that describe the kinetics of P-450 and other enzymes. Studies of isotope effects on the rates of metabolite formation from various substrates can provide valuable information on the rigidity of the enzyme substrate complexes. Other investigators have derived equations that describe the isotope effects on Vmax and Vmax/Km parameters, but these frequently are inadequate to describe the isotope effect on these parameters for cytochrome P-450 systems. During the past year, we have derived equations for several plausible models of the mechanisms of cytochrome P-450 systems. The equations predict that if the

putative heme oxene intermediate of the substrate-cytochrome P-450 complex is formed irreversibly, there should be no apparent isotope effect on the Vmax/Km parameter for total metabolite formation, provided that all of the oxene intermediate is committed solely to the formation of metabolites. During the past year, however, we have found isotope effects on the total metabolism of testosterone by P-450_a without any isotope effect on Km. The findings thus indicate the presence of decoupling mechanisms, but stoichiometric studies of the rates of oxidation of NADPH and the rates of formation of H_2O_2 and metabolites suggest that water formation can account for only part of the decoupling. Nevertheless, most of the isotope effects on the pattern of metabolite formation is due to branched pathways.

The Vmax and Km parameters for a substrate in a mixture of substrates, such as racemic mixtures, cannot be obtained by standard techniques, such as Lineweaver-Burk plots. During the past year we have derived equations, based on the integration of the rate equation for competitive inhibitor models. These permit estimates of the Vmax and Km for each of the components in the mixture. The equations are also useful in describing the pharmacokinetics of racemic mixtures <u>in vivo</u>.

Quantum mechanical models. Last year we initiated quantum mechanical studies to estimate the tendency for hydrogen abstraction by cytochrome P-450. Our initial theoretical studies of the properties of various radicals suggest that hydrogen abstraction from ethane by p-nitrosophenoxy radical possesses the closest similarity to the ω-oxidation of fatty acids by cytochrome P-450. Estimates based on the quantum mechanical studies of the ability of the p-nitrosophenoxy radical to abstract hydrogen atoms from 18 putative substrates suggest that the activation energy may be described as a mathematical function of both heats of reaction and ionization potential of the product radical. Last year we reported a theoretical model that appeared to predict compounds which could be desaturated by P-450 systems. This year we used the model to predict that some isozymes of cytochrome P-450 might catalyze desaturation of 10,17-dihydrocarbanazepine, 9,10-dihydrophenanthrene, 10,11-dihydro 5H dibenzazepine, and 1,2-dihydronaphthalene. Studies on the metabolism of the first three of these substances by various P-450_b preparations verified the predictions.

Studies of theoretical models led to the prediction that pentachloroethane might undergo a dehydrochlorination reaction by some isozymes of cytochrome P-450. Since hypochlorous acid should be a product of this reaction, our finding that 2,6-dimethylphenol is converted to its p-chlorophenol derivative during the metabolism of pentachloroethane supports the validity of the prediction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00937-07 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

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	Special Volunteer	LCP	NHLBI
Other: Hydar Ali	Vist. Fellow	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. Penny Jones, LNP, NINCDS. 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:
1.4	1.4	

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.)*

The breakdown of membrane inositol phospholipids, the increase in concentration of cytosolic Ca2+and the secretionof histamine and other substances induced by antigens in RBL-2H3 cells were all highly dependent on the presence of high external concentrations of Ca2+. This dependency on external Ca2+ appeared to be related to the of Ca2+-influx through a nonselective cation channel that could carry Na+, Ca2+ and Mn2+ but was effectively blocked by low concentrations of Zn2+ and La2+. 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. In addition to the elevation of cytosolic Ca2+ levels, antigen stimulation resulted in a slow progressive increase in cytosolic pH which may serve as an additional synergistic signal for secretion by reducing the requirement for an elevated level of Ca2+.

Other studies have indicated that adenosine receptors in RBL-2H3 cells are coupled to the inositol phospholipid-specific phospholipase C via a G-protein that is altered by pertussis toxin and cholera toxin. The studies in total indicate the recruitment of several G-proteins in activated RBL-2H3 cells which in turn activate various phospholipases and ion channels to provide the necessary signals for secretion by these cells. Mapping of mRNA for G-proteins in these cells reveal the expected array of G-proteins except that the mRNA for Gi2α was particularly abundant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00962-07 LCP

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.)*
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: Lynn E. Butler, Ph.D., IRTA Fellow, LCP, NHLBI
John W. George, Chemist, LCP, NHLBI
Lance R. Pohl Pharm.D., Ph.D., Chief, Pharmacological Chemistry Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Dr. Brian Martin, NSB, NIMH

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:
2.25	1.25	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.)*

Our previous research has demonstrated that antibodies in the sera of halothane hepatitis patients react with trifluoroacetylated rat liver microsomal proteins of 100 kDa, 76 kDa, 59 kDa, 57 kDa , and 54 kDa. These findings suggest that similar trifluoroacetylated proteins are the immunogens responsible for the formation of the patient's antibodies and perhaps may lead to the subsequent development of hepatitis. In addition, the 59 kDa protein and the 57 kDa TFA protein have been identified as a carboxylesterase and protein-disulfide isomerase, respectively. During this year, another trifluoroacetylated protein of 58 kDa has been purified from rat liver and shown to react in an ELISA assay with serum from halothane hepatitis patients. The purified TFA proteins of 100 kDa, 76 kDa, and 57 kDa have also been employed as antigens in an ELISA assay for detecting patients sensitized to halothane. Preliminary results indicate that the assay is more sensitive and specific than is previously reported methods for the detection of antibodies in the sera of halothane hepatitis patients. This assay will be more completely evaluated in the future using each of the TFA proteins and their respective unaltered native forms. Polyclonal antibodies have been raised against the 76 kDa and 58 kDa proteins and will be used to characterize these proteins further in order to determine their role in halothane hepatitis and their physiological functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00967-07 LCP

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.)*
Regulation of cytochrome P-450 turnover

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

P.I.: Yoichi Osawa, Ph.D., Visiting Fellow, LCP, NHLBI
Others: Lance R. Pohl, Pharm.D., Ph.D., Chief, Pharmacological Chemistry Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Dr. Robert Highet LCH, NHLBI; Dr. Brian Martin, NSB, NIMH; Dr. Robert Feldman, DIR, DCRT; Angela Murphy, LC, NHLBI; Dr. Adriaan Bax, LCP, NIDDK

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: 1.25	PROFESSIONAL: 1.25	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 metabolism-based covalent bonding of the heme prosthetic group to its apoprotein has been shown to be an important event involved in the suicide inactivation of P-450 cytochromes, a family of hemoprotein monooxygenases that play a vital role in the metabolism of a variety of xenobiotics, including drugs and environmental pollutants, as well as endogenous compounds, such as steroids, prostaglandins, and fatty acids. Recently, we have developed a model system for this reaction consisting of ferrous myoglobin and $BrCCl_3$ to aid in delineating the mechanism of formation of these heme-protein adducts. It was found by amino acid sequence analysis, mass spectrometry, and ^1H-NMR spectrometry that histidine residue 93 of myoglobin, which normally binds to the heme-iron, and a CCl_2 moiety derived from $BrCCl_3$ become covalently bound to the prosthetic heme in this reaction. It is believed that this heme-adduct arises from the initial attack of a trichloromethyl radical on a specific vinyl group of the prosthetic heme. The altered heme-protein has greater reducing activity towards substrates, including molecular oxygen, as well as enhanced susceptibility to hydrolysis by trypsin. Both of these altered activities are apparently due to a change in the tertiary structure of the protein. This study has shown for the first time that the self-catalyzed alteration of the prosthetic group of hemoproteins does not always have to lead to an inactivated protein, but instead may result in the formation of a more reactive catalyst, a process we have called metabolism-based activation. This kind of activation of hemoproteins may play a role in the formation of toxic oxygen radicals in oxygen reperfusion injury in the heart, liver, and other organs and be responsible at least in part for the toxicities produced by many xenobiotics that are metabolized to radicals products, such as lipid peroxidation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00973-05 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

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	Special Volunteer	LCP	NHLBI
Other:	Michael A. Beaven	Chief, Section	LCP	NHLBI
	Wilford Saul	Chemist	LCP	NHLBI

COOPERATING UNITS *(if any)*
Drs. K.P. Huang and F. Huang, ERRB, NICHHD

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Cellular Pharmacology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

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 previous reports we described studies with activators of protein kinase C such as phorbol myristate (PMA) and Ca2+ ionophores that support the notion that activation of protein kinase C synergizes with a Ca2+-signal for secretion in RBL-2H3 cells. However, this was clearly not the only signal that synergizes with Ca2+ in antigen stimulated cells. For example, staurosporine, a potent inhibitor (Ki, 2nM) of protein kinase C, completely blocked secretion induced by the combination of A23187 and PMA but only partially blocked the antigen-induced secretion at high concentrations (>20nM). It was also apparent that the isozymes of protein kinase C were recruited differently in response to antigen or PMA. In addition, antigen stimulation results in the hydrolysis of not only the inositol phospholipids but phosphatidylcholine as well. The products from these reactions result in both common (e.g. the activation of the protein kinase C by diacylglycerol) and diverse (e.g. release of intracellular Ca2+ by inositol phosphates and production of lytic agent actions such as lysophosphatidic acid).

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00975-05 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Signal cascade mechanisms in histamine releasing and nonreleasing RBL clones

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

P.I. Hydar Ali	Vist. Fellow	LCP	NHLBI
Other: Michael A. Beaven	Deputy Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. Joel Moss, LCM, NHLBI and Dr. Reuben Sirganian, Natl. Institute of Dental Research

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SECTION
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INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	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.)*

Antigen mediated histamine release form RBL-2H3 cells is associated with substantial hydrolysis of membrane inositol phospholipids, mobilization of intracellular and extracellular Ca2+ the activation of protein kinase C and the phosphorylation of the light and heavy chains of myosin by protein kinase C. The relative importance, however, of each of these events in promoting the secretion of inflammatory substances of these cells has not been clearly defined. Studies with variants of the RBL-2H3 cells revealed several variants that were completely unresponsive to antigen but did release histamine when challenged with a combination of ionophore and phorbol ester. One such variant showed no or very little phosphoinositide hydrolysis in response to stimulants of GTP-regulatory proteins such as sodium fluoride in intact cells and GTPγS in permeabilized cells. The studies indicate that these variants will be useful in further defining the relationship between the stimulatory events and secretion in RBL-2H3 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00983-04 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of MPTP-induced cell death

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

P.I.: R. Krishnan Kutty	Sr. Staff Fellow	LCP	NHLBI
Other: Gopal A. Krishna	Section Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*
None

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SECTION
Drug Tissue Interaction

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	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.)*

1-Methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) is a well known inducer of Parkinson's disease in humans and monkeys. MPTP and its toxic metabolite MPP+ induced cell death in neuroblastoma-glioma hybrid, NG 108-15, cells as indicated by morphological changes, by the leakage of the cytosolic enzyme lactate dehydrogenase(LDH), and by the release of adenine nucleotides from cells prelabeled with [14C]adenine. The cell death was preceded by a marked reduction in ATP. The ATP depletion and cell death was prevented by the presence of high concentrations(25 mM) of glucose in the culture medium. 2'-Methyl MPTP, an analog of MPTP, was highly toxic to NG 108-15 cells. This could possibly be due to the ability of this compound to serve as substrate for both MAO-A and MAO-B and the fact that these cells contain a larger proportion of MAO-A. Both clorgyline and deprenyl, inhibitors specific for MAO-A and MAO-B, respectively, protected the cells from the toxic effect of 2'-methyl MPTP. Glucose was also found to have same protective effect against the cytotoxicity of 2'-methyl MPTP as MPTP. The conversion of MPTP and 2'-methyl MPTP into corresponding pyridinium metabolites, MPP+ and 2'-methyl MPP+, by NG 108-15 cells was shown to occur by analysis of the incubation media by plasma desorption mass spectrometry. Thus, the cell death is resulting from the depletion of ATP, the main energy source in the neuronal cells, by the inhibitory action of the pyridinium metabolites on the mitochondrial oxidative phosphorylation. The protective action of glucose could result from its ability to generate ATP via anaerobic glycolytic pathway and compensate for the ATP loss produced by the inhibition of oxidative phosphorylation by pyridinium metabolites. A similar mechanism involving ATP depletion could be responsible for the specific loss of dopaminergic neurons in MPTP-induced Parkinson's disease.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00985-04 LCP

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.)*
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; Dr. Alan Buckpit, Univ. California, David, CA

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:	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 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 must undergo activation by an isozyme of cytochrome P-450 to a reactive metabolite that causes destruction of pneumocytes. During the past year, we have partially purified an isozyme of cytochrome P-450 from rat lung that appears to be the principal isozyme in rat lung that bioactivates 4-ipomeanol. This isozyme belongs to the class of isozymes of cytochrome P-450 that are potentiated by cytochrome b5. Mouse lungs also contain an isozyme of cytochrome P-450 that activates 4-ipomeanol. But this isozyme appears to differ from the isozyme in mouse lung that converts naphthalene to a pneumotoxin. Whereas an antibody we have prepared against the mouse liver cytochrome P-450 mN inhibits the activation of naphthalene in mouse lung, it stimulates the activation of 4-ipomeanol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00987-03 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

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	PRAT 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)

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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 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. Both of these characteristics are being explored with the ultimate goal of predicting how changes in composition and structure of drugs will alter metabolic pathways. Methods used in the project include recombinant DNA techniques, determination of enzyme and isotope effect kinetics, and derivation of equations for plausible kinetic models which can be used to provide mechanistic interpretations for the observed isotope effects. During the past year, we have derived several equations for comprehensive kinetic models to describe the observed kinetic isotope effects on cytochrome P-450 catalyzed oxidations. The models include those in which the active oxygenating species is formed irreversibly with several metabolites being formed from rapidly or slowly interchangeable substrate orientations. They also include models in which the rate of substrate metabolism is uncoupled from the rate of electron flux through the system. We also have studied the metabolism of testosterone by clones of P-450a and P-450b and several chimeras developed by Dr Frank Gonzales and his associates (LMC,NCI). These studies have revealed that modification of a few amino acid residues in critical positions can markedly affect not only the turnover number but also the pattern of metabolites. Thus, minor alterations in the amino acid sequence may affect the orientations of the substrate-enzyme complexes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00990-03 LCP

PERIOD COVERED
October 1, 1989 through September 30, 1989

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
	Hydar Ali	Vist. Fellow	LCP	NHLBI
	Wilford Saul	Chemist	LCP	NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
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SECTION
Cellular Pharmacology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	.03

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.)*

Long-term treatment of RBL-2H3 cells with dexamethasone resulted in suppression of antigen-stimulated inositol phospholipid hydrolysis, mobilization of Ca2+ and release of arachidoni C acid and histamine. The extent of suppression was dependent on the concentration and time of exposure to dexamethasone. The suppressive action of dexamethasone was immediately reversed upon permeabilizing the cells. Studies in intact and permeabilized cells indicated that dexamethasone treatment did not result in permanent alteration of the activities of phospholipase C, or its associated G-protein, phospholipase A2 and protein kinase C. Moreover, in intact dexamethasone treated cells, the responses to stimulants that bypass the receptor stimulation, such as the combination of NaF or calcium ionophore with phorbol ester were not impaired. Of particular note, dexamethasone produced a significant enhancement of all responses (PI hydrolysis, increase in [Ca2+]i, secretion and arachidonic acid release) induced by 5'-N-ethylcarboxylmidoadenosine (NECA), an agonist of the A2 adenosine receptor. All these findings point to selective regulation of receptor-mediated responses at the level of the GTP-binding protein rather than the induction of protein(s) that suppress the activation of effector enzyme systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00991-03 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

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. Fellow	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:
1.5	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.)*

5'(N-Ethylcarboxamido)-adenosine (NECA), a membrane impermeable analog of adenosine, transiently stimulated a rat tumor mast cell (RBL-2H3 cells) line to cause release of inositol phosphates and an increase in levels of Ca2+ in the cytosol. It failed, however, to stimulate either uptake of 45Ca2+ or secretion. The effects of other agents that act on P1 or P2-purinergic receptors, suggested that NECA and related agonists acted via a previously undefined subtype of adenosine receptor. Although the order of potency of agonists suggested the involvement of a subcategory of P1-receptors, the A2-adenosine receptors, this seems unlikely because there was no indication of the involvement of adenylate cyclase nor were the actions of NECA blocked by antagonists of either A1 or A2-adenosine receptors. The fact that stimulation of inositol phospholipid hydrolysis by NECA in washed, permeabilized RBL-2H3 cells was blocked by pertussis toxin as well as by cholora toxin suggested instead that the NECA-sensitive receptor acted via a G-protein to activate phospholipase C. In contrast pertusis toxin did not block the antigen induced stimulation of the hydrolysis of inositol phospholipids. The increase in free Ca2+, the influx of 45Ca2+ and secretion from RBL-2H3 cells. In combination with NECA, all responses to antigen were markedly enhanced and this enhancement was selectively blocked by pertussis toxin. The ability of antigen, but not NECA, to provoke secretion may be dependent primarily on a cholera toxin-sensitive Ca2+-influx pathway that serves to amplify and sustain stimulatory signals for secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00993-03 LCP

PERIOD COVERED
October 1, 1988 through September 30, 19898

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.: Russell Ludowyke — Vist. Assoc. — LCP — NHLBI

Other:
Michael A. Beaven — Deputy Chief — LCP — NHLBI

COOPERATING UNITS *(if any)*

Dr. Robert S. Adelstein, LMC, NHLBI

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SECTION
Cellular Pharmacology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md. 220892

TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.1	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.)*

Our previous studies indicated that IgE-mediated stimulation of rat basophilic leukemia (RBL-2H3) cells results in the secretion of histamine and the phosphorylation of the heavy (200 kDa) and light (20kDa) chains of myosin. In unstimulated cells two-dimensional mapping of tryptic peptides of the myosin light chain revealed one phosphopeptide containing the serine residue phosphorylated by myosin light chain kinase. Following stimulation a second phosphopeptide appeared containing a serine residue phosphorylated by protein kinase C. Maps derived from myosin heavy chains show that unstimulated cells contain three major phosphopeptides. Following stimulation a new tryptic phosphopeptide appeared containing a serine site phosphorylated by protein kinase C. This year the stoichiometry of phosphorylation was confirmed by additional experiments. Before stimulation myosin light chains contained 0.4 mol phosphate/mol light chain all confined to a serine not phosphorylated by protein kinase C. Cells that secreted 44% of their total histamine in 10 min exhibited an increase in phosphate content at sites phosphorylated by protein kinase C from 0 mol phosphate/mol myosin subunit to 0.7 mol phosphate/mol light chain and to 1 mol phosphate/mol heavy chain. When RBL-2H3 cells were made permeable with streptolysin O they showed a qualitatively similar pattern of secretion and phosphorylation. The time course of histamine secretion from stimulated RBL-2H3 cells paralleled that of myosin heavy and light chain phosphorylation by protein kinase C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00994-02 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

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
Others:	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
NIH, NHLBI, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	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 clinical usefulness of doxorubicin is often hampered by the elevated myocardial toxicity of this potent antitumoral agent. In the present study, we investigated the mechanisms of doxorubicin-induced cardiotoxicity in an in vitro model of spontaneously beating cardiomyocytes, isolated from 2-3 day old rats and cultured for 8 days. Incubation with either deferoxamine (1 mM), L-acetylcysteine (1 mM) or dithiothreitol (1 mM) failed to prevent cell death at 24 hr and 48 hr after addition of doxorubicin (10 μM, 30 μM and 100 μM). In the same model of cultured cardiomyocytes, deferoxamine, cysteamine and dithiothreitol protected the cells from the damage induced by menadione (0.25 mM), which is known to induce oxidative stress through the formation of hydrogen peroxide. On the basis of these results, it seems unlikely that the formation of oxygen radicals generated by redox-active quinones plays a major role in the course of the myocardial toxicity induced by doxorubicin.

The role of extracellular Ca2+ and the influence of calcium entry-blockers on the myocardial toxicity of doxorubicin were investigated by incubating the cells either in the presence of different extracellular Ca2+ concentrations ([Ca2+]o) (0.1 mM, 1.36 mM or 3.0 mM) or with the calcium-entry blockers nifedipine and flunarizine. Reduction of [Ca2+]o or incubation with nifedipine or flunarizine (1 and 10 μM) increased both the accumulation of doxorubicin (10 and 100 μM) and its toxicity (30 and 100 μM). These data show that extracellular Ca2+ inhibits accumulation and toxicity of doxorubicin in myocardial cells by a mechanism that may not involve the voltage dependent calcium channels (VDCC), since the calcium-entry blocker flunarizine, which has a low affinity for the VDCC, increased cardiotoxicity of doxorubucin to a similar extent as nifedipine, a selective blocker of VDCC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00995-02 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of maitotoxin-induced toxicity

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

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:
0.8	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.)*

Maitotoxin, a highly potent marine toxin,has the ability to modulate various calcium-dependent cellular processes by increasing calcium influx into the cells. Treatment of different types of cell types with maitotoxin resulted in their death as indicated by the leakage of the cytosolic enzyme lactate dehydrogenase(LDH). The TD50 of LDH leakage ranged from 60 - 800 pM when rat liver, Reuber hepatoma, rat glioma C6, and neuroblastoma-glioma NG 108-15 cells were incubated with maitotoxin for a period of 24 hours. The cell death was not observed when calcium was omitted from the medium indicating that the presence of extracellular calcium was essential for the toxic effect of maitotoxin. A massive influx of calcium[45Ca] was observed in hepatoma cells following treatment with the toxin. The calcium influx preceded cell death as measured by LDH leakage. The LDH leakage in all the cell types studied was preceded by the release of adenine nucleotides into the medium. This was measured by monitoring the leakage of radioactivity from cells prelabeled with [14C]adenine. This parameter which is a measure of ATP depletion exceeded LDH leakage. Thus, the maitotoxin- induced cell death appears to be the result of ATP depletion caused by the inhibition of oxidative phosphorylation produced by a large influx of calcium into cells. A rapid increase in free cytosolic calcium was elicited when Fura-2 loaded rat liver ARL-15 cells were incubated with the toxin. This increase was inhibited in a dose-dependent manner by verapamil, a well characterized calcium channel blocker. These results indicate that maitotoxin causes cell death in a variety of cell lines by depletion of cell ATP caused by massive influx of calcium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00996-02 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of maitotoxin-induced crdiotoxicity

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

P.I.:	Giovanni Santostasi	Special Volunteer	LCP	NHLBI
Others:	R. Krishnan Kutty	Sr. Staff fellow	LCP	NHLBI
	Gopal Krishna	Section Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Drug Tissue Interaction

INSTITUTE AND LOCATION
NIH, NHLBI, 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.)*

Maitotoxin, the most potent marine toxin, is known to induce the uptake and the accumulation of Ca2+ into cells. In cultured cardiomyocytes, we previously demonstrated that the addition of maitotoxin results in the rapid influx of extracellular Ca2+, which evolves in few minutes and is followed by signs of cell damage and death. In the present study, the effects of maitotoxin on the cytosolic levels of free Ca2+ ([Ca2+]i) and on the intracellular stores of energy were studied in cardiomyocytes isolated from 2-3 day old rats. In freshly isolated, suspended cardiomyocytes, loaded with the fluorescent Ca2+ probe fura-2, maitotoxin induced a dose dependent increase in [Ca2+]i (EC50 0.3 ng/ml), which occurred with a lag interval of less than a minute and represented the earliest detectable effect of the toxin. Maitotoxin (1 ng/ml) induced a marked, almost complete depletion of intracellular ATP, which reached its peak after 30 minutes and preceded the signs of cell damage and death. All the effects of maitotoxin were prevented by incubating the cells in a low-Ca2+ medium, or in the presence of high concentrations of the calcium channel blocker verapamil. It thus appears that the maitotoxin-induced cardiototoxicity is secondary to an inordinate influx of Ca2+; the depletion of ATP appears to play a main role in the irreversibility of the cell damage that follows an increase in the cytoplasmic Ca2+.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00998-02 LCP

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.)*
Molecular biology of a 59 kDa Carboxylesterase

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

P.I.: Rochelle M. Long, Ph.D., PRAT Fellow, LCP, NHLBI
Others: Lance R. Pohl, Pharm.D., Ph.D., Chief, Pharmacological Chemistry Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Dr. Brian Martin, NSB, NIMH; Dr. Frank J. Gonzalez, LMC, NCI

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:
1.0	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.)*

Sera from halothane hepatitis patients have been shown to contain antibodies that react with several trifluoroacetylated proteins (100 kDa, 76 kDa, 59 kDa, 57 kDa, 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 patient's antibodies and raise the possibility that their formation may result in hepatitis. Recently, the 59 kDa protein has been identified as a carboxylesterase. In order to further characterize this protein, we have begun to clone it. Polyclonal anti-59 kDa antibodies were raised in rabbits and were used for screening cDNA libraries constructed in the expression vector lambda gt11. Several positive clones were isolated. A cDNA from a rat liver library containing a complete protein reading frame and putative active site regions for a carboxylesterase was completely sequenced. Clones obtained from a human liver library are currently being analyzed and should yield complete genetic and protein sequence data for a human carboxylesterase isoenzyme. These clones will be used in the future to determine the distribution, regulation, and physiological function of the liver microsomal carboxylesterases and to elucidate the immunodominant domains and role in halothane hepatitis of the trifluoroacetylated 59 kDa carboxylesterase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00999-01 LCP

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.)*
Trifluoroacetylated 62 kDa 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.: Lynn E. Butler, Ph.D, IRTA Fellow, LCP, NHLBI
Others: Jackie L. Martin, M.D., Prat Fellow, LCP, NHLBI
Rochelle M. Long, Ph.D., Prat Fellow, LCP, NHLBI,
Lance R. Pohl, Pharm.D., Ph.D., Chief, Pharmacological Chemistry Section, LCP, NHLBI

COOPERATING UNITS *(if any)*

Dr. Brian Martin, NSB, NIMH; Dr. Frank J. Gonzalez, LMC, NCI

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:
1.5	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.)*

Sera from halothane hepatitis patients have been shown to contain antibodies that react with several trifluoroacetylated proteins (100 kDa, 76 kDa, 59 kDa, 57 kDa, 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 patient's antibodies and perhaps may lead to the subsequent development of hepatitis. In this project, a new trifluoroacetylated protein (62 kDa) has been purified from liver microsomes of halothane treated rats and partially characterized. Its isoelectric point, glycosylation state, and the amino acid sequences of its N-terminal region and several internal peptides have been determined. No homology has yet been found with any published sequences of other proteins, suggesting that the 62 kDa protein may be an uncharacterized liver protein. In this regard, rat liver cDNA libraries are currently being screened for a clone of this protein using antibodies and synthetic oligonucleotides as probes. After isolating a full length cDNA clone of the protein, it will be expressed in cultured cells. This should help to elucidate its physiological function and potential role in halothane hepatitis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01000-01 LCP

PERIOD COVERED
October 1, 1988 through September 30, 1989

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	PRAT 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.)*

Because of their importance in drug inactivation and toxicity, a long term goal of this laboratory is to develop procedures for predicting the metabolic profile of a new drug or xenobiotic by the different cytochrome P-450 isozymes. The development of predictive procedures would involve an understanding of the factors that govern both the binding orientations of the substrates with the various isozymes and the susceptibility of various functional groups of the substrates to be oxidized. This study focuses on the latter. Considerable evidence indicates that the initial step in the oxidation of an alkyl group by the active oxygenating species is the abstraction of a hydrogen atom. In addition, a recently characterized desaturation reaction involves a second hydrogen atom abstraction. We, therefore, have used molecular modeling techniques to estimate the ability of various substrates to undergo hydrogen abstraction reactions at various positions. Synthetic and analytical methods are then used verify proposed mechanisms and validate theoretical models. Major findings include: 1) Quantum chemical calculations suggest that the p-nitrosophenoxy radical is capable of abstracting hydrogen atoms from many functional groups. The resulting transition states satisfy the geometric requirements for use as a model for the cytochrome P-450 active oxygenating species. 2) A linear relationship (R2=0.94)is obtained when calculated energies of activation for 18 reactions are regressed on both heats of reaction and ionization potential of the resulting radicals. Thus, the tendency for a functional group to be oxidized can be obtained by the relatively simple procedure of calculating the stability and ionization potential of the resulting radical. 3) Theoretical models have been developed that predict the tendency for P-450 mediated desaturation to occur. With these models, we have predicted the desaturation of 1,2-dihydronaphthalene, 9,10-dihydrophenanthrene, 7,8-dihydrocarbamazepine and iminodibenzyl and have verified the predictions by demonstrating desaturation with reconstituted P-450 systems. 4) A novel P-450 oxidative mechanism for direct dehydrohalogenation has been identified.

ANNUAL REPORT OF THE
LABORATORY OF CHEMISTRY
SECTIONS ON CHEMICAL STRUCTURE AND STRUCTURAL
NUCLEAR MAGNETIC RESONANCE
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

October 1, 1988, through September 30, 1989

The Laboratory employs 5 senior investigators, 3 staff fellows and 3 technicians covering the areas of mass spectrometry, nuclear magnetic resonance, x-ray crystallography, and organic synthesis. Its function is to separate, identify, and synthesize compounds occurring in biological matrices, elucidate their structures, and study their biophysical relationships. In addition, the members of the lab provide general advice on chemical and biophysical matters to NHLBI and NIH personnel.

J. Ferretti's group continues their study by NMR and energy calculation of conformations of biologically important peptides. The aim is to elucidate relationships governing receptor recognition and antigen-antibody relationships. Two receptor-selective tachykinin agonists, senktide and septide have been studied in detail, the first revealing a turn structure possibly responsible for activity. The second reveals a hydrogen bond to the terminal amide as an important feature perhaps responsible for interaction with the tachykinin receptor.

Molecular dynamics simulations of the conformational behavior obtained from complete interproton distance maps (<3.5Å) on both human epidermal growth factor and human transforming growth factor (including their cyclic and linear fragments) have revealed several possibly immunoreactive sequences. These were then synthesized and tested biologically. Preliminary results are positive.

Amino acid substitution in a 17 amino acid peptide from the envelope glycoprotein associated with HIV causes it to fail to discriminate between healthy and ill seropositive patients. Ferretti's group has detailed the conformational changes brought about by these substitutions in the hope that a relationship between structure and activity can be found. All such studies depend on precision in estimating internuclear distances from NMR data. The group has now found a relationship between digital resolution and error that should allow significant improvement in these determinations.

Using NMR, R. Highet has elucidated the structure of the active component of a chinese herbal medicine used to regulate "menstrual disorders". Surprisingly, it is the highly aromatic 2,5,8-triphenyl-1,4,7,9b-tetraazaphenylene. In a xenobiotic study involving the renal toxicity of bromobenzene, Highet has also elucidated the structure of a product from the action of glutamyl transferase and air on 2-bromo-glutathionylhydroquinone as a benzothiazoline-glycine adduct. Further work on the oxidative degradation of cytochrome P-450 using trichloromethyl radicals on myoglobin as a model has shown that the ring I vinyl group of the hemin residue is linked to a histidine of the protein.

In x-ray crystallography, J. Silverton has completed structures of many thalidomide optical isomers and polymorphs, triorthothymotide, triphenylphosphine oxide hemihydrate, an inhibitor of 17-α demethylase and a synthetic nucleotide. Thalidomide is assuming new significance in suppressing graft rejection, but only one of its enantiomers is teratogenic. This work reveals that one racemic form closely mimics the optically active form, so establishing purity to avoid teratogenicty will be difficult. Triorthothymotide is being considered as a medium for optical resolution through clathration so the effects of asymmetry on structure must be understood.

Silverton continues his studies on molecular and quantum mechanics with a theoretical study of the pK values of a series of potent carcinogens. These results may assist both in the prediction of carcinogenicity and a better understanding of their mode of action.

H. Lloyd, using GC-MS has examined environmental samples from regions of China that are notorious for the number of people affected by Kachim-Beck disease, a severe musculoskeletal problem affecting 3 million Chinese children with 20 million at risk. Several unusual organic compounds were found, but none likely to be involved in the erosion of cartilage observed in such cases. Dr. Lloyd has also discovered and elucidated the structure of a new terpene, 2,3,6-trimethyl-5-heptenol and a series of components in the deadly yellow-bellied sea snakes that are a problem in the Pacific. A series of unusual O-alkyl glycerols have been discovered in the scent glands of the American rattlesnake and their significance as pheromones is now being studied elsewhere. Finally, a large series of new synthetic biphenyls prepared at Meharry Medical College, several of which are in preclinical trials as AIDS agents are being studied by MS to establish their structures.

T. Jones continues to elucidate the structures and synthesize ant toxins of the dialkylpyrrolidine and indolizidine classes. These compounds are very closely related to the important neurotoxin derived by J. Daly (NIDDK) from poisonous frogs. Synthesis of

the indolizidines requires considerable attention to the stereochemistry of crucial steps and new procedures involving addition of Grignard reagents to indolizidinium ions have

afforded the desired products. Jones has also synthesized Lloyd's new terpene (see above) and a sufficient quantity of a series of saturated and unsaturated methyl ketones to allow biological testing a pheromones in garter snakes (see below).

H. Fales using electron impact, ion trap, and plasma desorption mass spectrometry has assisted in the solution of a wide variety of structural problems. Last year, one project involved the structure of a neuropeptide of 3600 kDa responsible for production of the pheromone in the serious economic pest, the corn earwig moth (only 50 femtograms were available for analysis). This has now been synthesized (H. Jaffe, USDA) and found to be completely active. Others include the natural killer cell factor (J. Ambrus, NIAID) whose structure is not yet apparent, and characterization of the gamma-glutamyl/fluorescein adduct with its associated peptide by PDMS (R. Levine, NHLBI). Using GC-MS he has also elucidated the structure of a metabolite of 3-fluoroglucose used by Balaban and Berkowitz (NHLBI) to trace by NMR the activity of glucose *in vivo*.

In development of the mass spectral technique, Fales and Mason, with Pannell and Pu (NIDDK) have clarified the phenomenon of self-chemical ionization that has plagued the use of the ion trap mass spectrometer. As a result they have been able to delineate completely for the first time fragmentation of a series of simple ketones and esters. Now it should be possible to obtain complete fragmentation pathway maps on substances examined by mass spectrometry, making structural elucidation of unknowns much easier. Currently the group is retrofitting the plasma desorption spectrometer with a reflectron built to specification in France. It is anticipated that this will lead to improvement in resolution from 500 to 10,000, allowing improved definition of proteins and better study of fundamental processes occurring in the ionization of large molecules.

R. Mason, with Fales and Jones, has elucidated the structures of the garter snake pheromone as a series of unsaturated long chain ketones. Besides their behavioral importance, these pheromones may be useful in controlling snake populations. An important example is the Australian brown tree snake that is causing great environmental destruction in the Pacific basin and is a danger now to Hawaii. We have acquired small numbers of these snakes and are investigating their pheromones in a like manner.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01003-15 CH

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.)*
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, CH:NHLBI

COOPERATING UNITS *(if any)*

Fredrick Cassels WRAMC-WRAIR
Govind Kapida Howard University

LAB/BRANCH
Laboratory of 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 identification of two naturally occurring materials is outlined. The saccharide sequence and conformation of a hexasaccharide isolated from the cell wall of oral microbes Streptococcus Sanguis. The determination of the structure of several indole alkaloids is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01003-17 CH

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 Chemistry

SECTION
Chemical Structure Section

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.)*

A variety of compounds have been studied using electron impact, plasma desorption and ion trap mass spectrometry. In many cases a complete structural analysis was possible; in others, useful information was obtained which led to structure when combined with data from other sources.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01004-19 CH

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.)
Characterization of Natural Products

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

PI: Lloyd, Helen A., LC, NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of 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.)

The work involves the structure determination of physiologically active compounds of plant and animal origin. Various types of chromatography (gas, thin layer, ion exchange, liquid) are used to isolate pure samples of unknowns. Structures are determined by chemical methods (degradation and synthesis) and with the aid of spectrometry (infra-red, UV, NMR and mass spectrometry).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 01005-13 CH

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, LC, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of 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, configuration** and **conformation** of **biologically-important** compounds. Compounds relevant to **synthesis, enzyme action, carcinogenisis** and **drug** action have been investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01006-18 CH

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.)*

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, LC, NHLBI
OTHER: I. Victor Ekhato, Ph.D. Visiting Fellow

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Structural Nuclear Magnetic Resonance Section

INSTITUTE AND LOCATION

NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	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.)*

Spectral studies have established the structure of an alkaloid of cinnamon cassia, of the product of the action of glutamyl transferase on a glutathionylhydroquinone, and of a product of trichloromethyl radical on myoglobin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 01027-07 CH

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.)

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., LC, NHLBI
Others: Han, K.-H.

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of 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.)

Because knowledge of the three-dimensional structure of peptides and small proteins is one of the foundations of protein design, protein engineering, and drug design, we have been studying the conformation of a series of biologically active peptides. Nuclear magnetic resonance spectroscopy is the technique employed to carry out structural studies in solution and other noncrystalline states which may be similar to the physiological environment. The peptides we have studied include the tachykinins, analogs related to epidermal growth factor, the actinomycins, and AIDS related peptides. As a result of these conformational studies we have proposed relationships governing receptor recognition and antigen-antibody interactions.
We have developed and implemented new NMR pulse methods for studying these systems and we are continuing to evaluate the errors in the measurements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 01029-02 CH

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.)
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, LC, NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of 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.)

Research was directed at the structure elucidation and synthesis of a number of saturated nitrogen heterocycles, as well as biologically active terpenes and lipids. The goals of this work are the development of new preparative methodologies and the elucidation of structure in cases where insufficient materials present to make this possible by the usual spectrometric means.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01030-01 CH

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.)*

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

COOPERATING UNITS *(if any)*

Louis Pannell, Ph.D. Research Associate
NIDDK

LAB/BRANCH

Laboratory of Chemistry

SECTION

Chemical Structure Section

INSTITUTE AND LOCATION

NIH:NHLBI:Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	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.)*

A series of sugars, including branched chain polysaccharide such as the antennary polysaccharide found attached to important proteins have been successfully analyzed by PDMS. The ion trap mass spectrometer has been studied to resolve conditions under which ion molecule reactions occur. Several series of simple ketones and esters have been completely mapped in their collision activation fragmentation mode.

ANNUAL REPORT OF THE
LABORATORY OF KIDNEY AND ELECTROLYTE METABOLISM
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1988 to September 30, 1989

Our continuing goal is to analyze the function of the kidney as a basis for understanding its pathophysiology and treating its disorders. Since the formation of urine depends upon the transport of water and solutes by kidney tubules, understanding renal function requires analysis of these cellular processes and of their integration in the kidney. Therefore, we are studying transport by cells in general and kidney cells in particular, as well as the mechanisms, hormonal and other, that support and control transport and metabolism.

Isolated segments of renal tubules.

In order to understand kidneys at a cellular and molecular level the functions of the different types of epithelial cells must be identified. Progress in this direction has relied heavily on the direct study of individual nephron segments. Each nephron segment has a different cell morphology and function. An important method (which originated in this laboratory) for directly studying the nephron segments is to dissect them and perfuse them individually in vitro. The findings of Knepper and his colleagues during the past year using this method are as follows:

Controlled acid-base excretion is a vital renal function to which the nephron segments in the renal medulla make an important contribution. The most important products are bicarbonate and ammonia. Therefore, we are measuring ammonia and bicarbonate metabolism and transport by the different nephron segments in isolation and are combining the results in a mathematical model.

Mejia, Flessner and Knepper are developing a mathematical model of acid-base transport in the kidney similar to previous models of the concentrating mechanism. The model will be used as a tool for integration of data derived from single tubule experiments to obtain a general view of how ammonia and bicarbonate are handled in the kidney.

Flessner and Knepper are carrying out isolated perfused tubule experiments to measure ammonia permeabilities in each renal tubule segment of the renal medulla. Recent results of Garvin, Burg, and Knepper have demonstrated that ammonium is actively absorbed in the thick ascending limb, suggesting the presence of a countercurrent multiplier for ammonia. Packer and Knepper have demonstrated, using tissue slice analysis techniques in rat kidneys, that there is a corticomedullary ammonium gradient with a maximum concentration at the papillary tip. The gradient is enhanced by systemic acid loading and diminished by systemic alkali loading.

Wall and Knepper studied control of luminal acidification in isolated perfused terminal inner medullary collecting ducts (IMCDs). The rate of bicarbonate absorption is increased by in vivo acid loading, in vivo deoxycorticosterone

administration, and in vitro vasopressin. An apparatus for measuring intracellular pH, using the fluorescent probe BCECF, was constructed. Studies have begun to determine the mechanism of acidification and the mechanism of control by vasopressin.

Chandhoke and Knepper investigated luminal acidification by the rabbit papillary surface epithelium. The epithelium is isolated from the kidney and mounted in an Ussing chamber for in vitro study. Net acid secretion into the apical compartment is linked to glycolysis, but is not due to lactic acid secretion. Studies are continuing to determine the mechanism of acid secretion. The acidification is proposed to be a means of inhibiting in vivo calcium stone formation in the pelvic fornices and secondary pouches which are relatively poorly-mixed.

Wright and Knepper are developing fluorescence methods for measurement of ammoniagenic enzyme activities in single microdissected renal tubule segments. Regulation of acid-base excretion by the kidney results largely from control of ammonium production by the renal tubule. Ammonium production is regulated as a function of systemic pH, but it is presently unclear how changes in blood pH can selectively increase the activities of the enzymes involved in ammoniagenesis. Studies are underway to address this problem. Ultramicro-assays have been developed for the measurement of glutaminase and glutamic dehydrogenase (GDH) activities in single microdissected renal tubules from rats. GDH activities are extremely high in the proximal convoluted tubule, proximal straight tubule and the distal convoluted tubule. GDH activity is unaltered by systemic bicarbonate loading, but increases markedly in proximal convoluted tubules (and not in other segments) in response to acidosis.

Controlled excretion of NaCl is another vital renal function which is being studied by methods similar to those described above. Terada and Knepper are continuing to study the mechanism and control of NaCl transport in the rat cortical collecting duct. Previous studies demonstrated that bradykinin and atrial natriuretic factor inhibit net NaCl absorption by about 50% with no effect on the transepithelial voltage, suggesting inhibition of an electroneutral NaCl transport pathway. To study this phenomenon further, they developed a new continuous-flow ultramicro-colorimetric technique, based on macrocyclic ionophore technology, for measurement of sodium and potassium concentrations in nanoliter volume samples. Studies using this technique revealed that hydrochlorothiazide, a diuretic which inhibits neutral NaCl transport in other epithelia, decreases net Na absorption by about 50% without an effect on the transepithelial voltage. Amiloride, an inhibitor of the conductive pathway (Na channel), decreases the Na flux by about 50% and completely inhibits the voltage. Ouabain, which poisons the sodium pump, inhibits the flux and voltage completely. The results are compatible with two parallel Na transport mechanisms in the apical membrane, a Na channel and a neutral NaCl pathway.

The transport of urea in the kidney is important for the urinary concentrating mechanism. Chou and Knepper continued to investigate the mechanism of vasopressin-stimulated urea transport across the epithelium of the terminal

inner medullary collecting duct (IMCD). The reflection coefficient for urea is one, indicating a lack of significant urea transport via the vasopressin-stimulated water channels in this epithelium. Thiourea transport is inhibited by phloretin suggesting that thiourea moves across the epithelium via the urea pathway. Thiourea transport is saturable. The evidence accumulated so far demonstrates that urea penetrates the IMCD via a specialized facilitated pathway which is most likely an integral membrane protein.

Sands, Bernard, Terada and Knepper developed a micro-assay for measurement of aldose reductase activities in single microdissected tubule segments. High levels of aldose reductase are present in inner medullary collecting ducts, thin ascending limbs, and inner medullary thin descending limbs. The activity increases in each of these segments toward the papillary tip. There is little or no aldose reductase in outer medullary segments, cortical segments, and in glomeruli.

Transport in model planar epithelia.

The transporters in some planar epithelia such as toad bladders, toad skins, and Necturus gall bladders are similar to those in parts of the nephron. These planar epithelia are easier to manipulate than individual nephrons, making them valuable models for studying the transporters. Spring and his colleagues have been studying solute and water transport by Necturus gall bladders and toad skins. They developed and used a combination of light microscopic, video, computer, and electrophysiologic methods to study cell volume and intracellular ions.

Flamion and Spring have developed and utilized a computer controlled, video, light microscope technique to measure the size and shape of the cells in isolated perfused rat medullary collecting ducts. By following the rate of change of cell volume in the first seconds after a step change in the concentration of the bath, they measured the osmotic water permeability of the basolateral membranes. These are the first precise direct measurements of this important parameter in medullary collecting ducts.

Furlong and Spring have analyzed the mechanisms of volume regulation by the gallbladder epithelial cells of the Amphibian, Necturus Maculosus. They found evidence for K and Cl channels in the basolateral cell membrane which were activated by swelling. They also showed that transport of glucose by the gallbladder resulted in a cell volume increase and subsequent volume regulatory decrease. The glucose-induced volume increase and recovery are the first physiologically relevant examples of volume regulation in gallbladder.

Siebens and Spring completed their studies of sorbitol movement across the cell membranes of papillary epithelial cells in tissue culture. These cells accumulate the sugar alcohol, sorbitol, in high concentrations when exposed to high osmolality media. Sorbitol is rapidly released to the medium by these cells when the medium osmolality is reduced to plasma levels. They showed that sorbitol exit is mediated by a specialized transporter which prefers sugar alcohols, rapidly activates when the medium osmolality is reduced and

inactivates when the medium osmolality is increased. Activation of the sorbitol exit pathway is blocked by the inhibitor quinidine. Garty and Spring have extended this work by preparing vesicles of the plasma membranes of papillary cells and analyzing the flux of sorbitol in these vesicles.

Kachadorian and Spring have continued their studies of the control of cell volume and membrane water permeability of the granular cells of the urinary bladder of the toad. This tissue has been extensively studied both as a model for the distal nephron of the kidney as well as an epithelium responsive to the action of antidiuretic hormone. Kachadorian and Spring showed that agents which directly increase intracellular cyclic AMP lead to an increase of the water permeability of the luminal membrane of the granular cells. The structural correlates of the time course and magnitude of this increased water permeability response has been extensively analyzed by both light and electron microscopy. The results support the previous hypothesis that tissue water permeability is regulated both at the luminal membrane of the granular cells and a more distal site.

Organic osmolytes.

Bacterial, plant, and invertebrate animal cells are known to accumulate compatible, osmotically active, organic intracellular solutes when their environment became hyperosmotic. These "osmolytes" help maintain the intracellular milieu because they do not perturb vital intracellular macromolecules, in contrast to sodium and potassium salts which in abnormally high concentrations do perturb macromolecules. Most mammalian tissues are not normally hyperosmotic and presumably do not express osmolytes. The exception is the renal inner medulla which is hyperosmotic because of the renal concentrating mechanism. In previous studies Burg and his colleagues identified the principal osmolytes in rat and rabbit inner medullas as sorbitol, inositol, glycerophosphorylcholine, and betaine.

Control of the cellular accumulation of these osmolytes is most readily studied in tissue culture. Burg, Bagnasco, and Nakanishi previously screened several renal cell lines in hyperosmotic media and found that cells that survived accumulated the same organic osmolytes previously found in intact renal medullas. The cell lines that we are now studying in detail are 1) MDCK, a dog kidney line, whose cells accumulate GPC, betaine, and inositol and 2) GRB-PAP1, a rabbit renal medullary cell line, whose cells accumulate all of these and also sorbitol.

Sorbitol previously was found to accumulate in GRB-PAP1 cells by synthesis from glucose, catalyzed by aldose reductase. Sorbitol accumulation and aldose reductase activity increase greatly when medium osmolality is elevated. The additional aldose reductase activity is explained by an increase in aldose reductase protein. We purified the enzyme and raised antiserum against it. Using pulses of ^{35}S-methionine, Moriyama, Garcia-Perez and Burg now find a much greater rate of synthesis of aldose reductase protein in cells grown in hyperosmotic medium. Induction of aldose reductase by hyperosmolality correlates with increased intracellular ionic strength and not with cell volume.

In order to determine whether transcription of aldose reductase increases, we have cloned and sequenced cDNA for aldose reductase from induced GRB-PAP1 cells. Using a RNA probe, prepared from the cDNA, we find that the abundance of aldose reductase mRNA is greatly increased in cells grown in media with a high osmolality. We plan to do nuclear run-ons to establish whether increased transcription is responsible. Finally, we have obtained a rabbit genomic library, which we are screening in order to clone the aldose reductase gene. We anticipate that the 5' flanking region will contain sequences important for regulation of transcription and cloning it will enable us to define the mechanism involved.

In order to relate the control of sorbitol accumulation in vivo to the findings in tissue culture, Cowley, Ferraris and Burg decreased rat renal medullary extracellular NaCl concentration by administration of furosemide or increased it by dehydration. Also, they tested rats with congenital diabetes insipidus before and after correction of their condition with vasopressin. Inner medullary aldose reductase activity, protein and mRNA and cell sorbitol all change in agreement with the results in cell culture, confirming the applicability of the cell culture results to the living animal.

We previously found that inositol accumulates in MDCK cells when medium osmolality is increased. The mechanism is increased transport into the cells from the medium. No accumulation occurs unless inositol is present in the medium. The transport is sodium-dependent and is inhibited by phlorizin. Vmax increases, but Km does not change. We hypothesize that the number of transporters increases because more are synthesized. In order to test whether transcription and translation actually are involved, we will determine the effects of actinomycin-D and cycloheximide on induction of transport. In order to characterize the inositol transporter, Kwon, Handler, Garcia-Perez and Burg are attempting to clone its cDNA by expression of mRNA in frog oocytes. Using sucrose gradient centrifugation, we have identified in the mRNA from induced cells fractions that increase sodium-dependent ^{3}H-inositol uptake into frog oocytes. We have prepared a cDNA library from the most active mRNA fractions and are using the oocyte expression system to screen mRNA transcribed from the cDNA clones. After isolating the cDNA coding the inositol transporter, we intend to prepare antibodies against the transporter, based on the amino acid sequence and use the antibodies to study synthesis and degradation of the transporter. RNA probes, prepared from the cDNA, would be used to test for osmoregulatory changes in transcription of the transporter.

Betaine, like inositol, is accumulated by MDCK cells in hyperosmotic medium because of increased transport into the cells. There is a large increase in Vmax. The mechanisms involved may be the same as for inositol and Robey, Garcia-Perez, and Burg are proceeding with cDNA cloning along similar lines.

Accumulation of GPC by MDCK cells in hyperosmotic medium is due to several processes: 1) increased synthesis from choline, 2) increased uptake of GPC from the medium, and 3) decreased catabolism of GPC by GPC diesterase. In contrast to the other osmolytes, GPC accumulation is triggered by high urea, as well as by high NaCl. Zablocki, Garcia-Perez and Burg are tracing the osmoregulated

metabolic pathway from choline to GPC by measuring the likely intermediates and by pulse-chase with 14C-choline. Once the rate limiting step is identified, we will attempt to isolate the corresponding enzyme and proceed as for aldose reductase.

In order to test whether accumulation of one osmolyte in hypertonic medium might affect the accumulation of the others, Moriyama, Garcia-Perez and Burg have systematically varied the concentrations of their metabolic and transport substrates. When betaine accumulation in GRB-PAP1 cells is increased by increasing medium betaine, cell sorbitol falls. Conversely, when cell sorbitol is decreased by inhibition of aldose reductase, cell betaine increases. Neither inositol or GPC is affected, nor are the other osmolytes much affected when inositol or GPC is varied independently. Thus, betaine and sorbitol seem to respond to a common signal, but inositol and GPC do not respond to that signal.

Accumulation of organic osmolytes in response to osmotic shock is a basic biological phenomenon previously identified in virtually all cells from bacteria to those in lower vertebrates. The present recognition of its vital role in renal medulla is the first indication that it is more than a curiosity in mammalian cells. Possible disorders of this system have not yet been investigated, but there are a number of poorly understood diseases of the renal medulla that should be considered. Further, the aldose reductase system, whose function we are unravelling in the renal medulla, is implicated in complications of diabetes in eyes, nerves and kidneys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01266-07 KE

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.)*

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:	Arthur Siebens	Senior Staff Fellow	LKEM, NHLBI
	Bruno Flamion	Visiting Associate	LKEM, NHLBI
	Timothy 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:	PROFESSIONAL:	OTHER:
5.2	5.2	

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.)*

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 rat, the toad bladder, cultured renal cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01282-03 KE

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.)

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	MSF	P.A Wright	VF
Y. Terada	VF	P.S. Chandhoke	NRSA
S.M. Wall	SSF	R. Mejia	Math
C.-L. Chou	V	M.B. Burg	Chief, LKEM
R. Packer	GW		

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Kidney and Electrolyte Metabolism

SECTION:

INSTITUTE AND LOCATION: National Heart, Lung and Blood Institute, NIH, Bethesda MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.2	7.2	

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 that atrial natriuretic factor (ANF) directly inhibits active NaCl absorption, and that ANF appears to block selectively a thiazide-sensitive NaCl transport pathway. Isolated perfused tubule studies have demonstrated that urea transport in the rat inner medullary collecting duct is saturable, is inhibited by chemical analogs of urea, is inhibited by phloretin, and is independent of the vasopressin-stimulated water permeability pathway. These results support the view that the urea transport occurs via a specialized urea carrier or channel. Experiments in terminal inner medullary collecting ducts have demonstrated luminal acidification and bicarbonate absorption which is increased by in vivo acidosis and by vasopressin in vivo. Enzyme assays in microdissected rat collecting ducts have demonstrated that aldose reductase is present at high activity in the terminal inner medullary collecting duct, in the thin ascending limbs and in the inner medullary descending limbs. Experiments in isolated rabbit papillary surface epithelia have demonstrated proton transport into the luminal compartment which is coupled to glycolysis, but is not due to the appearance of lactic acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01283-02 KE

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.)*: 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

Annual Report
Laboratory of Molecular Cardiology
National Heart, Lung, and Blood Institute
October 1, 1988 through September 30, 1989

The Laboratory of Molecular Cardiology is primarily devoted to studying the function, regulation and expression of the contractile proteins in vertebrate smooth muscle and nonmuscle cells. While our primary focus has been on the contractile protein myosin, we have also investigated proteins that have been shown to modify the activity of myosin such as actin, tropomyosin, caldesmon, calmodulin and the enzymes myosin light chain kinase and protein kinase C. The purpose of these studies has been three-fold: 1) to elucidate the role of myosin in vertebrate cell function, 2) to study the various factors that regulate the activity of smooth muscle and nonmuscle myosins, and 3) to clone the cDNA and genes for vertebrate nonmuscle myosins in order to have new tools to study 1) and 2).

Elucidation of the role of myosin in vertebrate cellular function: Previous work has suggested that contractile proteins in vertebrate cells play a role in (among other things) cytokinesis, cell motility and changes in cell shape. Recent work in our laboratory has concentrated on a possible role for myosin in the release of histamine and serotonin from rat basophilic leukemia cells (RBL-2H3 cells). These cells contain numerous granules which can release their contents extracellularly following aggregation of the receptors for IgE which are present on the surface of these cells. Our work has shown that antigenic stimulation of these cells leads to the de novo phosphorylation of the 200 kDa nonmuscle myosin heavy chain and the 20 kDa myosin light chain by protein kinase C. The time course for the phosphorylation of myosin by protein kinase C correlated with the time course for the release of histamine from these cells. Based on these studies we have developed a model for the role of myosin phosphorylation (by both myosin light chain kinase and protein kinase C) in the release of histamine and other agents from nonmuscle cells. We are presently testing our model using RBL-2H3 cells made permeable by treatment with streptolysin O. (This work is a collaboration between our laboratory and that of Dr. Michael A. Beaven, LCP, NHLBI.)

In order to study factors that regulate the activity of myosin, Dr. James Sellers and his associates have characterized two in vitro motility assay systems, developed originally in other laboratories, so that they could be used for experiments with vertebrate nonmuscle and smooth muscle myosin. One of these assays makes use of actin cables which can be exposed by microdissection of the alga Nitella. Vertebrate nonmuscle or smooth muscle myosin can be bound to latex beads and the movement of the beads along the actin cables can be quantitated. Dr. Sellers has used this technique to show that phosphorylation of the 20 kDa myosin light chain by myosin light chain kinase is required for bead movement. He has also used the assay to show that phosphorylation of the 20 kDa myosin light chain by protein kinase C does not affect bead motility.

Drs. Sellers and Matsudaira (M.I.T.) used a second in vitro motility assay system to authenticate the myosin-like properties of a 110 kDa protein purified from the microvilli of chicken intestinal brush border cells. They demonstrated that this protein is capable of translocating fluorescently-

labeled actin filaments at a rate that is comparable to other more conventional nonmuscle myosins.

We have also been studying the expression of nonmuscle myosins in vertebrate cells. Vascular smooth muscle cells express low levels of an mRNA coding for the nonmuscle myosin heavy chain in addition to the mRNA coding for the smooth muscle myosin heavy chain. When these muscle cells are placed in tissue culture, the nonmuscle form of the myosin heavy chain becomes the predominant form of myosin in these cells, during the period of rapid cell proliferation. When these primary culture cells reach confluence the smooth muscle isoform of the myosin heavy chain reappears and becomes equal in amount to the nonmuscle isoform. In order to explore the switches that regulate the appearance of these two different myosin isoforms, as well as to understand the function of the nonmuscle isoform(s) during the period of rapid cell proliferation, we cloned the cDNA for the nonmuscle myosin heavy chain from chicken intestinal epithelial cells.

We derived the entire amino acid sequence of the myosin heavy chain and found that it was expressed in all nonmuscle cells assayed as well as in smooth muscle cells. The laboratory of Tomoh Masaki (University of Tsukuba, Tsukuba, Japan) generously shared their data obtained from two partial cDNA clones they had isolated from chicken fibroblasts, one of which was identical to the clone we had sequenced. This allowed us to construct oligonucleotide probes based on both clones in order to study the expression of these two myosin isoforms in nonmuscle and smooth muscle cells. We found that the mRNA for the cDNA we had cloned predominated in fibroblasts, kidney and spleen cells whereas the second form of cDNA predominated in brain and vascular smooth muscle cells. Only one cell type examined, the chicken intestinal epithelial cell, contained mRNA encoding only one of the two isoforms. Whether the presence of this isoform by itself is related to the ability of these cells to proliferate rapidly is presently under study.

We have also isolated cDNA and genomic clones for human nonmuscle myosin. For cDNA clones we screened a human lymphocyte library. Similar to the chicken, there also appears to be at least two genes encoding nonmuscle myosin in the human. We substantiated this by isolating cDNA clones that showed differences in their nucleotide sequences over several hundred kb. At least one of these human cDNA clones showed an unusual secondary structure at the 5' end of the cDNA suggesting that it might be regulated at the level of translation.

The presence of at least two genes for nonmuscle myosin in vertebrate cells raises a number of interesting possibilities, including the probable existence of at least two nonmuscle forms of the myosin heavy chain which appear to be differentially expressed and which may play distinct roles in cellular function both in health and disease. Below, we briefly summarize all of the ongoing projects in the Laboratory of Molecular Cardiology.

Growth and Differentiation of Smooth Muscle and Nonmuscle Cells (S. Kawamoto, Z01 HL 01655-14 MC). Using two oligonucleotide probes that are specific for two different mRNAs that encode vertebrate myosin heavy chains we studied the expression of the myosin heavy chains in a number of tissues and at different stages of development. Both nonmuscle mRNAs were present in all tissues examined (spleen, liver, kidney, brain, fibroblasts and muscle)

with the exception of intestinal epithelial cells. These cells only contained mRNA for myosin heavy chain-A, for which we have obtained a full-length cDNA clone encompassing the entire coding region (see Z01 HL 04208-03). Serum stimulation of cultured vascular smooth muscle cells and fibroblasts appears to increase the level of mRNA for myosin heavy chain A and decreases the level for myosin heavy chain B. These studies are important because they suggest that each of the two myosin isoforms may play a unique function in nonmuscle cells, for example, one isoform might play an important role in cytokinesis (e.g., the isoform up-regulated by serum) and the second isoform might regulate cell shape.

Myosin and Caldesmon Phosphorylation in Nonmuscle Cells (J.M. Hettasch, J.R. Sellers, Z01 HL 01785-10 MC). Caldesmon is a calmodulin and actin-binding protein that is thought to play a role in modulating contractile activity in smooth muscle and nonmuscle cells. This protein has been shown by others to be a substrate for protein kinase C in platelets that have been treated with phorbol esters. Dr. Hettasch investigated whether the phosphorylation of caldesmon was altered during activation and inhibition of platelet function by known physiological agonists and antagonists. She found that, although she could increase the state of phosphorylation of the myosin light chain and the 47 kDa protein (a known substrate for protein kinase C) by treatment of human platelets with thrombin, she did not alter caldesmon phosphorylation. These studies are important because they demonstrate that physiological agonists (and antagonists) which stimulate and inhibit human platelet function do not alter caldesmon phosphorylation and, thus, suggest that phosphorylation of this protein does not regulate actin-myosin interactions in human blood platelets.

Role of Phosphorylation as a Regulatory Mechanism in Muscle Contraction (S. Umemoto, J.R. Sellers, Z01 HL 01786-10 MC). Two different *in vitro* motility assay systems have been characterized for use with myosin from smooth muscle and nonmuscle cells. These systems appear to be *in vitro* models of muscle shortening under unloaded conditions. The systems are useful because they measure a parameter of myosin activity which is different from that measured by the actin-activated MgATPase activity. Dr. Umemoto has used the *in vitro* motility system to study the effects of multiple phosphorylations of the myosin molecule on the movement of latex beads coated with both smooth muscle and platelet myosin. Although he was able to confirm a role for myosin light chain kinase phosphorylation in bead movement, phosphorylation of myosin by protein kinase C did not appear to affect the movement of beads coated with myosin. He also studied the effects of using myosin that had been modified by the agent N-ethylmaleimide on bead migration. These studies are important because they add an important new parameter by which we can study factors that regulate the activity of smooth muscle and nonmuscle myosins.

Phosphorylation as a Regulatory Mechanism (M.A. Corson, Z01 HL 04202-08 MC). We wished to learn whether the neurotransmitter peptide, Substance P, is capable of causing a contractile response in tracheal smooth muscle strips that is independent of its ability to cause release of acetylcholine. Dr. Corson found that concentrations of Substance P of 10 μM or greater resulted in direct activation of tracheal smooth muscle strips. He also found that under conditions where Substance P activates smooth muscle strips directly, the tension and myosin phosphorylation responses qualitatively resemble those

observed for muscarinic cholinergic agonists. This work is important because it reconfirms the role of myosin light chain phosphorylation in initiating smooth muscle contraction using a non-cholinergic, non-adrenergic agonist.

Structure, Function and Expression of Myosin Light Chain Kinase (T.L. Cornwell, Z01 HL 04205-07 MC). We are interested in investigating the role of the enzyme myosin light chain kinase in the function of nonmuscle cells. Specifically, we wish to know if this enzyme is necessary for nonmuscle cells to undergo shape change, for movement of these cells and for cell division. Dr. Cornwell has prepared fluorescently labeled affinity-purified antibodies as well as fluorescently labeled myosin light chain kinase which, in collaboration with Dr. Lansing Taylor (Carnegie-Mellon University), she will introduce into living cells. The location of the injected material in moving cells will be monitored. In addition, modified enzyme forms, e.g., calmodulin independent myosin kinase, myosin kinase that has previously been phosphorylated by cAMP-dependent protein kinase etc., will be introduced into nonmuscle cells in an effort to see if modification of this kinase has an effect on cell function.

Regulation of Genes for Contractile Proteins in Muscle and Nonmuscle Cells (L. Weir, M. Simons, Z01 HL 04207-04 MC). We have been interested in obtaining the genes responsible for the expression of nonmuscle myosin in human cells. To this end Drs. Weir and Simons obtained genomic clones for two different nonmuscle myosin heavy chain genes as well as cDNA clones that appear to encode two different nonmuscle myosin heavy chains. At least one of these myosins seems to be regulated at the level of translation through an unusual secondary structure found in the 5' untranslated region.

The cloning of two different cDNAs for nonmuscle myosin may add to our understanding of the mechanism responsible for the onset of atherosclerosis. An early step in this disease is the proliferation of the smooth muscle cells that are present in the wall of the blood vessel. Since nonmuscle myosin is thought to be involved in cytokinesis it may be that an early step in smooth muscle cell proliferation requires an increase in the expression of one of the two nonmuscle myosin isoforms. We are now in a position to study the level of the two nonmuscle mRNAs present in the smooth muscle cells of normal and atherosclerotic blood vessels.

Cloning of the cDNA for a Nonmuscle Myosin Heavy Chain (M.A. Conti, S. Kawamoto, R.V. Shohet, D.A. Brill, Z01 04208-03 MC). We have completed the cloning of the cDNA encoding the amino acid sequence of a chicken intestinal epithelial cell myosin heavy chain. We now wish to produce mutated forms of the myosin heavy chain in an effort to study myosin function in nonmuscle cells. For example, as described elsewhere in this Summary, nonmuscle myosin heavy chains contain unique serine residue(s) that can be phosphorylated by protein kinase C *in situ*. The time course of this phosphorylation appears to correlate with the release of histamine from RBL-2H3 cells, suggesting, but not proving, a causal relationship. By producing and expressing an altered form of the nonmuscle myosin heavy chain that cannot be phosphorylated by protein kinase C we plan to test whether or not this phosphorylation is required for histamine release from RBL-2H3 cells.

Myosin Phosphorylation and Basophil Secretion (I. Peleg, Z01 HL 04209-03 MC). We have developed the following model that relates phosphorylation of

RBL-2H3 (rat basophilic leukemia) cell myosin to the release of histamine from these cells. RBL-2H3 cells grown in culture contain 0.4 mol phosphate/mol myosin light chain at serine-19, the residue known to be phosphorylated by myosin light chain kinase. Ordinarily, this extent of phosphorylation should be sufficient to transport the granules to the cell surface where they can release their granule content (i.e., histamine, serotonin, etc.). However, the presence of a cortical ring of contractile proteins may prevent the granules from reaching the cell surface. Activation of protein kinase C by, for example, aggregation of the receptors for IgE, results in phosphorylation of the myosin heavy chain and light chain by protein kinase C and the consequent dissociation of the cortical myosin filaments. The granules can now approach the cell surface and release their contents. We are presently carrying out experiments to test this model.

Myosin Phosphorylation and the Regulation of Contractile Activity (C.A. Kelley, Z01 HL 04210-02 MC). Although phosphorylation of the 20 kDa light chain of myosin by a number of kinases has been studied by numerous laboratories, it is only recently that phosphorylation of the smooth muscle myosin heavy chain has received attention. Recently, Dr. C. Kelley has demonstrated that both casein kinase II and the Ca^{2+}/calmodulin-dependent protein kinase II can phosphorylate aorta smooth muscle myosin heavy chain *in vitro*. Moreover, the sites phosphorylated by these two kinases appear to be the same as the sites phosphorylated *in situ* as determined by labeling of intact aorta smooth muscle cells with radioactive inorganic phosphate.

In an effort to understand the function of myosin heavy chain phosphorylation, Dr. C. Kelley has treated primary cultures of aorta smooth muscle cells with a number of agents, including growth factors. Preliminary results with one of these growth factors suggest that it may alter the level of phosphorylation of both the myosin heavy chain and light chain.

Characterization of a Vertebrate Myosin I (J.R. Sellers, Z01 HL 04212-01 MC). Myosin-like properties of the 110 kDa protein found in the intestinal epithelial cells of the chicken brush border microvilli have been demonstrated using an *in vitro* motility assay. Dr. J. Sellers has demonstrated that this protein is capable of translocating fluorescently-labeled actin filaments at 0.09 μm/s at a calcium concentration of 10 μM. This study is important in that it helps to define and characterize a small myosin-like molecule in vertebrate cells, similar to *Acanthamoeba* myosin I. It also raises the important question as to whether other forms of these "minimyosins" may exist in vertebrate nonmuscle cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01665-14 MC

PERIOD COVERED
October 1, 1988 through September 30, 1989

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:
0.9	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.)*

This laboratory has isolated cDNA clones for a nonmuscle myosin heavy chain (MHC), which encode the entire amino acid sequence, from a chicken intestinal epithelial cell library (cDNA size is 7.1 kb). The laboratory of Masaki (University of Tsukuba, Japan; (Katsuragawa et al. [1989] Europ. J. Biochem., in press)) has recently isolated two different cDNA clones (2.8 kb and 0.9 kb) which encode part of the amino acid sequence for two different nonmuscle MHCs from a chicken fibroblast library and provided evidence for two nonmuscle MHC genes. The 0.9 kb clone was a portion of the cDNA clone isolated by this laboratory, whereas the 2.8 kb clone showed differences throughout the sequence with 73% nucleotide identity (79% amino acid identity).

We synthesized two oligonucleotides complementary to the two different mRNAs in an area of relative sequence dissimilarity and confirmed that each hybridized to different DNA fragments on a genomic Southern blot. We studied the expression of the two nonmuscle MHCs in a number of tissues at different stages of development using Northern blots. Both nonmuscle MHC mRNAs were present in all tissues examined (spleen, liver, kidney, brain, fibroblasts and muscles) with the exception of intestinal epithelial cells. Intestinal epithelial cells appeared to express a single nonmuscle MHC mRNA and this mRNA (for MHC-A) was predominant in spleen and cultured fibroblasts. The mRNA for MHC-B was predominant in brain and aorta. The relative distribution of mRNAs among the different tissues is similar during development. The effects of serum on the expression of the two mRNAs were studied using cultured chicken vascular smooth muscle cells and fibroblasts. Serum stimulation causes an increase in the level of MHC-A mRNA whereas it causes a decrease in the level of MHC-B mRNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01785-10 MC

PERIOD COVERED
October 1, 1988 through September 30, 1989

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)*

Joann M. Hettasch, Ph.D., Staff Fellow, LMC, NHLBI
James R. Sellers, Ph.D., Research Biologist, LMC, NHLBI
Estelle V. Harvey, Biologist, LMC, NHLBI

COOPERATING UNITS *(if any)*

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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	1.3	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.)*

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 experiments investigating the possible regulatory role caldesmon plays in actin-based activities have demonstrated that purified caldesmon inhibits actin-activated myosin ATPase activity. This inhibition can be reversed in the presence of calcium and calmodulin. Although calcium may influence the ability of caldesmon to exert its effect, other evidence suggests that phosphorylation of caldesmon may be a regulatory mechanism.

In vitro studies have shown that the inhibitory effect of caldesmon on actin-activated myosin ATPase activity can be reduced following phosphorylation of caldesmon by Ca2+/CaM-dependent protein kinase. In addition, it was also demonstrated that caldesmon can associate with myosin and that this interaction is prevented when caldesmon is phosphorylated. More recently, it was demonstrated that protein kinase C phosphorylates caldesmon in intact platelets when these cells are stimulated with phorbol ester. These observations suggest that phosphorylation of caldesmon may play a role in the cytoskeletal and/or contractile changes that occur during platelet activation (i.e., shape change and secretion). In this regard, this project has examined whether phosphorylation of caldesmon is altered during activation and inhibition of platelet function. Caldesmon was immunoprecipitated from platelet tissue extracts prepared from cells which had been labeled with 32P and treated with thrombin, collagen or prostacyclin. It was found that phosphate incorporation into caldesmon was not altered during activation or inhibition of platelet functional change, suggesting that phosphorylation of caldesmon does not regulate actomyosin interactions in human blood platelets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01786-10 MC

PERIOD COVERED: October 1, 1988 through September 30, 1989

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

Role of Phosporylation as a Regulatory Mechanism in Muscle Contraction

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

Seiji Umemoto, M.D., Ph.D., Visiting Fellow, LMC, NHLBI
James R. Sellers, Ph.D., Research Biologist, LMC, NHLBI
Estelle V. Harvey, Biologist, LMC, NHLBI
William A. 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:
2.2	1.5	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.)

To understand the mechanism of smooth muscle contraction, we have been using two in vitro motility assay systems, the Nitella-based motility assay system and the fluorescently labelled actin assay. These in vitro motility assay systems measure the relative movement of myosin and actin and are thought to be correlates of the unloaded shortening velocity of muscle fibers. To establish these systems as quantitative assays for the movement of myosin and actin which are the two major proteins directly involved in smooth muscle contraction, we examined the effects of altering assay conditions such as ionic strength, pH, magnesium chloride and ATP concentrations on the relative movement of phosphorylated smooth muscle myosin and actin, and compared some of the results of the Nitella-based in vitro motility assay to those obtained using the fluorescently labelled actin assay. We also examined the capability of myosins to generate force in the Nitella-based in vitro motility assay using N-ethylmaleimide (NEM) modified skeletal muscle myosin as a load. We found that the relative movement of myosin and actin in the two assays was dependent on buffer conditions. The effect of ionic strength on the movement was similar in both assay systems. The effect of magnesium chloride on the movement was different in the two assays. We also found that more NEM-modified myosin was required to bring about 50% inhibition of velocity when mixed with phosphorylated smooth muscle myosin than when mixed with skeletal muscle myosin. In conclusion, using both in vitro motility assays allows one to discriminate not only direct effects on the interaction of smooth muscle myosin and actin, but also effects on the assay system itself and appears to be a useful tool in understanding the mechanism of smooth muscle contraction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04202-08 MC

PERIOD COVERED: October 1, 1988 through May 6, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Phosphorylation as a Regulatory Mechanism

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

Marshall A. Corson, M.D., Medical Staff Fellow, LMC, NHLBI (terminated 5/6/89)
Robert S. Adelstein, M.D., Chief, LMC, NHLBI
William A. Anderson, Jr., Chemist, LMC, NHLBI

COOPERATING UNITS (if any)

Mark Schoenberg, M.D., LPB, NIAMS
Mahtash Moussavi, Ph.D., LPB, NIAMS

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:
0.8	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.)

The contractile response of intact bovine tracheal strips was characterized, with attention to the pharmacologic mechanism of Substance P stimulation, and its effect on the contractile proteins. At low Substance P concentrations (≤1 μM) the isometric tension response is almost completely abolished by inclusion of atropine (0.3 μM), indicating mediation of contraction via Substance P-stimulated release of acetylcholine from pre-junctional nerve terminals; in contrast, at near-maximal concentrations (≥10 μM), the atropine-inhibited component of the tension response is <25%. When the muscles are activated by Substance P directly, half-maximal tension is reached in approximately 2.5 minutes, peak tension in approximately 11 minutes. Immunoblot analysis of the time course of phosphorylation of the 20 kDa myosin light chain reveals an increase to an incorporation of approximately 0.5 mol phosphate/mol, with subsequent slight decline while tension remains stable. Two-dimensional tryptic phosphopeptide analysis of phosphorylated light chain reveals a single major phosphopeptide at both early and late times, migrating identically to that produced by myosin light chain kinase phosphorylation of light chain in vitro. These results indicates that: 1) Substance P mediates contraction of bovine trachea both directly and indirectly (via acetylcholine), and 2) under conditions where activation is via the direct mechanism, the tension and phosphorylation responses qualitatively resemble those observed for muscarinic cholinergic agonists; however, following a Substance P mediated contraction there is only a slight decrease in phosphorylation while tension remains stable.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04205-07 MC

PERIOD COVERED
January 3, 1989 through September 30, 1989

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)*

Trudy L. Cornwell, Ph.D., Staff Fellow (PRAT, NIGMS), LMC, NHLBI
Robert S. Adelstein, M.D., Chief, 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:
0.9	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.)*

Myosin light chain kinase (MLCK) catalyzes the phosphorylation of the light chains of myosin in smooth muscle and nonmuscle cells. In smooth muscle, this reaction is considered to be the stimulus for actin-myosin interactions leading to contraction. In nonmuscle cells, the contractile events mediating cell functions such as migration, cytokinesis or shape change are thought to be similar to those occurring in smooth muscle. Although the reaction catalyzed by MLCK has been well characterized, little is known about the regulation of light chain phosphorylation and its importance in nonmuscle cell function. Recent studies by Lamb et al. (J. Cell Biol. 106: 1955-1971, 1988) support a role for MLCK in regulating actin microfilament integrity in fibroblasts. These authors hypothesize that cAMP-dependent protein kinase inhibits MLCK, leading to shape change in fibroblasts. Although phosphorylation of MLCK by the cAMP-dependent protein kinase in vitro decreases the sensitivity of the enzyme for the known activator, Ca2+/calmodulin, it is not clear that phosphorylation regulates MLCK in the intact cell. This project has been undertaken to further our knowledge of the function and regulation of MLCK in nonmuscle cells. Since inception of this project we have succeeded in labeling MLCK and anti-MLCK (with full retention of activities) using fluorescent analog cytochemistry. Microinjection of the analogs into 2 types of fibroblasts demonstrated the subcellular distribution of MLCK. This approach, combined with an approach utilizing biochemical, immunological, and molecular biological techniques, should allow us to determine the role and regulation of MLCK in nonmuscle cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04207-04 MC

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

Regulation of Genes for Contractile Proteins in Muscle and Nonmuscle Cells

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

Lawrence Weir, Ph.D., Visiting Associate, LMC, NHLBI (terminated 4/27/89)
Michael Simons, M.D., Medical Staff Fellow, LMC, NHLBI (terminated 6/22/89)
Robert S. Adelstein, M.D., Chief, LMC, NHLBI
Yvette Preston, Biologist, LMC, NHLBI

COOPERATING UNITS *(if any)*

O. Wesley McBride, DCBD, NCI

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Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.5	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.)*

We would like to understand the role of nonmuscle myosin in such diverse and vital contractile processes as cytokinesis, karyokinesis, secretion and organelle translocation. Molecular genetics offers the most fundamental and potentially fruitful approaches towards this goal. Accordingly, we have undertaken the cloning of the genes for human nonmuscle myosin heavy chain (NMMHC). cDNA probes for chicken NMMHC were used to isolate human cDNA clones from a T lymphocyte library. These cDNA clones were, in turn, used to isolate clones from human genomic libraries. Several lambda phage clones containing parts of a NMMHC gene were isolated. Because the gene was apparently very large, it was necessary to screen a cosmid library to try to obtain the complete gene. Genomic clones have been mapped with restriction enzymes and partly sequenced to reveal the exon-intron organization. This gene has been determined to be located on human chromosome 22. cDNA clones may be divided into two groups which apparently represent two different isoforms of the NMMHC with slightly different amino acid sequences. These two isoforms are not derived by alternative splicing, but are encoded by two different genes. We can now study the differential expression of these two genes and, by molecular manipulation of their expression, hope to elucidate how two types of myosin participate in the normal functioning of the cell. At least one of the myosins seems to be regulated at the level of translation via an unusual secondary structure at the 5' end of the messenger RNA. This structure clearly inhibits translation in vitro, but not in vivo which indicates that a mechanism must exist to either remove or melt the structure before translation is permitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04208-03 MC

PERIOD COVERED

October 1, 1988 through September 30, 1989

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)*

Mary Anne Conti, Ph.D., Research Chemist, LMC, NHLBI
Sachiyo Kawamoto, M.D., Ph.D., Visiting Associate, LMC, NHLBI
Ralph V. Shohet, M.D., Medical Staff Fellow, LMC, NHLBI (terminated 12/31/88)
David A. Brill, M.D., Medical Staff Fellow, LMC, NHLBI (terminated 12/31/88)
Robert S. Adelstein, M.D., Chief, LMC, NHLBI
Yvette 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:
2.9	2.1	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 complete amino acid sequence of a vertebrate cellular myosin heavy chain (1,959 amino acids, 226 kDa) has been deduced using cDNA clones from a chicken intestinal epithelial cell library. RNA blot analysis of kidney, spleen, brain, liver and intestinal epithelial cells, as well as smooth muscle cells from the aorta and gizzard indicates the presence of a 7.3 kb message. The chicken intestinal epithelial cell myosin heavy chain shows overall similarity in primary structure to other myosin heavy chains. The globular head domain is followed by an α-helical coiled coil region and like smooth muscle, but unlike vertebrate striated muscle, there is a short uncoiled sequence at the carboxyl-terminus of the molecule. Comparison of amino acid sequences in the rod regions between human and chicken cellular myosin heavy chains shows a remarkable 92% identity. Having completed the cloning of a cellular myosin cDNA we wish to use the information obtained to study the function of myosin in eukaryotic cells. Its ubiquitous presence indicates a general role in a basic cell process such as cell division, but it may also be important in specialized cell functions such as cell secretion, motility, or chemotaxis. The myosin heavy chain amino acid sequence from chicken intestinal epithelial cells, deduced from cDNA cloning, will be used as a basis for the expression of myosin and altered forms of the myosin heavy chain in various transfected cell lines. It is possible that, by point mutations, deletions and insertions in the cDNA sequence, we will be able to alter the functional properties of myosin and delineate its role in the cell. We are, at present, determining the site or sites of phosphorylation of the myosin heavy chain by protein kinase C. Phosphorylation of these sites may be a step linking cell-surface signals which activate protein kinase C to their effects on the cell's function. The sequences contributing to the ATP and actin binding sites as well as the noncoiled tail piece at the carboxyl-terminus are also targets for these functional studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04209-03 MC

PERIOD COVERED
October 1, 1988 through December 31, 1988

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

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

Itzhak Peleg, Ph.D., Visiting Fellow, LMC, NHLBI (terminated 12/31/88)
Robert S. Adelstein, M.D., Chief, LMC, NHLBI

COOPERATING UNITS *(if any)*

Russell Ludowyke, Ph.D., LCP, NHLBI
Michael Beaven, Ph.D., LCP, NHLBI

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Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

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☐ (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.)*

IgE-mediated stimulation of rat basophilic leukemia (RBL-2H3) cells results in the secretion of histamine. Myosin immunoprecipitated from these cells shows an increase in the amount of radioactive phosphate incorporated into its heavy (200 kDa) and light (20 kDa) chains. In unstimulated cells two-dimensional mapping of tryptic peptides of the myosin light chain reveals one phosphopeptide containing the serine residue phosphorylated by myosin light chain kinase. Following stimulation a second phosphopeptide appears containing a serine residue phosphorylated by protein kinase C. Tryptic phosphopeptide maps derived from myosin heavy chains show that unstimulated cells contain three major phosphopeptides. Following stimulation a new tryptic phosphopeptide appears containing a serine site phosphorylated by protein kinase C.

The stoichiometry of phosphorylation of the myosin light and heavy chains was determined before and after antigenic stimulation. Before stimulation myosin light chains contained 0.4 mol phosphate/mol light chain all confined to a serine residue phosphorylated by myosin light chain kinase. Cells that secreted 44% of their total histamine in 10 minutes exhibited an increase in phosphate content at sites phosphorylated by protein kinase C from 0 mol phosphate/mol myosin subunit to 0.7 mol phosphate/mol light chain and to 1 mol phosphate/mol heavy chain. When RBL-2H3 cells were made permeable with streptolysin O they still showed a qualitatively similar pattern of secretion and phosphorylation. Our results show that the time course of histamine secretion from stimulated RBL-2H3 cells parallels that of myosin heavy and light chain phosphorylation by protein kinase C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04210-02 MC

PERIOD COVERED: October 1, 1988 through September 30, 1989

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 A. Anderson, Jr., Chemist, 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:
1.7	1.2	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.)

Our laboratory has demonstrated previously that the reversible phosphorylation of the 20 kDa light chains of smooth muscle myosin regulates the actin-activated MgATPase activity of the molecule (Sellers et al., JBC 256: 13137, 1981). More recently, our laboratory observed that the heavy chains of smooth muscle myosin are also phosphorylated (Kawamoto and Adelstein, JBC 263: 1099, 1988). Our present goal is to understand the function of myosin heavy chain phosphorylation. We began by identifying the kinases that phosphorylate the myosin heavy chains in vitro and in intact cells. Our data shows that of a variety of serine/threonine kinases tested, only Ca2+/calmodulin-dependent protein kinase II (isolated from brain) and casein kinase II (isolated from bovine aortic smooth muscle) stoichiometrically phosphorylate the heavy chains of purified bovine aortic smooth muscle myosin in vitro. Two-dimensional tryptic phosphopeptide maps of myosin heavy chains phosphorylated by both of these kinases show that the major phosphorylated peptide in each case is identical suggesting that the major site of phosphorylation by the two kinases is the same. This site in the smooth muscle myosin heavy chain is also phosphorylated in intact bovine retinal pericytes and in intact bovine aortic smooth muscle cells. These results suggest that casein kinase II or Ca2+/calmodulin-dependent protein kinase II is responsible for this phosphorylation. Stimulation of a number of cultured cell lines with insulin-like growth factor 1 (IGF1) results in an increase in casein kinase II activity (Klarland and Czech, JBC 263: 15872, 1988). We have examined the effect of IGF1 on intact smooth muscle cell myosin phosphorylation. Preliminary results show that stimulation of cells with IGF1 results in a new phosphopeptide of the heavy chain as seen on two-dimensional tryptic peptide maps. We also observed changes in the phosphorylation of the 20 kDa myosin light chain following IGF1 stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04212-01 MC

PERIOD COVERED: October 1, 1988 through September 30, 1989

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

COOPERATING UNITS (if any):

Paul Matsudaira, Assoc. Professor, Whitehead Institute, MIT
Cathy 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:
0.85	0.25	0.60

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.)

The intestinal epithelial brush border microvilli contain a protein which migrates on SDS-polyacrylamide gels with a chain weight of 110 kDa. This protein appears to link the plasma membrane with the actin bundles. It has recently been shown to have myosin-like properties based on several criteria: 1) It has sequence homologies with both myosin I from Acanthamoeba castellani and cytoplasmic myosin II from vertebrate sources. 2) It has a MgATPase activity which can be activated by actin. 3) It is associated with several low molecular subunits which are thought to be calmodulin which may function similar to light chains. It appears to most closely resemble the single-headed myosin I type molecule based on its molecular weight and appearence in rotary shadowed electron microscopic images.

We have been studying 110 kDa protein which was isolated from chicken intestinal epithelial brush border. In order to more conclusively establish this protein as a myosin I class enzyme we have examined its ability to translocate fluorescently-labeled actin filaments in an in vitro motility assay. We found that it does translocate these filaments at a rate of about 0.06 um/s when the free calcium is very low. This rate is increased about 50% when the calcium concentration is raised to 10 uM. Increasing the calcium concentration to 100 uM results in total inhibition of the movement. We are also studying the actin-activated MgATPase activity of this molecule.

Annual Report of the
Laboratory of Molecular Hematology
National Heart, Lung, and Blood Institute
October 1, 1988 to September 30, 1989

The Laboratory of Molecular Hematology (LMH) is composed of three sections: the Section on Molecular Genetics is primarily involved in developing the basic knowledge and technology for carrying out gene therapy for human diseases; the Section on Molecular Cloning is primarily concerned with understanding the nature of transcriptional control elements; and the Section on RNA and Protein Biosynthesis is primarily concerned with understanding the mechanism and regulation of eukaryotic gene expression at both the transcriptional and translational levels.

SECTIONS ON MOLECULAR GENETICS AND MOLECULAR CLONING

The diseases chosen as the initial candidates for human gene therapy are cancer, AIDS, and adenosine deaminase (ADA) deficiency, a cause of severe combined immunodeficiency (SCID). Retroviral techniques and recombinant DNA technology have been used to construct retroviral vectors containing the human ADA gene, the human soluble CD4 gene, and/or a selectable gene, NeoR (the latter codes for a phosphotransferase enzyme that confers resistance to the drug G418, a neomycin analogue that can kill mammalian cells). An efficient procedure for transferring functional genes into mammalian tissue culture cells in vitro and into bone marrow cells of mice and monkeys in vivo has been developed over past years using these retroviral vectors as a delivery system.

Previously we demonstrated that when murine hematopoietic progenitor cells are infected in vitro with a vector carrying the NeoR gene and then reinjected into a lethally irradiated recipient mouse, 85-90% of the stem cells (CFU-S) can be shown to carry an intact copy of the NeoR gene. The majority of these cells can be shown (by analyzing spleen foci in the CFU-S assay) to produce the NeoR phosphotransferase (NPT). Using the knowledge gained from the murine system, an autologous bone marrow transplantation (BMT)/gene transfer protocol was developed for nonhuman primates. These results have now been extended to several additional cell types: tumor infiltrating lymphocytes (TIL), endothelial cells, hepatocytes, and fibroblasts.

During the past year, these two Sections have achieved the following results:

(1) A clinical protocol is underway in which NeoR-gene marked TIL are being used to study adoptive immunotherapy for malignant melanoma. A clinical protocol has been developed with Dr. Steven A. Rosenberg, Chief, Surgery Branch, NCI, and Dr. R. Michael Blaese, Chief, Cellular Immunology Section, Metabolism Branch, NCI, which is designed to provide information on the trafficking of TIL during adoptive immunotherapy of patients with advanced cancer. The protocol was approved by the NIH and the FDA. It is the first use of gene transfer technology in human patients.

(2) The technology has been developed for inserting genes into hepatocytes, fibroblasts, and a range of other cell types. The gene-engineered cells are grown on three-dimensional collagen-coated pads (a technology developed last year in

this lab), and the cell-containing pads are implanted into animals either into subcutaneous or intraperitoneal sites. The gene-engineered cells have been recovered from pads after four months in vivo and they still express the inserted gene.

(3) Vascular endothelial cells have successfully been used for the insertion of genes which produce secretable proteins, specifically t-PA. The gene-engineered endothelial cells have been grown on vascular grafts and shown to continue to secrete the gene product in vitro. In vivo studies are being initiated.

SECTION ON RNA AND PROTEIN BIOSYNTHESIS

To understand the regulation of expression of genes transcribed by RNA polymerase II, promoter elements of the Adenovirus 2 major late transcription unit and the eukaryotic translation factor eIF-2 are being used to characterize and purify individual factors required for correct initiation.

The mechanisms by which adenoviruses and influenza ciruses take over the translational machinery of the infected cell, and the defense mechanisms used by cells to prevent viral takeover are being studied.

The role of eIF-2B during protein synthesis initiation in normal and viral infected cells is being studied using antibodies directly against eIF-2B and factors whose activities it modulates.

During the past year this section has:

(1) The promoter region of the eIF-2α gene has been sequenced and analyzed. This gene is characterized by an unusual 4 kb long intron within the 5'-UTR. The promoter region of this housekeeping gene contains neither a TATA box nor a CAAT box. by S1 nuclease and primer extension analysis, 10 to 12 transcription start sites are identified within a 43 bp region.

(2) The eIF-2α promoter region has been examined by in vivo and in vitro footprinting, as well as consensus binding site analysis. Although several previously characterized binding sites such as those for AP-1 and SpI are identified, 6 new binding sequences are found. Their effects on transcriptional activity of the eIF-2α gene are under investigation.

(3) Several new promoter elements for the Adenovirus 2 major late promoter have been identified by DNA-affinity techniques and functional transcription assays. One (DTF) binds to a downstream promoter sequence extending from +146 to +165 (relative to the cap site at +1). A second binds just downstream of the TATAA factor binding site. In addition, several specific topoisomerase I binding sites map within the Ad2 MLP.

(4) By UV cross-linking, the DPS binding factor is identified as a new 40kDa transcription factor. In vivo functional analysis of DPS mutations introduced into the intact Ad2 genome is currently being analyzed.

(5) The mechanisms by which influenza virus prevents shutoff of protein synthesis by the eIF-2α specific protein kinase activated during viral infection was studied. Influenza virus was found to encode a gene product which directly blocks kinase autophosphorylation and kinase activity. Suppression of kinase

activity occurs within 2 hours post-influenza infection and requires viral gene expression. In vitro mixing experiments also show that the influenza viral inhibition can act in trans to block kinase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02213-12 MH

PERIOD COVERED

October 1, 1988 through September 30, 1989

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

RNA Polymerase II Transcription Factors

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

PI: B. Safer, Medical Officer, LMH, NHLBI
Others: R. Cohen, Staff Fellow, LMH, NHLBI
T. Silverman, Staff Fellow, LMH, NHLBI
W. Jacob, Staff Fellow, LMH, NHLBI
S. Garfinkel, Bio. Lab. Tech., LMH, NHLBI
T. Boal, Biol. Lab. Tech., LMH, NHLBI
L. Yang, Biologist, LMH, NHLBI
W. F. Anderson, Chief, LMH, NHLBI

COOPERATING UNITS *(if any)* Michael Katze, U. of Wash. Med. School, Seattle, WA; Tom Shenk, Princeton University, Princeton, NJ; Rosemary Jagus, University of Pittsburgh, Pittsburgh, PA; John Hershey, University of California, Davis, CA

LAB/BRANCH

Laboratory of Molecular Hematology

SECTION

Section on RNA and Protein Biosynthesis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	4.1	2.4

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 major goal of the Section on Protein Synthesis is to characterize the mechanisms which regulate expression of genes which encode protein synthesis initiation factors. Towards this end we have cloned and characterized the promoter region of the gene for the α subunit of eIF-2. Typical of many housekeeping genes, the promoter region is G+C rich and contains neither a TATA nor CAAT element. In vitro and in vivo footprint analysis, in conjunction with DNaseI hypersensitive site mapping, however, identify 8 novel DNA-protein binding sites. We have purified to near homogeneity a transcription factor required for eIF-2α gene expression whose binding site at the CAP region contains both direct repeats and palindromic sequences. The function of this element in promoting α transcription is being studied.

A second major goal is to understand the importance of the adenovirus 2 major late promoter CAP-proximal sequence. We have identified by mutational analysis and DNaseI footprinting a CAP-proximal sequence required for efficient transcriptional initiation. By conventional and sequence-specific DNA affinity chromatography we have extensively purified a factor distinct form TFIID which interacts with this region.

A third major project concerns the mechanism(s) of translational activation following mitogenic stimulation of Go T-lymphocytes. Within 24 hours, cellular levels of eIF-2α mRNA, a rate-limiting factor which catalyzes the first regulated step of translation initiation increase 50 to 100-fold. However, neither an increased rate of transcription nor an increased mRNA stability can account for this accumulation. Rather, increased processing of the primary transcript and/or efficient transport is responsible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02216-10 MH

PERIOD COVERED

October 1, 1988 through September 30, 1989

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)*

PI: W. French Anderson, Chief, LMH, NHLBI

J. Mason, Staff Fellow, LMH, NHLBI
L. Baltrucki, Staff Fellow, LMH, NHLBI
E. Karson, Med. Staff Fel., LMH, NHLBI
R. Morgan, Ad. Sci., LMH, NHLBI
J. McEachlin, VA, LMH, NHLBI
S. Freeman, Ad. Sci., LMH, NHLBI
K. Cornetta, NRSA Fellow, LMH, NHLBI
S. Sturm, VA, LMH, NHLBI
R. Anderson, A.S., LMH, NHLBI
L. Shu, A.Fel. LMH, NHLBI

COOPERATING UNITS *(if any)*

A. Nienhuis, Chief, CHB, NHLBI
M. Blaese, Chief, MET, NCI
S. Rosenberg, Chief, SB, NCI
M. Eglitis, Genetic Therapy, Inc., Gaith. MD
R. Moen, Genetic Therapy, Inc., Gaith., MD
J.A. Thompson, Genetic Therapy, Inc., Gaith., MD

LAB/BRANCH

Laboratory of Molecular Hematology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
11.5	9.8	1.7

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.)*

A highly efficient procedure for transferring functional genes into mammalian cells has been developed using retroviral vectors as a delivery system. Retroviral vectors containing the human gene for the enzyme adenosine deaminase (ADA) and/or the NeoR gene have been made. These vectors have been used to introduce exogenous genes into human tumor infiltrating lymphocytes (TIL) T lymphocytes, fibroblasts, endothelial cells, and other cell types. These studies are preliminary to attempting human gene therapy in patients suffering from ADA Deficiency and advanced cancer. A human gene transfer protocol using NeoR-gene marked TIL to study adoptive immunotherapy for malignant melonoma is underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02218-01 MH

PERIOD COVERED
October 1, 1988 through September 30, 1989

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)*

PI: R. Morgan, Adjunct Scientist, LMH, NHLBI
W. F. Anderson, Chief, LMH, NHLBI

COOPERATING UNITS *(if any)*

F. Wong-Staal, Staff Fellow, NCI
R.C. Gallo, Chief, NCI
J.A. Thompson, Genetic Therapy Inc., Gaithersburg, MD
R. Moen, Genetic Therapy Inc., Gaithersburg, MD.

LAB/BRANCH
Laboratory of Molecular Hematology

SECTION
Section on Molecular Cloning

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.2	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.)*

Retroviral vectors have been developed which produce a secreted form of the helper/inducer T-cell antigen, CD4. Amphotropically packaged vectors were used to transduce cells and these cells were shown to express the secreted CD4 (sCD4) gene product. The sCD4 produced by the viral vectors is immunoprecipitated by monoclonal antibodies against CD4 which specifically block HIV infection of helper/inducer T-cells. A direct physical interaction of vector-produced sCD4 and HIV-1 gp120 was demonstrated by coprecipitation of sCD4/gp120 with antiserum directed against HIV gp120. Further, transduced cells producing sCD4 can protect HIV susceptible cells from infection by HIV. We have demonstrated that retroviral vectors can be constructed which express sCD4 and that cells transduced by these vectors can protect cells from HIV infection in culture. sCD4 retroviral vectors could potentially be used to engineer the cells of an AIDS afflicted individual, and thus these data are a model for 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 02219-01 MH

PERIOD COVERED

October 1, 1988 through September 30, 1989

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)*

PI: David Dichek, Medical Staff Fellow, LMH, NHLBI
W.F. Anderson, Chief, LMH, NHLBI
K. Anderson, Adjunct Scientist, LMH,NHLBI
K. Newman, Adjunct Scientist, LMH, NHLBI
O. Nussbaum, Staff Fellow, , LMH, NHLBI

COOPERATING UNITS *(if any)*

J. Thompson, Genetic Therapy, Inc., - Gaithersburg, MD
R. Bowman, TDL, NHLBI
R. Clark, Chief, SB, NHLBI B. Brewer, MDB, NHLBI

LAB/BRANCH

Laboratory of Molecular Hematology,

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
3.7	2.7	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.)*

During the past few years, investigators have described and cloned several genes which play important roles in the etiology and treatment of cardiovascular diseases. Two prime examples of these genes are the tissue plasminogen activator (t-PA) gene and the gene coding for the low density lipoprotein receptor (LDLr). Many of the recent advances in the treatment of hypercholesterolemia and coronary thrombosis are based on our current understanding of the physiological roles of these genes and their protein products. The development of gene transfer technology has occurred simultaneously with, but separate from, these advances in cardiovascular research. Our goal is to bring these two fields together in order to create in vitro and in vivo models for the treatment of human cardiovascular disease.

We are using the Watanabe Heritable Hypercholesterolemic Rabbit,an established animal model of human hypercholesterolemia, as a target for gene therapy using the low density lipoprotein receptor gene. In so doing we hope to establish the feasibility of using gene transfer for the treatment of some types of hypercholesterolemia.

In an effort to develop new treatments for intravascular thrombosis, vascular graft failure, and coronary restenosis, we have achieved the insertion and overexpression of the human tissue plasminogen activator gene in cultured sheep endothelial cells. We have also developed protocols for seeding these cells onto vascular grafts and intravascular stents. We plan to proceed with the implantation of these cells and devices in animals.

ANNUAL REPORT OF THE
LABORATORY OF TECHNICAL DEVELOPMENT

OCTOBER 1, 1988 TO SEPTEMBER 30, 1989

Pulmonary and Cardiac Assist Devices

We have shown that partial and/or total cardiac assistance, can be provided through means that do not require thoracotomy, and the implantation of blood pumps. Assistance can now be provided through percutaneous cardiopulmonary bypass using a membrane artificial lung. The left heart is uniquely decompressed through a small helical coil passed percutaneously to rest within the lumen of the pulmonary artery valve and the lumen of the tricuspid valve. Such a cardiac assist system is called for when intraaortic balloon counterpulsation alone for cardiac failure is not sufficient, and when consideration is given for the implantation of cardiac assist devices, or as a bridge to cardiac transplantation. Because of its great effect in reducing left heart filling pressure, it may find application in the management of acute myocardial infarction, or to reduce the volume of myocardium at risk of infarction.

We have reproduced in a laboratory model the evolution of highly lethal acute respiratory failure (ARF) with multiorgan system failure, which in great part can be blamed on the effects of the use of a mechanical ventilator. These adverse effects, we believe, can be prevented, and reversed, by the judicious use of extracorporeal membrane lung gas exchange and cardiac assist, which allows us to avoid, and to discontinue the use of injurious ventilator settings.

Separation Science Instrumentation

The third prototype of the cross axis synchronous flow-through coil planet centrifuge was constructed. Preparative capability of the apparatus was successfully demonstrated by the efficient multigram separation of 2,4,-dinitrophenyl amino acids, indole auxins, and bacitracin in a pair of large multilayer coils with a total capacity of 1.5L.

A compact portable model of a high-speed countercurrent chromatograph with a 2.5cm revolution radius was constructed. Analytical capability of the apparatus was successfully demonstrated in separation of flavonoids from a crude sea buckthorn extract in a multilayer coil with a total capacity of 8 ml.

The horizontal flow-through coil planet centrifuge with multilayer coil set holds a set of four identical multilayer coils around the column holder. These columns are interconnected in series, have a capacity of about 200 ml and provides universal application of two-phase solvent systems including aqueous-aqueous polymer phase systems used for partition of macromolecules and cell particles. The capability of the apparatus was successfully demonstrated in separation of cytochrome c and lysozyme in an aqueous polymer phase system.

Foam countercurrent chromatography (CCC) was successfully applied to separation of bacitracin components with nitrogen gas and distilled water free of surfactant or other additives. The method may be extremely useful in enrichment of bioactive minor components in biological fluids such as urine, blood and blood dialysate, cell culture medium, etc.

Biophysical Instrumentation

The oxygen equilibrium curve analyzer we have been developing has been completed. The analytical spectroscopy of ATP, ADP, and PO4 has progressed to the point that we have demonstrated distinct spectral differences of each species in the NIR, 900-1800 nanometers. Analysis has been done using single-valved decomposition (SVD) and partial least squares (PLS) with some success. Better results have been obtained by the use of Neural Nets.

A polypropylene flow cell suitable for insertion into the batch microcalorimeter required the development of a Diamond-Like-Carbon (DLC) coating of the polypropylene to stop water evaporation through the plastic cell wall. This proved very successful and led to the setting up of an Argon-Ion Depositon and Milling Apparatus to carry out DLC biocompatibility studies on a variety of plastics and to permit the construction of new types of very sensitive semi-conductor thermopiles.

Time Resolved Fluorescence Spectroscopy

Time-Resolved Fluorescence Spectroscopy is a method that measures the submicroscopic environment of molecules inside proteins, membranes and DNA. Our effort have focused on devising new laser-driven instruments that provide better speed, accuracy and sensitivity. For example, we are now able to study the folding processes of miniscule amounts of protein in a few seconds; similarly, the binding of protein to DNA (for control) on the lipid disruption caused by virus binding to membranes is apparent to our instruments. We are continuing instrument development while collaborating with dozens of our biochemical and medical colleagues.

Special Devices

A mock circulatory system to evaluate vascular grafts bearing gene-engineered endothelial cells has been constructed and tested. If these altered endothelial cells can adhere to the grafts and function, a new avenue of therapeutics is expected to emerge from this work.

Fluorescence Spectroscopic Studies

Spectroscopic investigation of tryptophan dipeptides showed differences between isomers having tryptophan at the N- or C-terminus. Blue shifts in electronic spectra and a lower pKa occurred when the indole group was near the protonated amino group. The findings explain some spectral shifts in proteins.

A study showing the mechanism of concentration quenching of dyes, principally 6-carboxyfluorescein, was published. Additional work showed that the dye-liposome system could be used to assay lytic peptides and the kinetics of lysis.

Serotonin, melatonin, and other 5-alkoxy and 5-hydroxy indoles in acid exhibit a new fluorescence band because of excited-state protonation. This effect is sensitive to small anions and represents a new way to study counterion association in solution.

In the enzyme, glyceraldehyde-3-phosphate dehydrogenase, the method of decay-associated spectra showed energy transfer from 1 of 3 tryptophans to groups attached to the active site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01404-21 LTD

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.)*

Membrane Lungs for Long Term Respiratory, Cardiac and Cardiorespiratory Assist

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

P.I.	T. Kolobow	Medical Officer	LTD:NHLBI
	G. Foti	Visiting Fellow	LTD:NHLBI
	G. Vitale	Visiting Fellow	LTD:NHLBI
	S. Mandava	Special Volunteer	LTD:NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Technical Development

SECTION

Section on Pulmonary and Cardiac Assist Devices

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	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.)*

We have shown that partial and/or total cardiac assistance can be provided through means that do not require thoracotomy, and the implantation of one or more blood pumps. Instead, such assistance can be provided through percutaneous means through cardiopulmonary bypass using a membrane artificial lung, and while the left heart is uniquely decompressed through a small helical coil passed percutaneously to rest within the lumen of the pulmonary artery valve and the lumen of the tricuspid valve. Such a cardiac assist system is called for when intraaortic balloon counterpulsation alone for cardiac failure is not sufficient, and when consideration is given for the implantation of cardiac assist devices, or as a bridge to cardiac transplantation. Because of its great effect in reducing left heart filling pressure, it may find application in the management of acute myocardial infarction, or to reduce the area of myocardium at risk of infarction.

We have elucidated some factors that we believe contribute to the syndrome of severe acute respiratory failure (adult respiratory distress syndrome = ARDS). Prime among them is the use of the mechanical pulmonary ventilator at high peak airway pressure. We have reproduced in a laboratory model the evolution of highly lethal multiorgan system failure, which in great part can be blamed on the effects of the use of a mechanical ventilator. These adverse effects, we believe, can be prevented, and reversed, by the judicious use of extracorporeal membrane lung gas exchange and cardiac assist, which allows us to avoid, and to discontinue the use of injurious ventilator settings.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01407-26 LTD

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.)*

Fluorescence Spectroscopic Studies

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

P.I. Raymond F. Chen	Sr. Investigator	LTD:NHLBI
Jay R. Knutson	Sr. Staff Fellow	LTD:NHLBI
Chen-Lu Tsou	Fogarty Scholar	NIH
Robert Highet	Sr. Investigator	CH:NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Technical Development

SECTION

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 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.)*

Spectroscopic investigation of tryptophan dipeptides showed differences between isomers having tryptophan at the N- or C-terminus. Blue shifts in electronic spectra and a lower pKa occurred when the indole group was near the protonated amino group. The findings explain some spectral shifts in proteins.

A study showing the mechanism of concentration quenching of dyes, principally 6-carboxyfluorescein, was published. Additional work showed that the dye-liposome system could be used to assay lytic peptides and the kinetics of lysis.

Serotonin, melatonin, and other 5-alkoxy and 5-hydroxy indoles in acid exhibit a new fluorescence band because of excited-state protonation. This effect is sensitive to small anions and represents a new way to study counterion association in solution.

In the enzyme, glyceraldehyde-3-phosphate dehydrogenase, the method of decay-associated spectra showed energy transfer from 1 of 3 tryptophans to groups attached to the active site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01413-27 LTD

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.)

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 LTD:NHLBI

NIA, Lab. Molecular Biology (J. Froehlich); U. of Penn. (L. Thiebault); DRS,

COOPERATING UNITS (if any)

Biomedical Engineering & Instrumentation Branch (H. Cascio); Commonwealth Technology, Alexandria, VA; Div. Blood Resources, LAIR (R. Winslow), DCRT (D. Shrager), NASA Houston (Paul Baffes and Robert Shelton), NIDDK (Ira Levin).

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Laboratory of Technical Development

SECTION

Biophysical Instrumentation Section

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

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

The development of a blood substitute, while of primary concern to the Dept. of Defense for Combat Victims, has important implications for the civilian population since it can be sterilized and rendered virus free. Scale up to 50 l/run is being started at the Div. of Blood Resources, Letterman Army Inst. of Research under Col. Robert Winslow. The development of various instruments, for the biophysical study of hemoglobin begun in this section many years ago, are now in demand at NIH, LAIR, and various univ. labs. The oxygen equilibrium curve analyser we have been developing with the considerable assistance of Horace Cascio, BEIB Elec. Eng., and the Fabrication Section of BEIB, has been finished and is presently being debugged. Utilizing summer personnel, we plan to thoroughly test the new all Hastoly C Stainless Steel cells on a new cross-linked hemoglobin, called BAFF, which is sterile and has a biological half-life of 25 hours in humans, and is furnished by LAIR. Effects of temperature, and chloride binding to hemoglobin on the equilibrium curve will be studied both manometrically and spectrometrically to establish a standard curve. A calorimetric determination of the heats of binding will be done with C. Mudd, BEIB Applied Clinical Engineering.

The analytical spectroscopy of ATP, ADP, and PO4 has progressed to the point that we have demonstrated distinct spectral differences of each species in the NIR, 900-1800 nanometers. Analysis has been done using single-valved decomposition (SVD) and partial least squares (PLS) with some success. Somewhat better results have been obtained by the use of Neural Nets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01414-17 LTD

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.)

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	LTD:NHLBI
Other:	C. P. Mudd	Biomedical Engineer	BEIB:DRS

COOPERATING UNITS (if any)

LAIR (N. Davids); Commonwealth Scientific Inc., Alexandria, VA.; Commonwealth Technology Inc., Alexandria, VA.; Mario Pace, U. of Milan, Italy; Dario Agnellini, U. of Milan, Italy.

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Biophysical Instrumentation Section

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 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.)

A polypropylene flow cell suitable for insertion into the batch microcalorimeter required the development of a Diamond-Like-Carbon (DLC) coating of the polypropylene to stop water evaporation through the plastic cell wall. This proved very successful and led to the setting up of an Argon-Ion Depoaition and Milling Apparatus to carry out DLC on a variety of plastics and to permit the construction of new types of very sensitive semi-conductor thermopilea. System inatallation, check out, and preliminary coatings on several materials have been carried out and some analysis of the type of carbon bonds formed were carried out for us at NIST. A test calorimeter is being set-up to measure water movement through the coated wall.

A heat capacity-calorimeter is being set up to study phospholipids in collaboration with scientists in NIADDK.

Kinetic and enthalpy measurements have been carried out on the enzyme NADase, breaking down NAD to Nicotinamide, Adenosine Diphosphate Ribose and H+. This reaction is of the order of 6 kilocalorie/mole endothermic, indicating it is entropy driven. Considerable further work on this enzyme is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01452-06 LTD

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.)*

Time Resolved Fluorescence Spectroscopy

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

P.I.	J. R. Knutson	Sr. Staff Fellow	LTD:NHLBI
Other	R. F. Chen	Sr. Investigator	LTD:NHLBI

COOPERATING UNITS *(if any)*

Cooperating units: L. Davenport (CUNY); M. Han (LB:NHLBI,Johns Hopkins Univ.) D. Walbridge, J. Wages, S. Roseman, C. Anfinsen, L. Brand (JHU); D. Sackett, J. Wolff (NIDDK); S. Green (Georgetown); Preston Hensley (Smith, Kline, and French); Sue Scarlata (Cornell); A. Russo (C:ROB);J. Beechem, E. Gratton (UI-U/C); M. Clague (C:MB); E. Korn, A. Attri (H:LCB), E. Miles (NIDDK).

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	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.)*

A pulsed laser: time-resolved fluorescence facility was developed to provide rapid collection and analysis of fluorescence data related to macromolecular size, flexibility, folding and fluctuations. This type of measurement provides insight into the ever-changing structure of proteins and membranes in solution. Further, it provides hundredfold sensitivity increases over competing methods like nmr.

Our main time-resolved spectrofluorometer was utilized to study the structure and dynamics of many different proteins, including: gramicidin, a "pore-forming" peptide; tubulin and actin, parts of the cell "skeleton", arginase and OTCase, enzymes whose metabolic "feedback" is controlled by shape changes identified on our equipment; enzyme I of the phosphotransferase system, and TF3A, a protein that binds DNA to control it.

Protein folding continued to grow as a priority topic in our lab. The structures of arginase, alcohol dehydrogenase, interleukin 1, PGK, and other enzymes were perturbed so our rapid-collection instrument could chronicle structural change vs. time.

We also continued our inquiry into lipid packing fluctuations, using a unique probe (coronene) that is sensitive to submicrosecond "melting" of membrane patches on cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01462-03 LTD

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.): Cross-Axis Synchronous Flow-Through Coil Planet Centifuge

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

P.I.	Yoichiro Ito	Senior Investigator	LTD:NHLBI
Other:	Hisao Oka	Visiting Fellow	LTD:NHLBI
	Molina Bhatnagar	Research Aid	LTD:NHLBI

COOPERATING UNITS (if any):

LAB/BRANCH: Laboratory of Technical Development

SECTION:

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, Maryland 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.)

The third prototype of the cross axis synchronous flow-through coil planet centrifuge was constructed. The apparatus holds a pair of large coil holders symmetrically, one on each side of the rotary frame, at a lateral position 12.5 cm from the center of the holder shaft held 10 cm from the centrifuge axis. Mathematical analysis of acceleration generated by the planetary motion of the apparatus revealed a unique centrifugal force field which promises high retention of the stationary phase in the multilayer coil to perform efficient preparative-scale countercurrent chromatography.

Performance of the apparatus was evaluated in terms of stationary phase retention, partition efficiency and sample loading capacity. Preliminary studies with short coils revealed high retention of the stationary phase under a proper combination of the head-tail elution and planetary motion. Preparative capability of the apparatus was successfully demonstrated on efficient multigram separations of 2,4-dinitrophenyl amino acids, indole auxins, and bacitracin in a pair of large multilayer coils with a total capacity of 1.5L.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01463-03 LTD

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.)

Analytic High-Speed Countercurrent Chromatography with 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	LTD:NHLBI
Others:	Hisao Oka	Visiting Fellow	LTD:NHLBI
	Fumie Oka	Guest Researcher	LTD:NHLBI

COOPERATING UNITS (if any)

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NHLBI, NIH, Bethesda, Maryland 20892

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

A compact portable model of a high-speed countercurrent chromatograph with a 2.5cm revolution radius was constructed for performing analytical countercurrent chromatography. The capability of the apparatus was evaluated with short coils in stationary phase retention and with a multilayer coil for analytical separations. The results indicated:

1. The system is capable of retaining a satisfactory volume of the stationary phase for a variety of solvent systems in a coil with a helical diameter of around 3.75 cm.

2. Analytical capability of the apparatus was successfully demonstrated in separation of flavonoids from a crude sea buckthorn extract in a multilayer coil with a total capacity of 8 ml.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01467-01 LTD

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.)*

Horizontal Flow-through Coil Planet Centrifuge With Multilayer Coil Set.

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

P.I.	Yoichiro Ito	Senior Investigator	LTD:NHLBI
Other:	Hisao Oka	Visiting Fellow	LTD:NHLBI

COOPERATING UNITS *(if any)*

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NHLBI, NIH, Bethesda, Md. 20892

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 apparatus holds a set of four identical multilayer coils around the column holder. These columns are interconnected in series to make up a total capacity of about 200ml. Because of the eccentric orientation of the multilayer coils with respect to the axis of the holder, the radially acting centrifugal force from the holder axis retains the stationary phase, either the lighter or the heavier phase, in each helical turn of the coil while the planetary motion of the coil produces efficient mixing of the two solvent phases. The system provides universal application of two-phase solvent systems including aqueous-aqueous polymer phase systems used for partition of macromolecules and cell particles. The capability of the apparatus was successfully demonstrated in separation of cytochrome c and lysozyme in a polymer phase system composed of 12.5% polyethylene glycol 1000 in 1M potassium phosphate aqueous solution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01468-01 LTD

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.)
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	LTD:NHLBI
Other:	Hisao Oka	Visiting Fellow	NHLBI:LTD

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NHLBI, 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.)

The new high-speed countercurrent chromatograph eliminates the use of counterweight and accommodates two or more column holders symmetrically around the rotary frame to achieve perfect balancing of the centrifuge system. The multiple columns mounted on the holders can be interconnected in series to increase the partition efficiency and sample loading capacity of the system. Two prototypes were constructed: The first prototype holds a pair of column holders, one on each side of the rotary frame, and the second prototype has a set of three holders symmetrically arranged around the rotary frame. High performance of these prototype centrifuges was demonstrated in separations of various test samples which include DNP amino acids, boswellic acids, indole auxins, flavonoids, bacitracin and tetracycline derivatives.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01469-01 LTD

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.)*

Foam Countercurrent Chromatography with a 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	LTD:NHLBI
Other:	Hisao Oka	Visiting Fellow	LTD:NHLBI

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION

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.)*

By the use of the coil planet centrifuge reported earlier (Z01 HL 01455-02 LTD) foam countercurrent chromatography (CCC) was successfully applied to separation of bacitracin componenta with nitrogen gas and distilled water free of surfactant or other additives. In the batch foam separation, the bacitracin componenta were separated according to their hydrophobicity. In continuous enrichment of the diluted sample solution (50 ppm), the foam fraction showed over 1000 to 2000 fold enrichment of hydrophobic components such as bacitracin A and F while in the liquid fraction no hydrophobic components were eluted. The method may be extremely useful in enrichment of bioactive minor components in biological fluids such as urine, blood and blood dialysate, cell culture medium; etc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01470-01 LTD

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.)*

Surrogate Host for Testing Genetically Altered Cell Grafts.

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

P.I.	R. L. Bowman	Chief, LTD	LTD:NHLBI
Other:	F. Anderson	Chief, LMH	LMH:NHLBI
	James Zweibel	Medical Staff Fellow	LMH:NHLBI
	David Dichek	Medical Staff Fellow	LMH:NHLBI
	Nga Nguyen	Biologist	LMH:NHLBI

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 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 those that would be encounted in a graft implanted in a living animal.

The mock circulation is designed to maintain a biochemical milieu consistant with optimal tissue growth and survival while challenging the adhesive and secretory activity through the mechanical forces tending to dislodge the grafted cells.

Cells on various graft materials are being tested with some success in the form of adherent cells detected by a dye marker test of the expression to distinguish the altered cells.